

ABDEL – SATER, M. A.

Antagonistic interactions between fungal pathogens and leaf surface fungi of onion

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The role of the OHO-31 protein, an importin- α homologue, in the oogenesis of *Drosophila melanogaster*

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The importin- α (I- α) protein plays a crucial role in the import of proteins into the nucleus as an adaptor, which binds cargo proteins to the importin- β receptor. The I- α homologue gene, *oho31*, was identified by P transposon-mediated mutagenesis in *Drosophila*. In the wild type, OHO31 is mainly expressed in the ovary and the early embryo. The intragenic deletion *oho31*^{D14} abolishes OHO31 protein production and causes female sterility, while the nuclear protein import remains normal in the somatic tissues. Sterile females show numerous abnormalities in egg chamber development. The eggs are ventralized, the oocytes are smaller than normal with signs of degeneration, material transport from the nurse cells to and maternal mRNA localization in the oocyte are defective, etc. In wild-type egg chambers, OHO31 protein is abundant in stage 10 oocytes, concentrating along the cortical cytoskeletal network, but not in the nucleus. After disrupting the actin cytoskeleton with cytochalasin B, OHO31 enters the nucleus. These results suggest that this I- α homologue has a specific function in egg chamber development, which is distinct from the protein import into the nucleus.

ALFÖLDI, L.

The megacin story

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In 1953, Professor Ivánovics was occupied with the isolation of *Bacillus megaterium* strains from natural habitats and their screening for vitamin B12 producers. He was puzzled by the behaviour of one strain (No. 216), which often lysed spontaneously. This strain proved to be the first bacteriocin-producing Gram-positive bacterium. He found the phenomenon so interesting that he studied megacin and megacinogeny for the next 25 years, until the end of his active life. Even so, some aspects of megacinogeny remain unresolved at the present time.

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Investigation of the mitochondrial DNA organisation of *Trichoderma harzianum* strain

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Trichoderma isolates are known as potent biocontrol agents. Some isolates were successfully used against phytopathogenic fungi such as *Pythium debaryanum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Mitochondrial DNA organisation is less studied in *Trichoderma* species. For this reason, mitochondria were isolated from a mycoparasitic *Trichoderma harzianum* strain and nucleic acids were purified by lysing the mitochondria, with the aim of constructing a detailed physical and functional map of the mtDNA in this filamentous fungus. The mtDNAs were found to be circular, 32 kb in size. A physical map was constructed through the analysis of double-digestion patterns obtained by using three restriction enzymes, *EcoRI*, *EcoRV* and *CfoI*. Heterologous gene probes were used for the construction of a functional map, which derived from *Aspergillus nidulans* (L-rRNS, S-rRNS, cobA, ATP-ase subunit 9), or *A. carbonarius* mitochondrial DNA (NADH dehydrogenase subunit 5). All gene probes hybridized to the mtDNA of *T. harzianum*. The gene order was similar to that observed in *A. nidulans*.

ANTMANN, K.¹, P. ANDERLIK², Á. GHIDÁN², F. ROZGONYI²

Characterisation of *Staphylococcus aureus* strains isolated from air and patients during a hospital surveillance

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In a previous air bacteriological investigation in Törökbálint Pulmonary Hospital 37 *Staphylococcus aureus* strains have been isolated from the air sample of the wards and from the nasopharyngeal swabs of patients nursed in these wards. Primary characterisation of the strains by clumping factor reaction, lipase, lecithinase tests and antibiotic sensitivity patterns indicated two clusters of the strains. Further studies on minimal inhibiting concentration (MIC) of methicillin for the strains showed 16 strains to be methicillin resistant. The degree of virulence and the presence of above mentioned properties strongly indicate a common origin of methicillin resistant strains isolated from both the ward air and the patients being nursed in these wards. The results indicate a possibility for air-mediated nosocomial methicillin resistant *Staphylococcus aureus* infection.

ARZUMANIAN, V. G.

New synthetic media for cultivation of lipophilic yeast *Malassezia* spp.

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The lipophilic yeast *Malassezia* spp. (the recent name of genera - *Pityrosporum*) are the only yeast human saprophyte. Their role in some skin pathologies - pityriasis, seborrheic and atopic dermatitis, psoriasis intensively studied. Some metabolites of *Malassezia* can be strong allergens. Cultivation of *Malassezia* on usual lipid-containing media is not difficult but some components of these media are allergens themselves. In this case synthetic media would be more useful. Since 1976 (Nazzaro-Porro) we do not know other publications concerning the growth of *Malassezia* on synthetic media but we could not use these recipes. So the aim of present research was to construct the synthetic media for *Malassezia* spp. Growth capacity of the *Malassezia* spp. (*Pityrosporum* spp.) on the synthetic nutritional media has been studied. Isolates of *Malassezia* have been obtained from healthy human skin and identified by method of Guillot and Gueho (1996). The modified Dixon's medium was taken as a prototype for preparing the synthetic media. New media contained asparagine, Tween 40 and emulsifier as carbon and energy sources. The isolates of *Malassezia sympodialis* have demonstrated relatively fast growth on such media, in contrast to *M.globosa*. The duration of exponential growth phase was 1 - 3 days depending on the isolate.

AUCKENTHALER, R.

Diagnosis of mycobacteria: new developments

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Tuberculosis (TB) has increased in certain parts of the world due to war, socioeconomic insufficiency, HIV and lack of TB surveillance. Today one third of the world's population is infected with *M. tuberculosis* and according WHO there are 8-10 million new TB cases each year. Multidrug resistant strains (MDR) are emerging and laboratories are challenged to provide rapid identification and antimicrobial susceptibility testing for effective treatment. Various methods have been recently developed in the view to increase the sensitivity and to speed up diagnosis: 1) Direct examination including cyto centrifugation, auramine staining and amplification of *M. tuberculosis* complex in direct specimens. 2) Culture including solid and liquid media using the semi-automated methods BACTEC 460 TB or automated BACTEC 9240, BACTEC 960 with Mycobacteria growth indicator tube MGIT (Becton Dickinson), MB/BacT (Organon Teknika), ESP automated test systems (Accumed). 3) Detection of positive cultures by probes or amplification. The PCR products can be automatically detected (Cobas Amplicor) or analyze by sequencing. 4) Susceptibility tests using the automated culture systems or other technologies such as detection of resistant genes to rifampicin and isoniazid by PCR. Today, microbiology laboratories should be able to ensure that results of acid-fast examinations of specimens are available promptly (ideally, within 24 hours of specimen collection), TB control programs should have access to adequate

mycobacteriology laboratory services. Reports of isolation and identification of *M. tuberculosis* should be available within 10-14 days, and reports of drug-susceptibility tests should be available within 15-30 days of specimen collection (Centers for Disease Control and Prevention, MMWR 1995 ; 44 : RR-11)

AWAD-MASALMEH, M.

Virulence factors of VTEC bacteria isolated from animals, meat and meat products

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VTEC strains of the serotype O157 (n = 15) and of other serotypes (n = 70) were tested for harbouring genes encoding for the virulence factors *hly*, *pCVD419*, *eaeA*, *astA*, *katP*, *espP*, *etpD*, *ileX* and *colD157*. Gene detection was performed by PCR. Furthermore, the adhesion properties and sensitivity of all VTEC strains were investigated on HeLa-cells-monolayer and in Etest (AB BIODISK, Sweden). Genes encoding for *astA*, *katP* and *colD157* virulence factors were not detected in any strain of the serotype O157. VTEC of this serotype isolated from human, meatproducts and intestinal contents from ruminants showed a very similar gene-profile (*hlyA*, *astA*, *eaeA*, *espP*, *etpD* and *ileX*). Strains of the serotype O157 showed a weak and not reproducible adherence to HeLa-cells-monolayer and all of them were sensitive to enrofloxacin, ampicillin and amoxicillin in vitro. The gene encoding *katP* virulence factor was not found in any strains of the isolated non-O157-VTEC serotypes. The non-O157-VTEC isolates from meat products showed genes encoding for *hlyA* in 40 %, *eaeA* in 20 %, *astA* in 40 %, *etpD* and *ileX* in 10 % each. Similar results were also observed in cases of VTEC isolates from fecal samples of ruminants. They possessed the genes for *hlyA*, *eaeA*, *espP*, *colD157* and *ileX* in 40, 20, 16, 14, 6 and 4 % respectively. These isolated VTEC strains showed a broad spectrum of gene compositions. 10 % of them have attached to HeLa-cells-monolayer and were resistant to tetracycline (40 %) and ampicillin (20 %).

BÁCSI¹ A., J. ARANYOSI², Z. BECK¹, J. SZABÓ¹, K. SZARKA¹, F. D.TÓTH¹

Syncytiotrophoblast cells are permissive to the complete replicative cycle of human cytomegalovirus by contact with placental macrophage

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Maternofetal transmission of human cytomegalovirus (HCMV) is the most common cause of congenital viral infection. How HCMV crosses the syncytiotrophoblast (ST), the barrier between maternal blood and fetal tissue in the villous placenta is unknown. Although syncytiotrophoblasts can be infected by human cytomegalovirus (HCMV), ST cells do not support the complete viral reproductive cycle or HCMV replication may occur in less than 3% of ST cells. On the basis of these observations we investigated whether placental macrophages might enhance activation of HCMV carried in ST cells and infected ST cells would be capable of

transmitting virus to neighbouring macrophages. For this purpose, we studied HCMV replication in ST cells grown alone or cocultured with uninfected placental macrophages. Our results demonstrated that HCMV gene expression in ST cells was markedly upregulated by coculture with macrophages, resulting in release of substantial amounts of infectious virus from primarily infected cells. After having become permissive for viral replication, ST cells delivered HCMV to the cocultured macrophages as evidenced by detection of virus-specific antigens in these cells. The stimulatory effect of coculture on HCMV gene expression in ST cells was mediated by marked interleukin 8 and transforming growth factor β 1 release from macrophages, an effect caused by contact between the different placental cells. Our findings indicate an interactive role for the ST layer and placental macrophages in the dissemination of HCMV among placental tissue. Eventually, these interactions may contribute to the transmission of HCMV from mother to the fetus.

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Heavy metals transport in *Zea mays* – *Ustilago maydis* system and airborne fungal spores

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Among the seventeen sites of East-Hungary studied, considered as non polluted, were chosen to illustrate the main results. Chemical analyses of various components of the samples: soil, plant (maize) organs, theliospores (chlamydo spores of *Ustilago maydis*) revealed, according to site, an accumulation of metals, mainly Cu and Zn in fungal propagules. The Zn load (mg/kg) on 5 sites, taken as example, was close to the soil load. On site 8 it largely passed the concentration obtained in various plant organs. This unaccountable difference might be due to the absence or presence of mycorrhizal fungi of maize. The range of Zn content in different soil were 23 - 100 mg/kg, in plants organ 2 - 69 mg/kg and the *Ustilago* spores 19 - 70 mg/kg. Concerning Cu, variability between sites was enormous, but the load of this metal always was higher in chlamydo spores than in plant organs. In site 6 it also largely passed the soil load. The range of Cu content in different soil were 0.5 - 30 mg/kg, in plants organ 1 - 470 mg/kg and the *Ustilago* spores 5 - 78 mg/kg. Distribution of Cr, Cu, Ni, Zn and Cd in components of system (soil, roots, stems) revealed, for instance in site 15, a clear aptitude of chlamydo spores to accumulate metals, especially Cu and Zn. Morphological analyses of 0.01g chlamydo spores contains a mean of 150,3 millions units, grossly echinulated and with a diameter little variable between samples. Propagule germination varies according to sites, for unexplained reasons, and takes place through a germ tube, which may grow longer or give directly birth to «luftsporidie», followed by formation of typical yeast colonies. Microbiological analyses of chlamydo spores revealed an associated microflora, quantitatively and qualitatively variable between samples. So association with *Cladosporium* spp., *Fusarium* spp., *Acremonium* spp., *Mucor* spp. and *Penicillium* spp. The *Cladosporium* spp and *Acremonium* spp. were typical of some samples. We isolated on the surface of 0.01g chlamydo spores 5 - 15 millions associated yeast. The range of differences, voluntarily chosen wide, of geographical origins, sampling

dates, maturity, etc.. may be cause of observed variabilities. However it demonstrates the existence of chlamydospore populations which contain and disperse variable amounts of heavy metals. These populations are also different by their associated microflora, and probably by the proportion of metalloproteins formed during their genesis. Thus it may be concluded that *Ustilago maydis* chlamydospores are totally different according to their origin. Their impact on environment is thus predetermined by the quality of the place from which they come. The implication of this established fact is wide. An identical allergizing ability of these populations, for instance, cannot be conceived. This might explain in the field of allergology, where the significance of the relationship «dose-effect» is restricted, the notorious failure of commercial provocative tests, prepared in well-determined conditions.

BÁNSZKY, L., A MARÁZ

Genotypic characterisation of strains belonging to the *Schizosaccharomyces* genus by RAPD fingerprinting and ribotyping

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In this work several strains belonging to the *Schizosaccharomyces* genus were characterised by molecular typing methods such as RAPD-PCR analysis and ribotyping (RFLP of amplified rDNA sequence). These PCR-based molecular typing technics can be useful in the identification and comparison of yeasts species and strains. By the application of the ribotyping method, an rDNA sequence was amplified flanked by the specific primers ITS4 and NS1. The amplified sequence contained the whole nuclear small rDNA, the 5.8S rDNA, two ITS regions and a small part of the nuclear large rDNA genes. The amplified rDNA sequence was digested with different restriction enzymes (*Hae* III, *Msp* I, *ScrF* I, *Sau* 3 A I) of four- and five-base recognition site. RAPD-PCR fingerprinting was carried out with four different 10-base random oligonucleotide primers of 50, 70, and 80 % G+C contents. Dendograms based on the RFLP of rDNA sequences and RAPD-PCR showed three distinct clusters of strains with low degree of similarity, which corresponded to the currently accepted three species of the genus *Schizosaccharomyces* as follows: *Schizosaccharomyces pombe*, *Schizosaccharomyces octosporus* and *Schizosaccharomyces japonicus*. Any of the four used restriction enzymes was suitable for differentiation of *Schizosaccharomyces japonicus* and the other *Schizosaccharomyces* strains, while restriction enzymes *Hae* III and *Sau* 3 A I resulted also differences between *Schizosaccharomyces octosporus* and *Schizosaccharomyces pombe* strains. Within each of these species ribotyping did not show any difference, indicating that this method is excellent for identification at the species level. RAPD-PCR analysis resulted also the previous three clusters of species, but showed more differences at intraspecies level. We could not find however, any clustering of the formerly delineated varieties of *Schizosaccharomyces pombe* and *Schizosaccharomyces japonicus*.

This work was supported by the Hungarian Research Grant OTKA No. T 22744.

BARABÁS, É., K. NAGY, V. VÁRKONYI, R. GONZALEZ, A. HORVÁTH

Disregulated mRNA expression of type 1 (IFN γ) but not type 2 (IL-10, IL-4) cytokines in asymptomatic phase of HIV infection

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The spontaneous expression of mRNA of IFN γ (type 1), and IL-10, IL-4 (type 2) cytokines in asymptomatic phase of HIV infection were analyzed and correlation between altered mRNA levels and disease progression were studied. In separated PBMCs of 1.) HIV infected individuals with < 200 CD4+ cells/ μ l; 2.) 300-400 CD4+ cells/ μ l; 3.) > 500 CD4+ cells/ μ l; 4.) their HIV-negative homo/bisexual partners; 5.) uninfected atopic patients; 6.) healthy male controls; relative expression of mRNA for IFN γ and IL-10, IL-4 with respect to β -actin were determined by semi-quantitative RT-PCR analysis. Spontaneous IFN γ mRNA in all of HIV infected groups (independently of CD4+ cell count) was significantly elevated compared to that of non-infected groups ($p < 0.015$, Student's test). There was no significant difference in IFN γ mRNA expression between HIV infected groups as well as between non-infected groups. Transcriptional level of IL-10 mRNA was moderated in all of groups studied and there were no significant difference. IL-4, however, showed significantly increased expression in atopic group ($p < 0.01$). Based on increased IFN γ mRNA expression these results suggest an elevated antiviral activity in immune system in asymptomatic phase of HIV infection. Elevation in type 2 cytokines mRNA levels was not observed in cross-sectional study. However, type 2 cytokines may have significant role in the progression of disease which could be analyzed by longitudinal study.

BARABÁS-HAJDÚ, E.¹, Z. PÁVAI², N. OPRIS³, Á. GÁLL³

In vitro study on the *Giardia lamblia* cysts' behaviour at different pH-s of the environment

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Giardia lamblia infection occurs in humans by ingestion of the infectious cysts present in contaminated water or food. Excystation begins in the stomach due to its acidic pH. The released trophozoites colonize the upper part of the small intestine where they attach to the apical edge of the epithelial cells and multiply by binary fission. Some of the trophozoites encyst, producing a rigid cyst wall that protects them after being excreted with the feces. We try to reproduce in vitro the steps of the life cycle of this primitive eukaryote starting with the excystation process. In our study we show that *Giardia lamblia* cysts obtained from persons with symptomatic giardiasis, after being purified by using the sucrose-gradient technique and

concentrated by centrifugation at 600 g/min for 15 minutes, are counted in a haemocytometer and 10^5 cysts are exposed to different pH-s of the environment, using 1 ml of HCl sol. 1N, pH-s 0,5; 1,0; 1,5; 2,0; 2,5; 3,0; 3,5; 4,0; 4,5; 5,0; 5,5 and 6,0. After incubation in cell culturing chambers at 37°C for 5, 10, 15, 20, 25, 30, 35, 40, 45 minutes and 1h, samples are taken and carefully examined under the microscope, magnified by 400. In spite all our efforts excystation was obtained only at pH 3,5 where excystation rate was very low (2%). We continue our study to obtain enough trophozoites to be able to cultivate these important microorganisms.

BAYOUMI HAMUDA, H. E. A. F.¹, H. ABDORHIM², I. JEVCSÁK¹, H. GODWAR², M. KECSKÉS¹

Ecotoxicological tests of Cd²⁺, Cu²⁺, Ni²⁺ and Pb²⁺ on the growth and symbiosis of *Vicia faba* - *Rhizobium leguminosarum* bv. *viciae*

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In soil ecotoxicological studies, the toxicity of heavy metals is usually investigated by mixing metal salts at different concentrations into the soil. In such tests, no attention is paid to the possible effect of the anionic partner of the investigated metal, which may itself has an adverse effect on soil microbiota. Investigations were carried out using four different techniques (paper disk, hole, streak and microfermentor) to study the effects of different polluted materials (Cd²⁺, Cu²⁺, Ni²⁺ and Pb²⁺) applied to cultural medium (*in vitro*) and to soil (*in vivo*) in five concentrations (10, 20, 40, 80, and 160 mg l⁻¹ medium or mg kg⁻¹ soil) on growth of *Rhizobium leguminosarum* bv. *viciae* and the symbiotic interaction with *Vicia faba* L. plant. The results showed that, there were no differences among the four used techniques. Therefore, the results of microfermentor method are summarized here. Strain of Lóbab Z proved to have the highest tolerance among the strains to tested chemicals ions followed by Bükköny 75/4, HB-3841*str*⁺, and E1012. The ions of Pb²⁺ was the least toxic and Ni²⁺ and Cu²⁺ were the most toxic ions *in vitro* followed by Cd²⁺. An agroecosystem symbiotic model of mesocosm experiments was also conducted in greenhouse using sterile acidic (pH 4.7) brown forest soil of Gödöllő to study the effects of tested ions *in vitro* on the growth of horse bean seedlings and its symbioses with *Rhizobium leguminosarum* bv. *viciae* (E1012, HB-3841*str*⁺, Lóbab Z, and Bükköny 75/4) strains. Results indicated that, Ni²⁺ and Cu²⁺ ions highly inhibited plant growth, nodulation and N₂-fixation at 40 - 80 mg⁻¹ soil the applied dose. The plants inoculated with Lóbab Z, and Bükköny 75/4 were more tolerants to the polluted ions than others. These inhibitions were reduced when the soil was treated with Cu²⁺ - containing fungicide (Cobox) before the plantation and rhizobial inoculation by three weeks. Also, the results proved that, the chloride forms compared to sulfate forms of metals had more ecotoxicological effects in both investigations *in vitro* and *in vivo* as well.

BAYOUMI HAMUDA, H. E. A. F.¹, A. KHALIF², L. KÖDÖBÖCZ¹, M. KECSKÉS¹

Effect of dicyandiamide, nitrapyrin, 2,4-D and thiourea on the growth and

symbiosis of *Vicia faba* - *Rhizobium leguminosarum* bv. *viciae*

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Decreasing the time of nitrification and increasing the time of nitrogen availability for plant absorption should increase the efficiency of soil fertility as well as the useful consumption of biological N₂-fixation. Inhibiting nitrification offers the availability of nitrogen in the reduced form and thus may prove to be a useful tool in maximizing soil bioproductivity and minimizing water pollution with oxidized nitrogen forms. The effects of nitrification inhibitors: Dicyandiamide (1-cyanoguanidine), Nitrapyran (2-chloro-6-(trichloromethyl)-pyridine), 2,4-D (2,4-Dichlorophenoxyacetic acid) and Thiourea at 0, 0.1, 1, 10, and 100 mg l⁻¹ on the growth and the respiratory activities of the *Rhizobium leguminosarum* bv. *viciae* strains (E1012, HB-3841^{str+}, Lóbab Z, and Bükköny 75/4) were studied *in vitro* (in yeast extract mannitol broth medium using microfermentor and Warburg's respirometer methods). Plant seedlings inoculated with *Rhizobium* strains in acidic (pH 4.7) Gödöllő brown forest soil with low humus (1.22 %) content treated with Dicyandiamide, Nitrapyran, 2,4-D and Thiourea at 0, 0.1, 1, 10, and 100 mg kg⁻¹ soil was also studied *in vivo*. The results of *in vivo* documented the *in vitro* observations, in which Dicyandiamide had relatively low effect on the growth and respiratory activities of microsymbionts and symbiotic parameters (plant growth and dry weight, nodulation potential and N₂-fixation). However, Thiourea was most toxic inhibitor, but 2,4-D and Nitrapyran had moderate effects in both ecosystems. The results showed that, the symbiotic parameters of the plant seedlings inoculated with Lóbab Z, and Bükköny 75/4 strains were more higher than of those inoculated with E1012 or HB-3841^{str+} strains in all treatments.

BEZNER, J.¹, I. VIDÁCS¹, J. TELEGDI², Gy. KANYÓ-PRINCES³

Bacterial attachment and growth on surfaces in the poultry processing industry

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Adhesion of microbes to different metals and their propagation on the surfaces is well known. The biofilm consists of extracellular microbial products, mainly polysaccharids, and embedded microbes and other materials. The biofilm is cause of a number of difficulties, i.e. energy loss in industrial cooling water systems, microbiological corrosion, contamination with pathogen and spoilage microbes in the food industry. In the medical practice biofilm formation on catheters, implantatums, pacemakers and other materials in the body may cause health problems. Within the biofilm the physico-chemical environment, the distribution of nutrition change, which – considering the survival - might be useful for the

microbes. From practical point of view, the most important change is the increase of bacterial resistance toward disinfectants and other factors. The culturing of microbes from the biofilm is difficult and non-reliable. Investigating the poultry processing line the places where the biofilm formation is the most probable were detected. The most critical places were selected and investigated during processing and after cleaning and desinfestation using traditional methods (total microbial count, *Enterobacteriaceae* and *Streptococcus faecalis*). Next to the microbiological investigation the adhesion and propagation of microbes were proved by microscopic investigations, too (epifluorescence microscopy, atomic force microscopy).

The investigations are part of the OTKA project No. T 020792.

BÉLÁDI, I.

Avian and human interferon studies initiated by György Ivánovics

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One of the interests of Professor György Ivánovics in 1959-60 was the kinetics of inactivation of the Aujeszky virus by ultraviolet (UV) irradiation. Accordingly, we studied whether the formation of small plaques was due to the increased capacity of interferon (IFN) induction by the UV-treated virus. Human adenoviruses were applied as negative control, because at that time adenoviruses (in contrast with other viruses) were considered to be unable to induce IFN. It turned out that adenoviruses, while not multiplying in chick embryo fibroblasts, are effective inducers of IFN. This was the beginning of a long-lasting study on the IFN-inducing ability of adenoviruses, and mainly that of human type 12. On the basis of our intensive IFN work, we joined in with the human leukocyte IFN production programme of the EGIS Pharmaceutical Company. Technology for the production of human leukocyte IFN and of the inducer Sendai virus has been developed on a laboratory scale. The quality and the biological properties of the produced IFN (Egiferon) have been studied. The antigenicity has been compared with those of the recombinant IFNs.

BELÁK, S.

Molecular epizootiology of pestiviruses

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The genetic variability and the molecular epizootiology of pestiviruses were studied by our group and the observations are summarised in this article. The *Pestivirus* genus in the *Flaviviridae* family comprises four genotypes, namely classical swine fever virus (CSFV), bovine viral diarrhoea virus (BVDV) types I and II and Border disease virus (BDV), major pathogens all over the world. The current nomenclature and classification based on host-species, should be revised, because crossing of host-species was observed in case of several genotypes. The aim of the present study

was to obtain data for molecular characterization and epidemiology of pestiviruses. The analysis of the 3' untranslated region (3'-UTR) of the viral genome provided a clear separation of the four genotypes. Inter-genotypic recombination was not observed, but BDV and BVDV I showed great intra-genotypic variability. Various deletions and insertions were observed in the 3'-UTR, further demonstrating that the 3'-UTR was less conserved than the 5'-UTR. In studies on the genetic variability of CSFV, a large collection of viruses obtained from 20 countries over a period of a half-century was analysed by RT-PCR and direct sequencing. When parts of the E2 and the NS5B (the putative polymerase gene) coding regions were analysed, two main phylogenetic groups were separated, indicating that the virus apparently evolved in two ancestor nodes. Investigating the genetic variability of CSFV in the rather restricted geographic area of Central Europe, comparative sequence analysis of the 5'UTR, the E2/NS2 and NS2 gene regions was performed. The viruses were separated into subgroups that largely coincided with their regions of origin. Another study showed that a simple restriction endonuclease cleavage assay of the 5'NC PCR products was useful to discriminate vaccine virus strains and recent field virus variants in Europe. The collected sequence data allow the rapid classification of newly emerging pestiviruses. For example, a pestivirus termed Frijters, which was spreading in swine populations in Europe, was identified as not of the CSFV genotype, but rather as a variant of an ovine pestivirus. This information prevented the unnecessary slaughter of large numbers of pigs. By studying the genetic variability of BVDV, two new subgroups of type I virus, distinct from subgroups Ia and Ib, were detected when viruses from Southern Africa were analysed. Additionally, pathogenicity markers in cytopathogenic BVDV strains were identified and different types of genetic rearrangements were described within the NS2-NS3 gene region. *In vivo* studies were performed with two genetically distinct isolates of African BVD viruses. In New Zealand, the presence of BVDV I in cattle and BDV in sheep was proved, while BVDV II was found in fetal calf serum samples of USA origin. By studying a number of relatively small farms in Sweden, a strict, herd-specific genetic clustering of BVDV was observed. The molecular characterization of ovine pestiviruses at the 5'-UTR and the N^{PTO} region revealed that sheep may naturally be infected not only with BDV, but also with BVDV types I and II. This indicates the inadequacy of the current host-species based nomenclature and classification of pestiviruses. In summary, the genetic studies provided novel data for characterization and classification of pestiviruses. The sequences were deposited in the GenBank and the obtained findings contributed to the establishment of molecular epizootiology. By means of rapid sequence analysis, the viruses are promptly identified during the new outbreaks. Molecular epizootiology provides powerful novel means to control the diseases caused by pestiviruses.

BENEDEK, O., J. KNURR, B. VINSON, C. L. TURNBOUGH JR.

Identification of peptide ligands that bind *Bacillus subtilis* spores

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Using a phage display screening system based on a combinatorial library of random

7-amino acid peptides individually displayed on the surface of the filamentous coliphage M13, we identified peptide ligands that bind tightly to the surface of *Bacillus subtilis* spores. The tight-binding phage were selected from the library by several rounds of biopanning, after which individual phage were isolated, amplified, and their genomic DNA extracted. The peptide-encoding region of the genome was sequenced to determine the amino acid sequence of the tight-binding peptide. These peptides were chemically synthesized and tested for use in spore capture and identification. The tight-binding peptides identified contain a consensus sequence found near the amino terminus of the SpsC protein, which apparently binds to the surface of the *B. subtilis* spore and is involved in spore coat polysaccharide synthesis. Presumably our peptides mimics the coat-binding region of SpsC. Several tight-binding peptides were fluorescently labeled and shown to be capable of species-specific identification of spores by fluorescence activated cell sorting (FACS). One of these peptides was also attached to a water-insoluble polymer, coated on a glass slide, and shown to specifically capture *B. subtilis* spores in aqueous suspension. Fluorescently-labeled monoclonal antibodies against M13 phage are currently being used to detect the *B. subtilis* tight-binder phage on the surface of the spore to improve FACS analysis.

BEREK, I.

The story of the biochemical and genetic analysis of porphyrin biosynthesis in *Bacillus subtilis*

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The story of studies of porphyrin biosynthesis in *Bacillus subtilis* started in 1965 with the activities of Professor György Ivánovics and his staff. Numerous porphyrin auxotrophic mutants were isolated from the 168 trp C2 strain of *Bacillus subtilis* by selection with streptomycin. Some of them could be supplemented with ALA, while the majority grew only in the presence of hemin. Among the latter strains, the syntropism test allowed the distinction of two groups differing in phenotype, viz. feeders accumulating ALA and non-feeders accumulating not ALA but other porphyrin intermediates. On the basis of transductional studies, feeders and non-feeders could be divided into two and four groups, respectively. Biochemical investigation revealed that, with one exception, one enzyme of the porphyrin biosynthesis was coordinated to each hem locus. The following genes were identified: hem A - ALA synthetase, hem B - ALA dehydrase, hem C - PBG deaminase, hem D - uroporphyrinogen cosynthetase, hem E – uroporphyrinogen decarboxylase, hem F – coproporphyrinogen oxidase, and hem G - protoporphyrin iron chelatase. Mapping of the hem genes demonstrated that the hem A, hem B, hem C, and hem D loci are located on the left replication arm of the *Bacillus subtilis* chromosome, and the other known hem loci on the right arm.

BERCOFF, R. P.

The molecular basis of recombination of poliovirus: Implications for

eradication programmes

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BERTSCHINGER, H. U.¹, E. MEIJERINK², P. VÖGELI²

Prevention of infectious diseases of animals through breeding for genetic resistance

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Most information on genetic resistance to infectious diseases has so far been obtained from laboratory mice and from poultry. The impact on animal production has been minimal, whereas vegetable farming and horticulture have derived considerable advantage from the development of genetically resistant plant breeds. Research into microbial virulence factors has in many cases revealed an absolute need for adhesion to highly specific receptor structures on host tissues. Genetic polymorphisms may cause variation in these receptor structures. Examples of economical relevance have been found in the field of porcine enteric *Escherichia coli* infections. In the case of fimbrial type F4, many pigs have no enterocyte brush border receptors for enterotoxigenic *E. coli* strains with this adhesin. The receptor status is inherited in a simple Mendelian fashion, presence of receptor being dominant over its absence. The receptor phenotype can only be identified by means of a microscopic enterocyte adhesion test, i.e. after death of the pig. The second common fimbrial type, F18, is associated with porcine *E. coli* strains producing Shiga-like toxin 2e and/or enterotoxin(s). The F18 receptor is also inherited as a dominant trait. Our group has found that it is coded for by a gene on chromosome 6 which is perfectly linked to - and probably identical with - the gene for fucosyltransferase 1 (*FUT1*). The genotype at *FUT1* can be determined by PCR starting with DNA extracted from a blood or tissue sample. This allows the selection for breeding stock of homozygously resistant pigs, a method which is already practiced in Swiss pedigree herds.

BESZTERI, B., É. ÁCS, J. MAKK, G. KOVÁCS

Application of molecular methods in diatom taxonomy

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Molecular biological methods are becoming widespread tools also in phycology recently. Until now the most commonly used technique is sequence analysis of 18S rDNA, most frequently with the aim of determining relationships of large taxonomical units (divisions, classes, orders) of algae and other eukaryotes. The

purpose of our work started recently is to use this tool in diatom taxonomy on a lower taxonomic scale.

Diatom taxonomy is a good example of morphology-based taxonomy because several morphological characters found on the finely structured silica valves serve as key differential characters. Generally electron microscopic techniques are used in these examinations, and the number of species described and identified by small differences in valve structure is increasing fast. Much less investigations can be found on non-morphology based (e.g. biochemical, molecular biological, ecological) differences between and within diatom species. We're trying to use 18S rDNA and ITS sequence comparisons to determine genetic differences between some "morpho" species.

As the first step, cultivation and purification experiments were made. For purification of the cultures both streaking and single-cell isolation methods and their combinations were used. For sequence determinations the widely used methods of PCR amplification and direct sequencing of the product were used. As the first step of our study we determined sequences of some well growing diatom species (e.g. *Melosira varians*, *Amphora montana*, *Gomphonema parvulum*, *Navicula minima*, *Navicula subminuscula*, *Phaeodactylum tricornutum*,) and compared them to those already available. The phylogenetic tree constructed does not fit exactly with the tree based on phenotypic characters.

BINTSIS, T.¹, R. DAVIES¹, E. LITOPOULOU-TZANETAKI², R. K. ROBINSON¹

Microbiology of Feta cheese brine

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The primary and secondary micro-floras of two batches of Feta cheese brine from two different factories in Northern Greece were investigated. The microbiological analysis included total count, coliforms, enterococci, *Staphylococcus* spp., yeasts, lactic acid bacteria, salt-tolerant bacteria, *Listeria* spp. and *Yersinia enterocolitica*. Chemical analyses (pH, NaCl, free fatty acids, ash, protein and lactose content) were also carried out; representative samples of brine were taken in duplicate at 0, 10, 20, 30, and 60 days from manufacture. The brines had low pH values (4.8-4.0) and high salt contents (5.7-8.0%) which along with nutrients leaching from the cheese, made them a special medium with a specific micro-flora. The total counts were 1.4×10^7 - 3.1×10^8 cfu/ml, the counts for lactobacilli and streptococci/lactococci were 1.5×10^6 - 1.8×10^8 cfu/ml and 2×10^6 - 1×10^8 cfu/ml respectively, and yeasts were found to be 1.1×10^5 - 1.9×10^6 . Coagulase-negative *Staphylococcus* spp. declined from 2.8×10^4 to 30 cfu/ml over the 60 days, while coliforms were present in only one sample and decreased during maturation; enterococci were found only in one sample at low numbers (1.9×10^3 cfu/ml). The high numbers of lactobacilli, streptococci/lactococci and yeasts suggest that these microbial groups play an important role during the maturation stage of Feta cheese, but the survival of pathogenic bacteria could be a problem. Consequently, a novel decontamination system using UV/furocoumarins

was applied to simulated cheese brines in order to determine the effect of the system on the natural micro-flora and on selected pathogenic bacteria capable of surviving in cheese brines.

BIRÓ, B.¹, L. KÖDÖBÖCZ², M. KECSKÉS², P. L. EBERBACH³

Comparative *in vitro* study for the herbicide sensitivity of various authentic and Hungarian *Rhizobium* strains belonging to different species

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Compatibility of the legume cultivations by the various xenobiotics is the crucial point of developing the sustainable agricultural practices. Among the agrochemicals, the herbicide applications are the most frequent treatments throughout the World. The effect of the most abundant herbicides (of the *Rhizobium*-legume systems) with various active ingredients, such as 2,4-D, trifluraline, MCPA or N-phosphono-methyl-glycine has been examined among *in vitro* conditions. Four concentrations (0.1, 1, 10 and 100 $\mu\text{g l}^{-1}$) of these chemicals were used in a micro-fermentor (or in a bio-photometer). The cell number of the various strains was measured after 15 hours of incubation (or permanently in every 20 minutes), starting from the 10^6 CFU ml^{-1} in liquid YEM media. Six authentic *Rhizobium* strains of the Australian Type Culture Collection was examined, belonging to various species, such as *R. leguminosarum* bv. *trifolii* (NA-25, NA-14, NA-71) bv. *viciae* (NA-503) bv. *phaseoli* (NA-575), *R. meliloti* (NA-355), and also some other hungarian isolates from *Coronilla varia*, *Lupinus albus*, *Trifolium repens*, *Medicago sativa* and *Glycine max*, so as to compare the inter- and intra-specific variations in the sensitivity. Among the herbicides investigated, the glyphosate with N-phosphono-methyl-glycine content proved to be the less harmful for the *in vitro* growth of rhizobia. After 14 hours of incubation the cell number significantly was reduced by 0-31 % only at the 10 $\mu\text{g l}^{-1}$ concentration comparing to the untreated control. Due to the more frequent use of glyphosate in the clover-grass mixtures abroad, the authentic *R. leguminosarum* bv. *trifolii* strains was found to be less sensitive comparing to the home inoculum strains for clover. Importance of the effecting periods of pesticides in the adaptation and the natural selection of tolerant lines, therefore, is highly concluded. Sensitivity on the other hand proved to be positively correlated rather with the single isolates and the origin, than the species and hosts of the fast- and slow-growing *Rhizobium* (*Bradyrhizobium*) sp.

Sponsored by the Hungarian Research Fund (OTKA): T 023543 and T 030941.

BIRÓ, S.¹, Zs. BIRKÓ¹, A. SÜMEGI¹, A. VINNAI¹, G. WEZEL³, F. SZESZÁK¹,
S. VITÁLIS¹, P. SZABÓ², Z. KELE², T. JANÁKY²

Characterisation of the gene for factor C, an extracellular signal protein involved in morphological differentiation of *Streptomyces griseus*

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The gene encoding factor C (*facC*), an extracellular signal protein of cellular differentiation was cloned from *Streptomyces griseus* 45H and the complete nucleotide sequence was determined. The deduced amino acid sequence was also confirmed by high performance liquid chromatography/electrospray ionization - mass spectrometry analysis. The full length protein consists of 324 amino acids with a molecular mass of 34 523 Da. The mature extracellular 286 amino acid protein (M_r 31 038) probably produced by cleaving off a 38 amino acid secretion signal sequence. Southern hybridization revealed the presence of the gene for factor C (*facC*) in several other *Streptomyces* strains but searching of the databases failed to identify a protein with significant homology to factor C. The effect of the expression of factor C from low and high copy number vectors in different *S. griseus* strains on cytodifferentiation and antibiotic production and its possible role will be discussed.

BOIKO, N.¹, **Z. FÁBRY**², **V. LYTVYN**³, **M. MIHÁLY**⁴

Prophylactic effectiveness of a new bacterial biopreparation, "Monosporine-PK", against acute gastrointestinal infections of some agricultural animals and poultry

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It has been found that from the diseased calves and piglets, enterobacteria were regularly excreted, ranked by genera according to their excretion frequency as follows: *Escherichia* (45%); *Klebsiella* (16, 5%); *Citrobacter* (12, 5%); *Proteus* (9%); *Shigella* (6%); *Serratia* (4,5%); *Yersinia* (4%); *Hafnia* (1%); *Enterobacter* (0,5%). Certain peculiarities of the diseased chickens' intestinal microflora had been found main reflected by prevalence of *Escherichia*, *Salmonella*, *Proteus* genera enterobacterias. The biopreparation "Monosporine - IIK" manufactured in two medical forms according to the Ukrainian Technical Specification TY Y 46.15.275 – 97 in quantity of three million doses. It was applied to prevent acute gastric disturbance of the above etiology of the new born diseased animals, in compliance with the State Testing methods and Biopreparation Use Instruction approved by Ministry of Agriculture and Food Production of Ukraine. "Monosporine – PK" 's effectiveness was collated with that of "Bacterin – SL", "Lactosporine", "Bifidumbacterine" used as control. "Monosporine – PK" and "Bifidumbacterine" were observed to reveal the highest protective activity. Impact of the eubiotics applied upon the gastrointestinal microflora of animal and poultry, was studied for all the experiments as well as histological studies of the organs were carried out and haematological indices of blood were studied. Correlative dependence between the weight increase of the newly born calving and piglets and chickens and the thyroid

gland's functional state as revealed is worth most peculiar attention. Using the immuno-ferment analysis, we showed that during pathological processes (bacterial diarrhea, dyspepsia), thyroxin (T₄) triiodothyronine (T₃) and thyrotrophin (TTG) contents in the blood of the experimental animals changed ambiguously. Depending on seriousness of the disease, we observed both decrease of T₃ and T₄ contents with increase of TTG and increase of T₃ and T₄ contents with TTG decrease trend. The both trends are negative for they result in protein synthesis recession, dominants of catabolism over anabolism this being one of the reasons why the animals would lose their weight. Preliminary use of "Monosporine – PK" may ensure normalization of the thyroid gland's functional indices.

BORAS, E. A. M.¹, R. KISS², G. KOVÁCS¹, K. MÁRIALIGETI¹

Isolation and characterisation of *Listeria* spp. from food and environmental sources

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The results of our set of examinations showed that *Listeria* spp. however not frequent, can be isolated from environmental samples like food, sewage, sewage sludge cake, soil, and rotting leaf material. Clear results were obtained by using a two stage enrichment technique and three "selective" media. PALCAM, Oxford, and Blood agars were highly effective and differential.

Isolates were identified by the use of BBL Crystal Gram-positive technique as *Listeria monocytogenes* all except two (RIA, RIC) which were delineated as *L. grayi* and *L. murrayi*. Serotyping was also done for all 35 isolates of *Listeria*. The serotypes of food isolates consisted of (six) 1/2a, (four) 3a and (one) 4b strains, whereas strains from sewage, sewage sludge cake consisted of (four) 1/2a, (ten) 3a, (three) 4ab and (one) 4b serotype. Samples from rotting leaf material, and soil contained (four) 3a, (one) 4e and (one) 4c type strain.

Comparison of the BBL Crystal identification results with 16S rDNA sequence based identification showed a good correlation excepting two ARDRA groups. Group A strain (identified as *L. monocytogenes* by BBL Crystal test) proved to be *L. ivanovii* and group H, RIA strain (BBL identified as *Listeria grayi*) could be determined by sequencing as *L. innocua*.

The results of our survey suggest that there is a considerable reservoir of *Listeria monocytogenes* in human and animal populations. The organism exists either as commensal in the gut or as a causative agent of subclinical or clinical infections, occurring from time to time following ingestion. We have shown that *Listeria monocytogenes* is widely distributed in sewage, food and soil, and rotting leaf material. It is interesting to note that sewage sludge cake (used as an agricultural fertiliser) was found to harbour few listerias. We have to call attention to the use of CAMP test, because the lack of using it can led to improper identification with BBL Crystal.

BORSODI, A. K.¹, A. MICSINAI¹, I. MIKLÓS², O. ORAVECZ¹, Cs. ROMSICS¹

Bacterial communities participating in the biodegradation of *Phragmites* rhizomes

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Microbial activity contributing to the breakdown of organic matter plays a crucial role in the nutrient cycling of wetland ecosystems. In this study investigations were carried out on the microbial communities participating in the biodegradation of reed (*Phragmites australis* /Cav./ Trin et Steudel), which is a dominant species in the littoral vegetation of Hungarian lakes.

Following a reed harvest in winter, reed rhizomes were exposed in net bags floating on the water surface to biodegradation. Seasonal samples were taken. From the four samples bacterial strains have been isolated by taking scrapings of the reed rhizome surfaces and tested using classical microbial methods. The carbon-source utilisation pattern was investigated with BIOLOG plates. The results were subjected to numerical analysis and species level identification was carried out.

The results have shown that the late autumn and winter samples, and the late spring and summer samples were in closer relationship to each other, based on their bacterial community structure. In the former two samples primarily facultatively chemolithotrophic bacteria (*Xanthobacter*, *Ancylobacter*, *Alcaligenes*, *Hydrogenophaga*) were identified with the potential to establish a closed nitrogen cycle within the bacterial biofilm formed on decaying rhizomes and showing preference towards organic acids and not easily degradable biopolymers (cellulose). In the late spring – and summer samples, however, facultatively anaerobic fermentative bacteria (*Aeromonas*) came out as dominant utilising primarily simple sugars and easily degradable biopolymers. Bacteria possessing oxidative metabolism (*Pseudomonas*, *Bacillus*) were characteristic in the colder periods.

The PCA method applied to the carbon-source utilisation patterns of the strains of different seasons gives further evidence of a seasonal dynamics.

BOŽ ANIC , R I., ROGELJ², L. J. TRATNIK¹

The influence of inulin addition on sensory and microbiological quality of fermented beverages from cow's and goat's milks

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Prebiotics are dietary additives that encourage the selective growth of beneficial organism in the intestinal tract. Most prebiotics that have been studied up to date are nondigestible oligosaccharides. The most common of these are

fructooligosaccharides composed of β -fructan units; in this group inulin and its enzymatic hydrolysate are included. Beneficial properties of prebiotics are of interest in the production of fermented milk beverages. The type of milk is very important for the fermented milk production as well. Although goat's milk, according to published information, is used for the therapeutic purpose, the production of fermented goat's milk is not significantly investigated until now. In this work the fermentation of cow's and goat's milk with and without inulin addition is investigated. Each type of milk was divided in two parts and in one part inulin was added (1,5%). Milk was fermented with ABT4 culture, containing *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium* sp. Fermented samples were stored in refrigerator (5° C) for 28 days. On 1st, 7th, 14th, 21st and 28th day pH was measured and microbiological and sensory analysis were performed. The fermentation of cow's milk was about 1 hour shorter (5²⁷) than goat's milk fermentation (6²⁵). At the end of fermentation Str : Bif : Lb ratio in all samples was similar 40: 33: 27, respectively. Viable count of *Streptococcus* was the highest, log N = around 10⁹/ml, and remained unchanged during 4 weeks of storage. During the fermentation lactobacilli growth poorly and its survival during storage was inferior. After first week of storage viable count of lactobacilli in all samples was lower than log N=10⁶. Sensory taste of fermented products was excellent. Samples with inulin possessed better consistency and higher viscosity. The inulin addition showed no influence on fermented cow's milk taste, while the specific flavour of goat was less expressed. Sineresis in goat's fermented milk samples with inulin was negligible compared with samples without inulin. This observation has shown great effect of inulin on stability of coagulum. The same observation was noticed at 28th day of storage.

BOZSIK, A.^{1,2}, Á. GRALLERT¹, M. SIPICZKI^{1,2}

The cytological and genetical study of the dimorphic yeast *Schizosaccharomyces japonicus*

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The majority of microscopic fungi including several pathogenic species is able to proliferate either in unicellular or filamentous form. This dimorphism promotes their adaptation to the changes of environmental conditions. The unicellular yeast morphology is more advantageous in fluid media while the filamentous mycelial form benefits solid substrates. In case of pathogenic dimorphic species it is the filamentous form that is generally more destroying as it is easier for it to invade into the host tissues. The species *Schizosaccharomyces japonicus* possesses both morphology and can convert these shapes according to the changes of environmental conditions so serves as a good model organism in studying metamorphosis. We revealed that starving provokes the transformation of unicellular yeast cells into filaments in solid media; this metamorphosis is maintained by the food gradient and can take place only in specific temperature interval. This event involves several drastic changes. The polarity of growth alters: yeast cells grow bipolarly, filamentous hyphae grow unipolarly. Yeast cells divide exactly in the middle while mycelial cells form a smaller apical and a larger distal

daughter cell. Hyphae grow much faster than yeast cells due to the large vacuoles generate at the non-growth pole of the cell. The situation of interphase actin is bipolarly in yeast cells and unipolarly in hyphae. Even the organization of microtubules is different in mycelial hyphae. Yeast cells grow only on the surface of the solid substrate, hyphae, however, invade into the medium. The two morphology can convert into each other reversibly. The changes of shapes depend on the level of cAMP: metamorphosis can be prevented by raising cAMP level. We isolated several morphological and auxotrophic mutants from the wild type of the strain. With the help of these mutants we started genetic analysis. We intend to generate a cloning shuttle-vector.

BÖSZÖRMÉNYI, E., K. LENGYEL, H. PAMJAV, E. SZÁLLÁS, A. FODOR

Gnotobiological analysis of *Heterorhabditis/Photorhabdus* symbiotic complexes

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The RFLP patterns of the PCR amplified internally transcribed spacer (ITS1 - 5-8S rRNA and ITS2) region the 18S – 28S rRNA genes 13 strains of 6 entomopathogenic nematode (EPN) phylogenetic species belonging to the *Heterorhabditis* genus and those of amplified internally spacer region of their bacterial (*Photorhabdus*) symbionts were determined. Species specific patterns were identified reproducibly and the possibilities of cospeciation is discussed. We accomplished a large scale gnotobiology study by growing nematode strains on each other's symbiont. Both dauerlarvae and axenic J1s were grown on bacteria in Petri plates (TSY media), the next generation dauers were regained, *Galleria mellonella* larvae were infected, and the bacteria were regained from the next generation of dauers and then determined.

The results are discussed with the aspect of coevolution.

BURGYÁN, J.¹, H. SZUTORISZ^{1,2}, Gy. BISZTRAY²

The replication and 3'-end repair of tobacco necrosis virus RNA have different structural and sequence requirements

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Tobacco necrosis virus (TNV) as the majority of plant viruses have single-stranded RNA genomes which are frequently exposed to the action of cellular nucleases during their life cycle. Therefore, protective (active or passive) mechanisms that maintain the integrity of the 3'-terminal sequences would be very advantageous for viral RNAs. We showed that the deletion of up to 5 nt from the 3' end of TNV genome is repaired *in planta*, similarly to turnip crinkle and cymbidium ringspot viruses in the Tombuviridae family. In addition, we demonstrated that only one unit of 10 nt long repeated sequences located at the 3' terminus of TNV is required for

the genome replication. However, the RNA lacking one unit of 10 nt repeats was not repaired, because the 8 nt stem in the hairpin structure formed by the repeated sequences is essential for the repair. Using mutant RNA transcripts, We also showed that the structure and not repeated sequence itself are required for 3'-end repair of the TNV RNA. This result shows that the replication and repair of the viral RNA are different activity of the same enzyme (replicase). Alternatively, the 3'-end repair of viral RNAs is a result of cellular enzyme playing a role analogous to that of cellular telomerases.

CLUTTERBUCK, A. J.

***Aspergillus* chromosomes, maps and sex**

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In the early 1950s G. Pontecorvo chose the homothallic ascomycete fungus *Aspergillus nidulans* as a model genetic organism for investigating the relationship between recombination and gene function. Homothallicity means that any strain can be crossed with any other, and has resulted in a large collection of mutant strains in Glasgow and elsewhere, all derived from a single wild type. Another consequence of homothallicism is the possibility of building a self-consistent genetic map. There is now also a cosmid contig map, but unfortunately the two do not always agree. Almost certainly there are mistakes in both, but the genetic map has the advantage that each new linkage reinforces the pre-existing map; moreover, a framework for each chromosome is provided by mitotic recombination. While molecular genetics, based on cloning and gene replacement does not need to know the location of any gene, telomere-associated variegation and gene clusters pose two position-related puzzles, well exemplified in this fungus. Homothallicity in *A. nidulans* is itself something of a puzzle. Firstly, it is evident that two strains participating in sexual reproduction take up distinct male and female roles, e.g. in the donation of mitochondria. Secondly, the complex dance of nuclei in the formation of thousands of asci in one fruiting body must surely require mutual recognition by the partners involved. We now have circumstantial evidence that there may be a mating type switch mechanism, reminiscent of that in yeast, which occurs only in the sexual phase of this fungus.

COSTENOBLE, R.¹, T. BRANDBERG¹, L. ADLER², C. NIKLASSON¹, G. LIDÉN¹

Expression of the bacterial *mtlD* gene in a glycerol-3-phosphate dehydrogenase-deficient mutant of *Saccharomyces cerevisiae*

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Baker's yeast (*Saccharomyces cerevisiae*) can be used for the production of other

chemicals than ethanol, its primary industrial product. These products can be substances, which the yeast produces naturally, or substances, which it can produce only after genetic engineering.

Yeast cells frequently use biochemical reduction reactions in their metabolism. The various enzymes performing this type of reactions often need a specific substance, the NAD⁺/NADH co-factor, to perform properly. One of these enzymes is glycerol-3-phosphate dehydrogenase (GPD), which is involved in the synthesis of glycerol. In earlier research, it was shown that a mutant lacking this enzyme stops to grow under anaerobic conditions. Since these cells could not produce glycerol anymore, they could not regenerate NADH, which builds up under anaerobic conditions. This led to growth cessation because the NADH-excess strongly inhibits other enzymes.

The objective of the presented project is to use this anaerobically induced NADH-excess to drive bioreductions yielding other products than glycerol. If this were possible, NAD⁺ would be regenerated and anaerobic growth could (re-)occur. This would make the GPD-deficient mutant a basis for a powerful, anaerobic bioreduction system. The first bioreduction investigated, is the production of mannitol, an artificial sweetener.

A GPD-deficient mutant of *S. cerevisiae* was transformed with the *mtlD* gene, which encodes bacterial mannitol-1-phosphate dehydrogenase. This enzyme catalyzes an NADH-dependent reduction of fructose-6-phosphate to mannitol-1-phosphate, which after dephosphorylation forms mannitol. The transformed GPD-deficient mutant produced mannitol under anaerobic conditions and only small amounts under aerobic conditions. However, although extracellular mannitol concentrations of 0.5 g/l were reached, the strain

CVETNIĆ, Ž.¹, M. OCEPEK², B KRT², H. KOVAČIĆ¹, K. BRLEK³, J. TRSTENJAK³

Use of different tests in diagnosis of *Mycobacterium paratuberculosis* in infected cattle

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During a regular annual tuberculinisation on the farm of dairy cows of Frisian breed 12,1% of unspecific reactions to tuberculin was established. Some of the cows had chronic watery diarrhoea and were markedly skinny, thus such clinical symptoms indicated paratuberculosis. The blood samples of 205 cattle on the farm were serologically analysed using the following methods: enzyme-linked immunosorbent assay - ELISA (IDEXX), complement fixation (CF) test and agar gel immunodiffusion - AGID test (Bioveta), *Mycobacterium paratuberculosis* gamma - interferon test kit - γ -IFN (IDEXX). The repeated allergy test (P.P.D. – Johnin - Etlik) was also carried out as well as the DNA - test (IDEXX) from the dung of cattle reacting positively or suspectedly with one of the tests mentioned. Applying some of the tests mentioned above, positive reactions were established in 39 (19%)

out of 205 cattle examined. When using ELISA test positive reactions were established in 20 (9.7%) of cattle, with CF test 3 (1.5%) cows were positive and 9 (4.4%) of them suspected, with AGID test 4 (1.95%) of them were positive and with γ - IFN test reactions were established in 23 (11.3%) cattle. When using the allergy test in 22 (10.7%) cattle the swelling of skin wrinkle greater than 2 mm was established and by DNA probe positive reaction was established in 10 (25.6%) cattle out of 39 samples analysed. Combining several tests (ELISA, γ -IFN, DNA -probe) and using faecal culture reliable diagnostics of paratuberculosis in cattle can be obtained.

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Research aspects of the green pepper pathogen *Pseudomonas viridiflava*'s virulence factors

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Microbiological study among SIDS victims

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The potential role of microbiological agents was investigated in 15 cases of Sudden Infant Death Syndrome and in 15 non-SIDS cases in Budapest between September 1996 and April 1999. Autopsy, histological examination and producing *Staphylococcus aureus*-, Enterobacteriaceae and *Candida albicans* strains in large number and by the detection of *Parainfluenza Type 2 virus* antigen. *Staphylococcus aureus* microbiological tests were performed on samples of blood, cerebrospinal fluid, pharyngeal samples and lung tissue from infants under six months died suddenly without previous diseases. The multifactoral pathomechanism of SIDS was suggested by the isolation of toxin proved the predominant bacteria in the SIDS cases. Nasopharyngeal microbial flora and *Staphylococcus aureus* carrying state of 100 age matched healthy infants were tested during the same period. *Staphylococcus aureus* was isolated from 53% of SIDS cases and 37% from healthy infants (OR=1.986). The enterotoxin and TSST-1 toxin producing activity of *Staphylococcus aureus* showed a characteristic difference. The toxigenic *Staphylococcus aureus* was detected in 46% of SIDS cases and 16% of healthy infants (OR=4.5). The distribution of toxigenic and non toxigenic isolates was 85% in SIDS cases and 43% in healthy infants (OR=7.875).

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Investigations on the presence of *Mycoplasma mycoides* ssp. *mycoides* small colony type in Hungarian cattle herds by PCR

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Mycoplasma mycoides subspecies *mycoides* small colony type (MmmSC) is the etiological agent of contagious bovine pleuropneumonia (CBPP), a disease causing considerable economic losses. Using PCR method, we have investigated 1003 cattle originated from 110 farms located in 20 different districts of Hungary in order to attempt the detection of possible MmmSC infection with this very sensitive method. Lung tissues (953 samples) from the slaughtered cattle and nasal swabs (50 samples) were collected for DNA extraction. Examinations of the samples were performed with three different PCR assays described previously. Four hundred eighty samples were examined using *M. mycoides* cluster specific primers, 325 samples by MmmSC species specific primers and 198 samples were tested with MmmSC species specific primers in nested PCR. All the samples were negative regardless of the primers applied. Testing the sensitivity with DNA extracted from lung tissue of an artificially infected cow (used as positive control in the study) the nested PCR proved to be 100 times more sensitive than the one step PCRs. The same tissues and swabs were tested also by culturing and using selective media and were found to be negative for MmmSC. Our results strengthen the statement that CBPP is absent in Hungary.

DARABOS, G.

Bacteriological investigations on soil cover of karstic areas

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Some soil-microorganisms living in soil cover of karstic areas promote limestone weathering by produced inorganic or organic acids or ligands and by altered the chemical composition of the soil atmosphere and the infiltrating water. The aim of the present investigation was to get more information about microbiological communities in the soil of limestone areas and their role in the processes of karst corrosion. Soil samples were collected on the Aggtelek Karst, in Hungary. From the samples the CFU number of the main groups of microbes was determined. Gram-positive microbes dominated in all samples. Furthermore, all together 250 isolates were collected, from them 10 *Streptomyces* sp. and 10 other bacterial strains were selected to use in new laboratory model-system regarding the production of matters CaCO₃-aggressive. The identification all of the bacterial strains was facilitated by phenotypic methods. The shifting of pH-values and the amount of dissolved CaCO₃ effected by the selected microorganisms were measured under different ecological

conditions. It was clearly shown by the different tests that the various ecological factors (pH-value, the amount of acids etc) influence the activity of microorganisms that is decisive in the production of aggressive material and at the same time the microbiological products could change the ecological factors. Moreover, the earlier disregarded *Streptomyces* strains, are probably most important in the solution of limestone. These examinations were preliminary experiments to determine the circumstances of the CaCO₃-aggressive substance production by microbes and their role in the process of karst corrosion, in a more precise way.

DAVYDOVA, M. N., F. K. MUKHITOVA

Overproduction of extracellular hydrocarbons by *Desulfovibrio desulfuricans*

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It is known that sulfate reducing bacteria when growing on organic substrates can produce oxygen-containing and gaseous products. Hydrocarbons are present in small amounts in cultural media. It will be stressing state for bacteria when growing with lactate at low concentration of CO (up to 5%). The appreciable increase of reduced NADP is observed. Under these conditions the extracellular organic compounds have been found in the growth media. The long-chain hydrocarbons have been synthesised by cell suspensions *D. desulfuricans* in the presence of lactate or acetate in the atmosphere of 10% CO + 10% H₂. The results of inhibitor analysis have shown that synthesis was not limited by ATP. When the reduced equivalents (NADH or NAD(P)H) were added the yield of the reaction products increased. One can suppose that the excess of reduced equivalents in the cells of *D. desulfuricans* induced the processes directed towards their spilling.

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Follow-up study for determination of human papillomaviruses

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The human papillomaviruses (HPV) are regarded as biological precursors of cervical carcinoma. A follow-up study was organized to determine the prevalence of HPV in a population at low or high risk of HPV in cervical samples (n = 45). Two samples were collected at an interval of 8-18 months. Simultaneously with the HPV determination, cervix samples were collected for cytology and blood samples for the determination of serum antibody titers against sexually transmissible viruses and *Chlamydia trachomatis* from the HPV-tested women. The average reported prevalence of HPV in Hungarian asymptomatic women is 17.4%. 45.5% of initially HPV-positive symptomatic women were also positive in the second examination. In the first screening the HPV positivity was 43.4%. 6.8% of the patients had acquired

low-risk, and 34.1% high-risk types of HPV. 4.6% of the women were infected with both low- and high-risk types. CMV IgM and IgG antibodies were present in 0.0% and 73.3% of the patients, HSV 1 IgM and IgG in 8.9% and 80.0%, HSV 2 IgM and IgG in 0.0% and 77.8%, EBV IgM and IgG in 2.2% and 97.8%. There were no HCV-positives among the patients. *C. trachomatis* IgM and IgG were detectable in 60.0% and 51.1%. The present level of HPV positivity was only 45.5% in initially HPV-positive women. The HPV infection may be transient. The correlation of HPV positivity and sexually transmissible virus antibodies was relatively high. The follow-up of cytologically or molecular diagnostically HPV-positive women is very important as concern the prevention of cervical carcinoma.

DEÁK, R.¹, A. MARÁZ¹, H. AARTS²

Discrimination of *Candida glabrata* and *C. guilliermondii* clinical isolates by molecular typing

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Among the species belonging to the *Candida* genus several are capable of causing mycosis, named candidiasis. They are present on the skin and mucous membranes as the members of the normal flora and are also frequently isolated from natural and man-made habitats as saprophytic speciLast year we reported results of our molecular genotyping study on *Candida glabrata* clinical isolates (Deák et al, 1999**), which showed two distinct clusters of strains by RFLP analysis of rDNA gene sequences andRAPD-PCR fingerprinting methods. These results suggested the presence of a distinct species inside *C. glabrata* or consequent misidentification of strains belonging to other closely related species. Physiological and biochemical typing of the strains, which separated those from the other group included the type strain, made it probable that they belong to *C. guilliermondii*. Further molecular analysis by ribotyping and AFLP fingerprinting analysis confirmed it. These results indicate that simplified short tests, used for routine identification of clinical yeast isolates, can cause consequent misidentification, which can be avoided by using molecular typing methods.

DEGRÉ, M.

Cytomegalovirus interaction with blood and endothelial cells

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The mononuclear cells of the haemopoietic system are probably an important reservoir of latent human cytomegalovirus (HCMV) and also play a major role in

the pathogenesis of infections. HCMV DNA was detected in CD34+ cells (both HLA-DR- and +), from seropositive donors. Colony formation and single cell growth of progenital cells from bone marrow (BM), stimulated with growth stimulating factors were inhibited by HCMV, while progenitor cells from cord blood (CB) were not affected. Differences in the cell cycling activity might be at least part of the explanation. The mechanism(s) of inhibitory effect were examined in haematopoietic cell lines. HCMV inhibited the proliferation of KG-1, MO7 and U937 cell lines comparable to that of the primary haematopoietic cells. Uptake of virus was shown by the presence of pp65 lower matrix protein, but no replication was found and no transcription of the immediate early proteins was found. Transcription of the cells with pp71 tegument gene, but not with pp65 and pp150 or IE genes caused inhibition of the cell proliferation. Inoculation of cells with dense bodies, containing matrix proteins but no nucleic acid, also caused inhibition. HCMV infection of MO7 cells induced apoptosis in approximately 70% of the cells, indicating that the HCMV induced inhibition of the growth of haematopoietic cells is at least in part due to apoptosis. Matrix proteins may play an important part in this effect while IE and E proteins may have a minor role. Endothelial cells seem to play an important part in the pathogenesis of HCMV infections. HCMV have a strong tropism for vascular endothelium and microvascular inflammation, occlusion and ischemic injury are stages in the development of viral disease. HCMV productively infect endothelial cells, but cells from different organs have different susceptibility. Microvascular cells from intestinal endothelial cells (HIMEC) are more susceptible to HCMV infection than endothelial cells from umbilical vein (HUVEC) Also the IL-1 β -induced cellular adhesion molecules, E-selectin, VCAM-1 and ICAM-1, differed between HIMEC and HUVEC. This may explain the development of gastrointestinal HCMV disease in humans.

DEGRÉ, M.

Interaction of viral and bacterial infections

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Humans and animals are constantly being inoculated with various microorganisms, including potentially pathogenic viruses and bacteria. Some of these microorganisms may interact directly with other microorganisms, and many of these can interact indirectly by exerting a direct or indirect effect on the various factors of the defence mechanism of the host organism. Over the years we have studied such interactions both in the respiratory tract and in the intestinal tract in mouse model systems supplemented with in vitro studies. Several viruses, including Sendai virus, reduce the efficiency of some of the first line defence systems in the respiratory tract against superinfection by Haemophilus influenzae, such as the mucocilliary flow and the antibacterial effect of alveolar macrophages. A further effect on the macrophage activities is exerted indirectly by inducing production and of interferons, which in turn also influence phagocytosis and bactericidal activities. Several different viruses influence the process of internalisation of invasive bacteria into non-professional phagocytes. Both direct and indirect mechanisms are involved, and the effect is only partially dependent on replication of the viruses. Viral

infections may also influence the haematopoiesis and reduce the development and differentiation of monocytes/macrophages. Similar effect can also be observed by some by-products of the viral infections, as interferons and cytokines.

DELAŠ, F.¹, S. DURAKOVIC¹, I. DELAŠ², B. RADIC³

Relationship between biomass synthesis and ochratoxin A (OTA) production in mould *Aspergillus ochraceus* NRRL 3174 grown on a soil substrate in pure and mixed culture

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In this study, the growth of ochratoxicogenic mould *Aspergillus ochraceus* NRRL 3174 on a solid substrate (corn grains) was investigated, as well as the parameters which influence ochratoxin A (OTA) synthesis. The mould was incubated as pure culture, as well as mixed culture together with moulds *Trichotecium roseum* ZMPBF 1226 and *Fusarium* sp. ZMPBF 1215, which were found not to produce OTA. The biomass yield was measured by chitin method, while OTA concentrations were determined fluorodensitometrically. The growth monitoring was performed through 5 weeks during the cultivation in stationary phase, at incubation temperatures 15 °C, 20 °C, 25 °C and 30 °C, with 38% water content in the substrate. Inoculation was performed with 10⁶ spores g⁻¹ substrate. The highest level of OTA production was reached after three weeks of cultivation at 20 °C and was found to be 790 μg g⁻¹ and 290 μg g⁻¹ dry mycelium for pure and mixed culture, respectively. During the cultivation at 25 °C, under otherwise same conditions, OTA synthesis was reduced about 45% (355 μg g⁻¹) in pure, and 28% (210 μg g⁻¹) in mixed culture. At the same time, higher incubation temperature caused increased biomass production for 40% (12.40 mg g⁻¹) in pure and 20% (12.90 mg g⁻¹) in mixed culture. These results show the ability of mixed culture to inhibit OTA production, especially at lower temperatures (15 °C and 20 °C), where moulds *Trichotecium roseum* ZMPBF 1226 and *Fusarium* sp. ZMPBF 1215 prevail.

DEMAIN, A. L.

Importance of amino acids in regulating microbial secondary metabolism

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Amino acids have major positive or negative effects on the production of secondary metabolites. Methionine stimulates cephalosporin C production by *Cephalosporium acremonium* by acting as an inducer of several cephalosporin synthases; it also supplies the sulfur atom of the antibiotic. Lysine stimulation of cephamycin C production in *Streptomyces clavuligerus* is due to induction of lysine- α -aminotransferase, the first enzyme involved in bacterial conversion of lysine to the precursor, α -aminoadipate. Valine stimulates tylosin biosynthesis in *Streptomyces*

fradiae by inducing valine dehydrogenase, the first enzyme leading from valine to the small acid precursors of the macrolide ring. Other examples include stimulation of nikkomycin synthesis by branched amino acids, tryptophan induction of ergot alkaloid synthesis, phenylalanine stimulation of benzodiazapene alkaloid formation and leucine induction of bacitracin synthetase. On the negative side, lysine inhibits penicillin production by *Penicilliumchrysogenum* due to inhibition of homocitrate synthase, the first enzyme leading to formation of the α -amino adipate precursor in fungi. Also methionine inhibits biosynthesis of many antibiotics to which it contributes a methyl group, e.g., anthramycin, thienamycin, esperamicin and rapamycin. Recent experiments indicate that this is due to methionine repression of the methylating enzyme system. Leucine interferes with *Monascus* red pigment formation by a mechanism thought to involve enhanced decay of pigment synthase(s).

DIÓSI, G., L. G. GAZSÓ, GY. FARKAS

Study of microorganisms and biofilm development in connection with nuclear waste management

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In order to the correct planning of a nuclear waste disposal, the corrosive and migrative effects induced by the potentially developing biofilms on the surface of the container must be considered. Biofilms are defined as matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces. Each biofilm bacterium lives in a customized microniche in a complex microbial community that has primitive homeostasis, a primitive circulatory system, and metabolic cooperativity. Each of these sessile cells reacts to its special environment so that it differs fundamentally from a planktonic cell of the same species. Direct observations have clearly shown that biofilm bacteria predominate, numerically and metabolically, in virtually all nutrient-sufficient ecosystems. Therefore, these sessile organisms predominate in most of the environmental, industrial, and medical problems and processes of interest to microbiologists. Our work was to examine the samples (stone, water, technical water) taken from the uranium mine at the Mecsek Hill (a potential place for high level waste disposal) and also the water samples from the interim storage of the spent fuel at AEKI. Our aim was to isolate the microorganisms from these samples and to study the metabolism of them. We checked the isolated aerobic and anaerobic strains' gas and siderofore productivity and also examined the biofilms consisting of these bacterial strains and developed on the surfaces of stainless steel. We obtained useful information about the cell morfology, cellular metabolism, and the physical architecture of the biofilm matrix.

DIVÉKI, Z., D. SZILASSY, K. SALÁNKI, E. BALÁZS

Monitoring the movement of RNA viruses in plant tissue: the role of tomato aspermy cucumovirus (TAV; Bromoviridae) coat protein in cell-to cell movement

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Both cell-to-cell and long distance movement are crucial functions in the pathogenesis of plant viruses. In cell-to-cell movement, the viruses use the plasmodesmata connecting neighboring cells. This movement is primarily facilitated by different virus-encoded movement proteins, although several other gene products (e.g. coat proteins) and host factors are involved. Precise monitoring of the spread of virus infection in plant tissues is necessary for studying the function of different genes and non-coding sequences which could participate in this process. The jellyfish green fluorescent protein (GFP) is recently one of the most popular reporter gene used in molecular biology. The use of this reporter gene is also opened a new horizon in plant virology. In contrast with *in situ* hybridization, this method does not require the disruption of the plant material. Therefore, it facilitates studying the kinetics of virus movement directly in the intact plant tissue. TAV has a tripartite RNA genome; the coat protein (CP) is located on the RNA3 segment. Using GFP reporter gene, we constructed different fusion proteins with the TAV CP. Plants were coinoculated using *in vitro* transcripts of cucumber mosaic cucumovirus (CMV, *Bromoviridae*) RNA1 and RNA2 cDNA clones and the TAV RNA3 constructs. Virus movement was monitored by epifluorescent microscopy *in vivo*. Our results confirmed that the CP is essential for the cell-to-cell movement, and CP partly retained the ability to promote virus movement in fusion protein form in the examined host-virus systems.

DLAUCHY, D., J. TORNAI-LEHOCZKI, G. PÉTER

Identification of foodborne yeasts on the basis of 18S rDNA

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Yeast contribution to the production of alcoholic beverages and bread is widely known, however, their role as spoilage agents is often neglected and underestimated. The importance of yeast in the spoilage of foods is increasing. Process and quality control both require the reliable and rapid identification of foodborne yeasts. The yeasts are traditionally characterised and identified by 60 to 90 morphological, physiological and biochemical tests. The reliability of these criteria in yeast identification is often questioned. The determination of 60-90 characteristics is hardly feasible and time-consuming task for routine identification in the industrial laboratories and culture collections. The aim of our study was to develop a simple and rapid method to identify yeast strains on the basis of their 18S rDNA. We used the restriction patterns of 18S rDNA with the neighbouring ITS region, digested with four (*HaeIII*, *MspI*, *AluI*, *RsaI*) different four-base cutting enzymes, for differentiation and identification. Up to date we examined the restriction patterns of 18S rDNA of 130 frequent yeast species mainly associated with food, wine, beer and soft drinks. For constructing a database of restriction fragment patterns, the gels have been scanned and analysed using the Molecular Analyst Fingerprint 2.0 software (BIORAD). The use of four different enzymes proved to be sufficient in

strain identification except only the species of genus *Debaryomyces*.

DOBOLYI, Cs. ¹, I. KERESZTÉNYI²

Studies on availability of microbiological ecotoxicological methods in the biodegradation of hydrocarbons in aqueous phase

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Ecotoxicity gains great importance when the environmental hazard value of hydrocarbons that cannot be completely biodegraded is tested, or where the process of biodecay is slow. The availability of different methods based on the activity of microbes or their enzymes was studied for testing the ecotoxicity during biodegradation processes. We investigated the ecotoxicological effects of two crude oil derivates on the reproduction of the bacteria *Azomonas agile* and *Pseudomonas fluorescens*, the green alga *Raphidocellis subcapitata*, and the bioluminescence of *Vibrio fischeri*. The concentration ranges at which the inhibition of the reproduction of *A. agile* reached the level of 50 % were 150-200 mg/L at gas oil and about 1000 mg/L at lubricant fluid, and the 50 % inhibition of *P. fluorescens* was found in the same range of the tested chemicals. Both chemicals affected the reproduction of the test alga *R. subcapitata*, the change in reproduction was exactly quantified by the determination of cell counts. Both gas oil and lubricant compounds caused significant inhibition in bioluminescence (EC₅₀: 150 mg/L). The activity of the enzyme bacterial luciferase was rapidly affected by the substances which evolved in process of gas oil biodegradation: the level of 25-30 % inhibition was experienced at the start and more than 80 % could be measured after a ten-day-long incubation. In progress of biodegradation the value of EC₅₀ notably decreased in the case of the gas oil, while it showed a slight decrease in the case of the tested lubricant. The changes in ecotoxicity were proved with the reaction of all applied test organisms during the biodegradation of different hydrocarbons. The ecotoxicological monitoring in this field is proposed to be based on simultaneous determination with several different methods.

DOBOZY, A., L. KEMÉNY, R. GYULAI

Human herpesvirus type 8 in angiogenic tumors

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The discovery of human herpesvirus 8 (HHV8) in Kaposi's sarcoma (KS) tissue from AIDS patients has opened up new vistas in virology and oncology. HHV8 DNA has been found in all forms of KS, suggesting that it might be involved in the pathogenesis of KS. Additionally, HHV8 has been detected in both malignant and benign lymphoproliferative diseases, such as body cavity-based B-cell lymphomas and multicentric Castleman disease. The association of KS with HHV8 raised the question of whether HHV8 can also be associated with other endothelial cell-

derived vascular neoplasms. Benign vascular lesions (hemangiomas, lymphangiomas, pyogenic granulomas and hemangiopericytomas) were found not to contain HHV8 DNA. However, there are contradictory data concerning the presence of HHV8 in angiosarcomas. In contrast with previous reports, we detected HHV8 sequences in patients with angiosarcoma of the face. The seemingly contradictory findings might be due to differences in the samples examined. Additionally, we could detect HHV8 DNA in angiolymphoid hyperplasia with eosinophilia (ALHE). This benign disorder is characterized by multiple soft angiomatous tumors, usually appearing on the face, ear or scalp. The main histological feature of ALHE is the proliferation of atypical endothelial cells (as seen in KS), accompanied by eosinophilic infiltration in the dermal and subdermal connective tissue. The presence of HHV8 in both benign and malignant proliferations of endothelial cells suggests that the virus alone is not sufficient to produce a specific lesion.

DOMJÁN-KOVÁCS, H.¹, K. RÁSKY², A. FÁBIÁN¹

Chemiluminescence enzyme immunoassay, a screening method for selective detection of *E. coli* O157:H7 from food

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Verocytotoxin producing *E. coli* (VTEC) are now recognized as a major cause of haemorrhagic colitis and haemolytic-uremic syndrome. Although great variety of VTEC serogroups have been implicated in human disease, *E. coli* O157:H7 is the most prevalent strain. Since these pathogens may be present in food and environmental samples in only small numbers, sensitive methods are needed for their detection. This study was done for the evaluation of a Chemiluminescence Enzyme Immunoassay developed for the detection of *E. coli* O157:H7. For this aim different *E. coli* O157 serotypes were used. The sensitivity and specificity of the kit was determined from the decimal dilutions of the 24 hour broth cultures of the test strains. According to this trial the sensitivity of the kit is 10^3 - 10^4 cell/cm³, and it is specific for *E. coli* O157. Further on 25 g ground beef samples were prepared and inoculated with *E. coli* O157:H7 with different CFU g⁻¹. The samples were incubated in 225 ml mEC + n at 42 °C for 4 hour and the assays were performed. According to the results with the CLIA test 10^1 - 10^2 *E. coli* O157 g⁻¹ can be detected from the sample. So this kit seems to be suitable for screening the samples before selective cultivation of *E. coli* O157: H7.

DOW, M. A.

Validity of Gram reaction of fresh environmental isolates

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During the early days of bacteriology the detection of bacterial cells in tissues was difficult since most of the staining methods used coloured the bacterial cells equally. In 1884 Christian Gram attempted the development of a procedure that would

differentially stain "schizomycetes" from tissue cells, which is now known as the Gram staining procedure. This method of staining gained wide application because of its simplicity and quickness in the tentative classification of bacteria into two groups, Gram-positive and Gram-negative. In the age of molecular procedures to bacterial taxonomy one could argue that there is no need anymore for Gram differentiation. We have to realise, however that most of clinical microbiology laboratories base their diagnostic work on classical phenotypic characterisation, where the "everfirst test" is Gram staining. This situation will not change dramatically in the forthcoming decade.

In our work, different Gram staining methods were compared using authentic strains. KOH test and aminopeptidase were also used as confirmation. The best staining method was chosen, and applied on a broad set of freshly isolated strains. Altogether 216 strains were tested by KOH, aminopeptidase test, and Gram stain. Bacteria were isolated from the gills of eels caught by electric fishing in Lake Balaton. The eels were in part healthy, but in part eel nematode (*Anguillicola crassus*) infected. The bacteria were isolated from gill tissue macerate. All cultures were streaked and repeatedly reisolated to ensure purity and maintained on adequate slant agar. All the strains were subjected to ARDRA grouping, and representative members from each ARDRA group were identified by partial 16S rDNA sequence analysis.

Using the staining procedure on 18-to 24-h cultures, 151 (69.9%) of the strains were found to be Gram-negative, 64 (29%) to be Gram-positive, and only 1 (0.5%) to be Gram-variable. In case we compare the results with the result of sequence analysis, false gram staining reactions are encountered with genera *Acinetobacter*, *Microbacterium*, *Deinococcus*, *Bacillus* and *Aeromonas*. KOH test gave the best correlation with sequence results. In case of aminopeptidase problems arose with pigmented strains, but in a limited number (2-3%) nonpigmented strains gave false results similarly.

DÖMÖK, I.

Prospects in eradication of wild poliovirus

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The global eradication of poliomyelitis means the complete eradication of wild and potentially wild polioviruses. The essential activities are as follows. (1) Interruption of the transmission of wild polioviruses by (a) a strong routine immunization programme; (b) national immunization days; and (c) "mopping-up" immunizations. (2) Certification of the polio-free status by AFP and/or alternative surveillance. (3) Laboratory containment of wild polioviruses. (4) Discontinuance of immunization with OPV and containment of vaccine and vaccine-derived strains. Substantial results have already been achieved in the interruption of transmission and in the process of certification. In the American Region of WHO, the last indigenous polio case occurred in 1991 (Peru); in the Western Pacific Region, no polio case due to wild poliovirus has been detected since 1997; and in the European Region, all the

countries (50) have remained free from poliomyelitis, except Turkey in 1998. The South-East Asian and African Regions have remained the major reservoirs. The certification process is under progress in non-endemic countries controlled by the Global Commissions of WHO, with contributions from the Regional Commission and National Committees. The laboratory containment of polioviruses will be essential, since their possible introduction from laboratories into the community has been documented. Strategies to stop vaccinations with OPV depend on the persistence of vaccine-derived strains in the population and on the prevalence of long-term excretors among immunodeficient persons. At any event, after cessation of vaccinations, even vaccine-derived strains and stockpiled OPV should be under maximum laboratory containment.

DURAKOVIĆ, L., Z. PETROVIĆ, F. DELAŠ, M. GLANCER, S. DURAKOVIĆ

Dehydroacetic acid and the newly synthesised Schiff base to control ochratoxin A accumulation

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The potential for inhibition of ochratoxin A accumulation by ochratoxigenic fungus *Aspergillus ochraceus* NRRL 3174 was investigated using dehydroacetic acid (DHA) and the newly synthesized Schiff base 3-/2-Aminophenylimino(p-toluoyl)-4-hydroxy-6-(p-tolyl)-2H-pyran-2-one in yeast extract-sucrose (YES) medium at pH 5.5. YES medium was treated with various amounts of DHA and Schiff base after inoculation with *A. ochraceus*. Experiments were carried out in a stationary culture at temperatures of 20 °C and 28 °C during 28 days. Mycelial dry weights were determined gravimetrically, and concentration of ochratoxin A was measured fluorodensitometrically using a Camag TLC Scanner. DHA concentrations of 1.0 $\mu\text{mol L}^{-1}$ and 10.0 $\mu\text{mol L}^{-1}$, respectively, stimulated mould growth and ochratoxin A accumulation, but concentrations higher than 50.0 $\mu\text{mol L}^{-1}$ produced an inhibitory effect. In the presence of low Schiff base concentration, mould growth was decreased by 80% and toxin concentrations by 70% or completely.

EL-DEEB, B. A.

Isolation and characterisation of bacterial strain which is able to utilise a molluscicide bayluscide as a sole source of carbon and nitrogen and evidence for the involvement of plasmid in bayluscide degradation

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Egypt is considered the home of Schistosomiasis and also one of the most severe endemic areas. It has been the site of numerous control efforts. One of these efforts is the application of molluscicide bayluscide, *Pseudomonas* sp strain Bal 1 was isolated from a field contaminated with molluscicide bayluscide. This strain was able to utilize a bayluscide as a sole source of carbon and nitrogen. The degradation of bayluscide by Bal 1 strain is mediated by pBE1 (58 Kb) and pBE2 (110 Kb)

plasmids. The loss of these plasmids resulted irreversibly mutant which unable to degrade bayluscide. The transfer of these plasmids from wild type strain Bal 1 to Bal 1M mutant, restored completely its capability to degrade the molluscicide. It was proposed that pBE1 and pBE2 are conjugated plasmids and involved in the bayluscide degradation.

ELHOTTOVÁ, D.¹, J. TRÍSKA²

GC-MS/MS detection of poly- β -hydroxyalkanoates (PHA) as prokaryotic storage compounds

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Poly- β -hydroxyalkanoates (PHA) compounds are accumulated under conditions of nutrient unbalance by many bacterial species belonging to diverse taxa. They are considered as important markers of the growth and nutritional status of bacterial community. The PHA contain 3-hydroxyacyl monomer units with 3-14 C atoms. The most abundant PHA compound is poly- β -hydroxybutyrate (4 C) that has been often the only monitored PHA compound in majority of ecological studies. A lack of sensitive methods is a reason that the other PHA monomers are not observed in majority of environmental analyses. The goals of the study were (i) to modify the trace analysis for PHB determination (Elhottová et al., 1997) to complex determination of PHA composition, (ii) screening pure bacterial cultures to show distribution of individual PHA monomers. In principle the method is based on a single-phase extraction of lipids from the sample; release of monomer units (3-hydroxy fatty acids) from PHA by alkaline hydrolysis; monomer derivatization by N-tert-butyl-dimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA); and finally detection of derivatized products (MTBSTFA-3hydroxyacids) by GC-MS/MS method. Modification consisted in the final detection step of GC-MS/MS analysis. The analysis was divided to individual detection segments corresponding to retention time and m/z value of selected ions of individual derivatized PHA monomers. The fragment ions (M-57)⁺ and (M-15)⁺ were selected. This method allowed detection of all potential PHA monomers in one analysis on the trace level (10^{-15} mol μL^{-1} of injected volume). Screening of 13 bacterial strains showed this distribution of following PHA monomers: the most abundant were 3-hydroxyacids with 4 and 5 C (50 % of bacterial strains), less abundant were 3-hydroxyacids with 8 and 10 C (31 %), 12 C (23 %), 9 C and 6 C (15 %) and minority group represented 3-hydroxyacids with 7 and 11 C (7 %).

The work was supported by the Grant Agency of the Czech Republic as a part of the project 526/99/P033.

EL-KARAMITY, A. E.

Response of some lentil cultivars to inoculation and spraying with molybdenum

Agronomy Department, Faculty of Agriculture, Minia University, Egypt

ELMERICH, C.

Diazotrophs associated with cereal crops

Unit de Physiologie Cellulaire, Institut Pasteur, Paris, France

The biological process responsible of the reduction of molecular nitrogen to ammonia, catalyzed by the nitrogenase enzyme, is referred to as biological nitrogen fixation. Cereal crops are the most important sources of food. In particular rice is the major food for more than a third of the world's population. It is now recognised that plant growth promoting rhizobacteria (PGPR) play an important role in agriculture. Attempts to enumerate and to identify the nitrogen-fixing bacterial species associated with cereal crops and other grasses has revealed a large diversity and complexity. In general, nitrogen-fixing root-associated diazotrophs are soil bacteria able to colonize the root surface. Some, including *Azospirillum*, invade the superficial layers of the cortex. Obligate endophytes that inhabit the vascular system of the plant have been described. *Azospirillum* enhances the plant growth primarily by colonisation of the root surface that results in increasing the proliferation of the root hairs and of the root system of the host plant. This effect has been tentatively attributed to the production of auxins like compounds such as indole-3-acetic acid (IAA) by the bacterium. Surface colonization of wheat roots by *Azospirillum* involves the polar flagellum and the production of surface polysaccharides. Bacteria on the root surface are ovoid in shape, resembling differentiated cyst-like cells. Data on the genetic determinants involved in the colonization process will be presented.

Supported by ECOS and AFCRST.

ELNIFRO, E. M.¹, R. J. COOPER¹, P. E. KLAPPER², A. B. TULLO³

Multiplex PCR for the diagnosis of viral and chlamydial conjunctivitis

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EL-SAID, S. I. A.², M. M. ZAKI¹, N. G. EL-GAMAL²

***In-vitro* and *in-vivo* biological control for soil-borne fungi infected geranium plants (*Pelargonium graveolens* L.)**

¹Agricultural Microbiology Department, Ain Shams University, Cairo, ²Plant Pathology Department N.R.C., Dokki, Giza, Egypt

ENDRÉSZ, V.¹, K. BURIÁN¹, D. VIRÓK¹, Z. KIS¹, K. BODA², Z. PROHÁSZKA³, G. FÜST³, É. GÖNCZÖL¹

Human cytomegalovirus and *Chlamydia pneumoniae* specific antibodies in sera of patients with coronary heart disease

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Associations between a wide variety of infectious agents and atherosclerosis have been described in the literature. Most of the published studies relate to human cytomegalovirus (HCMV) and *Chlamydia pneumoniae* (*C. pneumoniae*) bacterium. DNA and antigens of these infectious agents have been localized in atherosclerotic lesions using PCR and immunocyto-chemistry. Seroepidemiologic data also support a relationship between HCMV and *C. pneumoniae* infection and the development of coronary atherosclerosis. We have evaluated the serologic evidence of this association in 4 four groups of patients with coronary heart disease in Hungary. Sera of 156 patients with signs of severe, 44 of mild coronary atherosclerosis, 41 with angina but without atherosclerotic coronary alterations and 96 regular blood donors were tested for antibodies against full HCMV antigens and HCMV immediate early-1 (IE1) antigen in ELISA tests, and against *C. pneumoniae* in ELISA and microimmunofluorescence (MIF) tests. By statistic evaluation of data no significant difference was detectable for the frequency of HCMV seropositivity and HCMV specific antibody level in the study groups. However, *C. pneumoniae* specific antibodies were detected in significantly higher proportion in both groups of patients with coronary heart disease than in control groups by using the MIF test. Among sera with high HCMV-IE1 antibody level the frequency of *C. pneumoniae* MIF positivity was significantly higher than in sera with low anti-HCMV-IE1 antibody level, suggesting an interaction between these two pathogens. These results indicate that *C. pneumoniae* infection might be related to the development of atherosclerosis.

This study was supported by grants of the Hungarian Scientific Research Fund ETT T-10 592/96. and MKM FKFP 2025/97.

FARKAS, E.

My always respected good friend György Ivánovics

The author briefly recalls those difficult decades following the Second World War, when he cooperated in many different ways with Professor Ivánovics. He speaks about the help provided to the Department under his leadership, in so many aspects by the mutual professional and moral support. He will always remember the very successful and high-level work of Professor Ivánovics in leading the Hungarian Society for Microbiology and as editor-in-chief of Acta Microbiologica Hungarica.

The author came to recognize Professor Ivánovics as an outstanding organizer and in all respects a totally dedicated human being.

FARKAS, J., É. ANDRÁSSY, K. POLYÁK-FEHÉR, L. MÉSZÁROS

Improvement of microbiological safety of vacuum-cooked meals by gamma irradiation

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Due to their good quality and convenience, production and trade of vacuum-packaged then cooked-chilled (sous-vide) meals are increasing in countries with well-developed cold chain. However, because of their minimal processing, the microbiological safety of sous-vide foods is vulnerable to contamination of their ingredients with spores of psychrotrophic pathogenic spore-formers such as non-proteolytic clostridia and *Bacillus cereus*, especially in case of temperature-abuse. Using a psychrotrophic strain of *Bacillus cereus* as test organism, inoculated packs of prepared meals such as cooked beef in tomato paste and smoked-cured pork with stewed beans were treated with combinations of pasteurizing heat treatments and gamma irradiation with 4 or 5 kGy. Before and after the treatments and periodically during storage at 10 oC, total aerobic and total anaerobic viable cell counts and, selectively, the viable cell counts of *Bacillus cereus* and sulphite-reducing clostridia have been determined. The effect of the treatment-order (first irradiation and subsequent heating, or, first heating and subsequent irradiation) was also studied. Sensory testing of uninoculated samples proved that the combination-preserved meals were of acceptable quality. The microbiological investigations showed that the medium-dose irradiation of prepared meals prior to their quality-friendly "sous-vide" cooking sensitized the surviving bacterial spores against the heat treatment and increased thereby considerably their microbiological safety and keeping quality.

FARKAS, S.¹, Gy. GUNICS², A. HEGEDÜS³, M. KECSKÉS¹

Susceptibility of the *Rhizobium* and *E. coli* strains to different antibiotics

¹Agricultural-, Environmental Microbiology and Soil Biotechnology Ph.D. Subprogram, Gödöllő University of Agricultural Sciences, Budapest, ²Albert Szent-Györgyi Medical University, Faculty of Health Sciences, ³Teachers Training College Gyula Juhász, Szeged, Hungary

Antibiotics susceptibility of two *Sinorhizobium meliloti*, five *Rhizobium leguminosarum* *bv.*, three *Bradyrhizobium* *sp.* and six *Escherichia coli* strains was tested with RESISTEST, BBL and E- test disk- diffusion, and Checkerboard methods. The interaction of Ampicillin and Gentamicin with Imipramine was studied on *S. meliloti*, *R. leguminosarum* *bv. phaseoli* and *E. coli* K12 RP4 strains. The *S. meliloti*, *E. coli* K12 *drd*⁺ and *E. coli* K12 RP4 strains were resistant against more than ten antibiotics. The other *Rhizobium* and *E. coli* strains were resistant against from five to ten antibiotics. Synergistic effect was found with Ampicillin

and Gentamicin on *E.coli* K12 RP4 and *S. meliloti* strains in the presence of Imipramine. The additive effect of Ampicillin and Gentamicin were tested with Imipramine on *R. leguminosarum* *bv. phaseoli* strain.

FAUST, I., B. JAKAB, J. HIDASY, M. PESTI

Parasexual recombination of *Candida albicans* morphological mutants

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A wild-type strain of *Candida albicans* (ATCC 10261) was used to obtain double auxotrophic mutants by nitrosoguanidine (NTG) treatment. Absence of complementation in hybrids for lysine auxotrophy of different origins proved that the three double auxotrophs possessed an allelic mutation for this selective marker. Repeated NTG treatment resulted in numerous colony morphological mutants. Colonies of various mutants were multiplied with the application of a single cell descendent method. In this way, four stable segregants were selected, characterised and used for complementation analysis by parasexual recombination. Examination of the hybrids of these colony morphological mutants of *C. albicans* is under way.

FENYŐ, É. M.

HIV biological phenotype and chemokine receptor usage

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Human immunodeficiency virus type 1 (HIV-1) biological phenotype, such as replication rate and cytopathology in culture, has long been recognized as marker for viral virulence. The ability to translate these phenotypic traits into molecular terms brought HIV biological variation into focus during the past three years. It has been recognized that HIV-1 uses members of the seven transmembrane domain chemokine receptor family as co-receptors to CD4 for membrane fusion and entry into cells. The two most well-defined HIV-1 co-receptors are CXCR4 and CCR5, members of the CXC (α) and CC (β) chemokine receptor subfamilies, respectively. Primary HIV-1 isolates previously termed rapid/high or syncytium inducing (SI) which readily infect activated PBMC and CD4+ T cell lines are defined by their use of the CXC-chemokine receptor CXCR4. Slow/low or non-syncytium inducing (NSI) viruses, which preferentially infect activated PBMC are defined by their use of members of the CC-chemokine receptor family, principally CCR-5. This allowed the introduction of a new terminology: CXCR4 using viruses have been termed X4 and CCR5-using viruses R5. In addition to CXCR4, some rapid/high viruses use CCR5, CCR3 or CCR2B and thus have a broader host range than slow/low viruses. The fact that the change in virus biological phenotype may occur in the same individual over time and is associated with progressive disease has suggested that CXCR4 using viruses are more virulent. We asked the question whether the pattern established with HIV-1 isolates of genetic subtype B holds true for subtype A, C, D and E. Of the 40 patients with non-AIDS 34 yielded CCR5-using (R5) virus (85%

vs 80% for subtype B). None of the viruses, including HIV-1 subtype B, were able to use any other chemokine receptor in this group. Conversely, many of the subtype A, B, D and E viruses derived from AIDS patients used CXCR4 (18 out of 40, 45%). Viruses using CXCR4 were in half of the cases able to use other receptors as well, most frequently CCR5 or CCR3, and were thus dual-tropic or multitropic. Interestingly, HIV-1 of subtype C differed from this general pattern in that CXCR4 usage was very infrequent. In fact, all of nine Ethiopian or four Swedish AIDS patients with subtype C virus infection yielded virus that used CCR5 only. Furthermore, we compared receptor usage of HIV-1 with the less pathogenic HIV-2. Similarly to HIV-1, some of the HIV-2 infected AIDS patients yielded viruses using CXCR4, instead of or in addition to CCR5. In contrast to HIV-1, the ability to use several coreceptors, particularly CCR1, CCR2 and CCR3 in combination with CCR5 and/or CXCR4, characterized 10 out of 11 HIV-2 isolates, regardless of the severity of infection. The results indicate that multitropism *per se* cannot explain the differences between HIV-1 and HIV-2 pathogenesis.

FILIPIC, B.¹, S. TOTH², S. KOREN¹

Modification of Eagle's medium to cultivate the adherent cells in suspension

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To cultivate the monolayer (adherent) cells in suspension, different techniques as well as different rather complex media were developed and more or less successfully used till recently. The presented experiments were aimed to develop a simple modification of Eagle's medium, that will make possible the cultivation of the normally monolayer-growing cells in the suspension conditions. Throughout the experiments the following normally adherent-growing cells were tested: (a) cell lines: HeLa, WiREF (Wistar rat embryonal fibroblasts), MDBK (Bovine kidney), PLA (Adult pig kidney); (b) primary cell culture: CF (Chicken embryonal fibroblasts). All of them normally grow in a monolayer in Eagle's medium supplemented with 5-10% of FCS (Foetal Calf Serum). To cultivate the monolayer cells in suspension, the Eagle's medium was changed as follows: glucose, phosphates and glutamine content was increased: glucose to 3000 mg/l, NaH₂PO₄ x 2H₂O to 1500 mg/l and glutamine to 3000 mg/l. The content of CaCl₂ was decreased to 40 mg/l. To enhance the cell growth to the medium 3.125 ml of insuline and 110 mg/l of sodium pyruvate was added. Instead of FCS, 15% of Tryptose-phosphate-broth: Peptone mixture was used. The obtained results show that HeLa, WiREF, MDBK, PLA and CF can be successfully adapted for the cultivation in suspension. The use of the proposed medium and various cultivation conditions for the more efficient multiplication of VSV virus on HeLa, WiREF, MDBK, PLA and CF and porcine interferon - β induction on PLA cells in will be shown.

FILIPIC, B.¹, O. ZORMAN-ROJS²

Biological activity of interferons in the sera of commercial poultry flocks

vaccinated under the standard immunoprophylactic programmes

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Commercial poultry flocks receive a number of vaccines to protect them from the environmental exposure to pathogens. Immunisation is the principal method used to control several virus infections. Immune response may be influenced by several host mechanisms, such as antibody production and cell-mediated reactions including interferons. The present experiments were aimed to test the biological activity of α -interferon in the sera of commercial poultry flocks in comparison to the antibody response. The sera were obtained from broiler flocks and from broiler chicks. Broiler breeders were vaccinated with the live vaccines against: Marek disease, Newcastle disease, infectious bursal disease, reovirus, infectious bronchitis, avian pox, avian encephalomyelitis and revaccinated with inactivated vaccine against Newcastle disease, reovirus, infectious bursal disease and infectious bronchitis. Broilers were vaccinated with live vaccines against infectious bursal disease and Newcastle disease. As a negative control, the sera from unvaccinated chickens were used. To determine the biological activity of interferon, sera were examined on the chicken embryonal fibroblasts with VSV as a challenge virus in comparison to the chicken IFN standard (50 I.U./ml). In broilers' sera an average titer of 600 I.U./ml was found, while in the sera from broiler breeders, the average titer was higher, around 784 I.U./ml. The titer found in the sera of nonvaccinated chickens was less than 50 I.U./ml. The possible biological role of serum interferons in connection to the immune status of the chickens will be discussed.

FÖLDES, J.

Effects of gyrase-inhibiting fluoro-quinolones on transformation and transfection in cells of *Bacillus subtilis*

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The quinolones inhibit gyrase in bacterial cells, disturb the equilibrium of the biological processes and at the same time induce RecA protein for repair. Transformation and transfection reactions appear useful for study of the mode of action of the quinolones. Transformation and transfection of competent cells of *Bacillus subtilis* 168 Γ were performed with the DNA of the wild strain and the SP50 phage. Ciprofloxacin, Ofloxacin and Pefloxacin were added at subinhibitory concentrations to the systems in different stages of the genetic process. The rate of transformation was calculated and the kinetic curve of transfection was constructed. The quinolones do not inhibit the uptake of DNA and the recombination process in the transformation. The number of transformants correlates with the decrease in growth rate of the bacterial cells. The gyrase inhibitors stop phage synthesis in any stage of transfection and continuously decrease the number of phages produced. The quinolones exhibit quantitative differences in their action on transfection.

FÖLDES, J.

From immunochemistry to molecular genetics. The nature of transfection in *Bacillus subtilis*. (Reminiscence of early days of scientific activity.)

Department of Clinical Microbiology, Albert Szent-Györgyi Medical University, Szeged, Hungary

FRIMAN, G.

Viral and bacterial heart diseases

Department of Clinical Microbiology, University of Uppsala, Uppsala, Sweden

FROLOV, I.², E. AGAPOV², T. HOFFMAN², B. PRÁGAI¹, M. LIPPA², S. SCHLESINGER², C.M. RICE²

Selection of RNA replicons capable of persistent non-cytopathic replication in mammalian cells

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Using engineered Sindbis virus RNA replicons expressing puromycin acetyltransferase as a dominant selectable marker, we identified mutations allowing persistent, non-cytopathic replication in BHK-21 cells. Two of these adaptive mutations involved single amino acid substitutions in the C-terminal portion of nsP2, the viral helicase-protease. This work also provides a series of alphavirus replicons for non-cytopathic gene expression studies (Agapov et al., PNAS USA 95: 12989-94, 1998) and a general strategy for selecting RNA viral mutants adapted to different cellular environments.

FURGANI, G., D. TRIGA, H. PAMJAV, E. SZÁLLÁS, A. FODOR

Gnotobiological analysis of *Steinernema/Xenorhabdus* symbiotic complexes

Department of Genetics, Eötvös L. University, Budapest, Hungary

The RFLP patterns of the PCR amplified internally transcribed spacer (ITS1 - 5-8S rRNA and ITS2) region the 18S - 28S rRNA genes 18 strains of 12 entomopathogenic nematode (EPN) species belonging to the *Steinernema* genus and those of amplified internally spacer region of their bacterial (*Xenorhabdus*)

symbionts were determined. Species specific patterns were identified reproducibly and the cospeciation is proved. We accomplished a large-scale gnotobiology study by growing nematode strains on each other's symbiont. Both dauerlarvae and axenic J1s were grown on bacteria in Petri plates (TSY media), the next generation dauers were regained, *Galleriamellonella* larvae were infected, and the bacteria were regained from the next generation of dauers and then determined.

The results are discussed with the aspect of coevolution.

FÜZI, A.¹, Zs. LANGÓ¹, L. G.-TÓTH², I. MUSKÓ²

Bacteriological data of the molluscs and the planktonic crustaceans of Lake Balaton

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The molluscs and the planktonic crustaceans are filter-feeding organisms and some of them are considered to be effective bacterial feeders. The aim of our work was to identify the bacterial communities of the hindgut content of three molluscs (*Anodonta* sp., *Unio* sp., *Dreicena* sp.), the gut content of two planktonic crustaceans (*Corophium* sp. and *Daphnia* sp.) and homogenized body mass of *Bosmia* sp. and *Eudiaptomus* sp. Up to the present we have not got any information on bacterial communities of these animals living in different aquatic habitats, and especially the Lake Balaton. We collected specimens of molluscs and planktonic crustaceans at two sampling sites (eastern part - Tihany and western part - Keszthely) of Lake Balaton. Seventy-two representative strains were studied in detail by their morphological, physiological and biochemical characters. Based on the phenetic data a dendrogram was generated by using the S_M coefficient and UPGMA algorithm with the help of the SPSS for Windows 6.0 software. Nine characteristic clusters were obtained. Representative strains of phena were identified by the Biolog metabolic fingerprint system too. The dominant clusters contain the following species: *Acinetobacter johnsonii*, *Pseudomonas diminuta*, *Aeromonas media*, *Aeromonas veronii*, *Klebsiella oxytoca*, *Enterobacter cloacae*, all Gram negative organisms (79% of strains). The species of family Enterobacteriaceae were characteristic only in the hindgut of the bigger size molluscs (*Anodonta* sp., *Unio* sp.). In the planktonic crustaceans no enterobacteria were detected.

GÁCSER, A., I. PFEIFFER, J. KUCSERA

Intraspecific polymorphisms among isolates of *Cryptococcus hungaricus*

Department of Microbiology, Attila József University, Szeged, Hungary

In our previous studies six *Cryptococcus hungaricus* strains derived from various geographic area proved to be polymorphic both in their nuclear and mitochondrial genome organisation. Thermo-sensitivity and ability of carotenoid pigment production are their common features. The morphological and physiological properties of six strains were investigated. Carbohydrate-assimilation were examined and five types of C-sources were found to be useful for differentiation. In the study of their nuclear organisation the ITS region of ribosomal DNA were amplified and double-digested. RFLP patterns of ITS sequences showed that only two strains were identical and the other showed striking differences. Physical and functional maps of the mitochondrial DNA of the six different strains were constructed. Results of the mtDNA organisation correlate with those of nuclear ITS organisation. On the basis of molecular characteristics one of the strains extremely differs from the others, not only in its restriction map but its native appearance on agarose gel. The native mtDNA sample is separated to eight bands with different sizes. The organisation of mitochondrial genome of this particular strain is in progress.

GATTINGER, A., L. ZELLES, J. C. MUNCH

Characterisation of methanogenic and methanotrophic microorganisms in agricultural soils by phospholipid analysis

GSF - National Research Center for Environment and Health, Institute of Soil Ecology, Neuherberg, Germany

We report on a method which enables the estimation of methanogenic (anaerobic) and methanotrophic (aerobic) microorganisms in soil ecosystems based on phospholipid analysis. Methanotrophs which are Gram negative bacteria contain ester-linked monounsaturated fatty acids in their polar membran lipids, whereas the phospholipids of members of the domain *Archaea* including methanogens lack the ester linkage. Their membrane lipids possess ether linkages. This biochemical differentiation can serve as a complement to molecular approaches of the domains *Bacteria* and *Archaea* in soil. Examples for the application of this phospholipid assay in a current research project are also contributed.

GAZDAG, Z.¹, N. FARKAS¹, Zs. FEKETE¹, N. HARTVIG¹, M. NYITRAI², J. BELÁGYI³, M. PESTI¹

Chromium(III) and (VI)-induced plasma membrane processes in fission yeast

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Laboratory, University Medical School, Pécs, Hungary

Auxotrophic and heterothallic strains of *Schizosaccharomyces pombe* were used to obtain chromium(VI)-sensitive and resistant mutants. The effects of the Cr(VI) anions on the plasma membrane were studied *in vivo* by applying electron paramagnetic resonance spectroscopy. The spin probes 5-doxylstearic acid (5-SASL) and 3-doxylobutyric acid (HO-185) spin probes were used to label the membrane. The order parameter (S) was calculated at different temperatures (0-25 °C). Addition of 225 µM K₂Cr₂O₇ significantly decreased the phase transition temperatures of the 5-SASL-membrane of the wild-type strain CRW-6 and the sensitive mutant CRS-6.51, but slightly increased the phase transition temperature of the resistant mutant CRR-6.66, as revealed by the HO-185 label. The general cellular oxidant dihydrorhodamine 123 (DHR) was applied to characterise the Cr(VI)-induced oxidative changes. The results suggested a strong, membrane-localised oxidation of DHR induced by Cr(VI). The Cr(III)-induced membrane alteration caused the loss of OD₂₆₀ materials from the cells.

GEML, J.

Utilisation of wild isolates of *Agaricus* spp. in mushroom breeding programs

Korona Spawn Plant and Research Laboratory, Demjén, Hungary

The mushroom produced in the greatest amount today is *Agaricus bisporus*. The importance of using wild varieties of this species in breeding new commercial strains has been realized by several researchers in the last decade. These wild types can be used to improve the commercial strains' growing and marketing characteristics, including better consistency, longer shelf life, flavour, resistance to pests and diseases. The objective of commercial mushroom breeding is to bring together these desired characteristics from two or more different individuals or strains. In addition to this, breeding programs include the creation and selection of desired traits.

This paper introduces the most important breeding methods used for *Agaricus* in the Korona Spawn Plant and Research Laboratory, including the preparation of tissue, mono- and multispore cultures from wild isolates, the method and criteria of selection in the laboratory and during grow-out trials.

GHIDÁN, Á¹, Cs. JENEY², K. CSISZÁR², F. ROZGONYI¹

Detection of vancomycin resistance in *Enterococcus faecalis* using the PCR method

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GÓRSKA E. B.¹, S. RUSSEL¹, B. TUDEK²

Effect of growth conditions on cellulolytic activity of *Bacillus circulans*

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Cellulolytic strain of *Bacillus circulans* was isolated from samples of typical lessive soil collected from long-term, static fertilization experiment at Łyczyn. The isolated strain of bacteria was cultured in mineral medium at temp. 28°C. The culture was incubated stationary and on rotary shaker. The culture medium was amended with carboxymethylcellulose, filter paper strips or Avicel cellulose. The ability of isolated strain of bacteria to degrade of above mentioned substrates was measured on the base of production of cellulolytic enzymes e. g. cellobiase, CMC-ase and FP-ase. The activity of cellulolytic enzymes was determined by Ghose and Mandels method, measuring level of reducing sugars in reaction mixture. The characteristic of investigated strain was done on the base of morphology and biochemical properties. The ratio of % GC in DNA was also determined. The isolated strain has been classified as *Bacillus circulans*. It was found that complex of cellulolytic enzymes produce by *B. circulans* doesn't contain cellobiase. Intensity of cellulolytic enzymes produced by *B. circulans* depended on aeration and carbon source in culture medium. Significantly higher cellulolytic activity was found in supernatants obtained from shaken cultures than stationary ones. The highest activity of CMC-ase was found in medium with CMC as carbon source. In shaken cultures the production of FP-ase was practically the same in growth media amended with CMC and Avicel cellulose as a sole carbon source. Optimum activity of both investigated cellulolytic enzymes was found at pH 7,0 and temp. 50°C.

GÓRSKA, E. B.¹, S. RUSSEL¹, J. LABETOWICZ²

The occurrence of mesophilic, cellulolytic bacilli in typical lessive soil under differentiated mineral and organic fertilization conditions

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In present paper the effect of organic and mineral fertilization and term of soil samples collection on occurrence of cellulolytic bacilli in typical lessive soil from Łyczyn was studied. The investigated soil samples were collected five times during vegetation season from experimental pots of long term, static fertilization experiment at Łyczyn. The most probably number (MPN) of mesophilic, cellulolytic bacilli was determined by dillution method using mineral liquid medium amended with strips of filter paper as a sole carbon source. Before inoculation of growth medium the proper dillutions of soil suspensions were pasteurized at temp. 80°C for 15 minutes. The cultures were incubated at temp. 28°C for two weeks. The presence of investigated bacteria were checked macro- and microscopically. During

macroscopic analysis the change of colour of filter paper strips, its looseness and the presence of slime was considered. The results were calculated statistically using two - way analysis of variance method. It was shown that occurrence of mesophilic cellulolytic bacilli in investigated soil samples depended on term of soil samples collection, climatic conditions and organic and mineral fertilization. The highest number spore-forming, cellulolytic bacteria was observed in July in soil samples collected from limed pots fertilized with manure and full mineral fertilization (NPK). From meteorological dates appeared that the July was the most wet and warm month of the year. The lowest value MPN of investigated bacteria was observed in June.

GRECO, I., P. ROMANO

Molecular biology strategies as biotechnological tools in wine quality

Dipartimento di Biologia, Difesa e Biotechnologie Agro-Forestali, Università degli Studi della Basilicata, Italy

To study the diversity present in a biological system is essential to determine its individual genotype and geographic characteristics. In this context the biological source of the genetic information allows to individuate any genetic traits, without environmental effects, and also to determine the degree of genetic polymorphism. As we are focusing our attention on the conservation of individual characteristics of Aglianico of Vulture, the typical grape variety of Basilicata region, we have chosen to investigate molecular and genetic variability in vine clones of Aglianico of Vulture cultivar and in autochthonous strains of *Saccharomyces cerevisiae*. The development of the polymerase chain reaction (PCR) has created fascinating possibilities in biotechnology, since virtually any target DNA can be amplified from complex samples and the use of RAPDs primers allows to analyse a great part of the genome in a single population. We applied these molecular techniques to assess genetic diversity of vine clones and yeast strains. In this study, thirteen vine clones of Aglianico of Vulture, collected on the basis of morphological characteristics and tested by RAPDs analysis, exhibited a certain variability. Fifty strains of *S. cerevisiae*, isolated from different spontaneous fermentation of Aglianico of Vulture, were characterised phenotypically for technological traits, such as resistance to SO₂ and copper, ethanol tolerance, production of some fermentation by-products. Of these, twenty strains, representing the different phenotypes found, were selected and characterised by molecular analysis. A strong polymorphism was found with the expression of biotypes, differing in size and/or number of bands. The preliminary results have demonstrated a high genetic variability in biological natural sources both in vine plant and in yeast strain. The goal of our study is to identify suitable associations between vine clone genotype and yeast strain genotype that, besides imparting desirable organoleptic qualities, can also preserve the individual characteristics of the wine under consideration.

GRISHKO, V.

Changes in bacterial numbers of microbial cenosis in soils contaminated with

fluorides

Krivorozhsky Botanical Garden NAS of Ukraine

Qualitative and quantitative characterizations of soil microbocenose are often used for diagnostics of biological processes taking place in soils contaminated with heavy metals, nitrogen compounds, organics. But, effects of fluorine containing effluents of industrial factories upon bacteria numbers of microbial cenosis in soils are small investigated. The study of bacteria numbers grown on meat-peptone agar (MPA) and sporulating bacteria showed considerable changes in their amounts at soils with various levels of fluorine contamination. Thus, when fluorine contents were enhanced from 1.1 to 5.2; 86.2, 120.2 and 169.4 mg/kg in top soil layer (0-10 cm), numbers of MPA-drown bacteria were decreased accordingly by 6.1, 2.5, 64.5 and 86.2%. Examination of samples from deeper soil layers (15-30 cm) resulted in fluorine content exceeding that in the control soil samples. Increased fluorine content in soil layer of 15-30 cm reduced numbers of microorganisms grown on MPA by 3.2 times. Enhanced amounts of sporulating bacteria indicated, in our opinion, forming of less favourable conditions in soil contaminated with fluorides. Thus, in soils with strong, middle and weak contamination levels sporulating bacteria were 64.8, 53.3 and 48% from total number of bacteria grown on MPA. In control soil bacilles number was 21%. In the top soil layer (0-10 cm) with higher toxicant level sporulating bacteria number exceeded by 9.3% in average, than in deeper layer (10-30 cm) with lower level of fluoride contents. In control soils it had been found tendency to increasing of sporulating bacteria number in lower soil horizontes. Fulfilled investigations showed, that in soils contaminated with fluorine significant changes in bacterial cenosis took place.

GUNICS, Gy.¹, S. FARKAS¹, N. MOTOHASHI², A. SHAH³, M. KAWASE³, S. SAITO⁴, J. MOLNÁR¹

The modification of antibiotic resistance in some Gram negative bacteria

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The Verapamil as a well known resistance modifier and its newly synthesized derivatives were tested for synergy with some antibiotics. The compound had no direct antibacterial effect on various *E. coli* strains. The antibacterial effect of Ampicillin was enhanced in the presence of the majority of Verapamil analogues on a laboratory strain. Compound [G10] and Verapamil were antagonistic with Ampicillin. Compounds [G2, G3, G8, G9, G11, G13, Nifedipine] among eleven Verapamil analogues were synergistic after 24hr incubation. When Verapamil analogues were tested on clinical isolates of *E. coli* Ampicillin^s, Erythromycin^r, Verapamil had moderate synergistic effect with Ampicillin. Compound [G1] antagonized the antibacterial effect of Ampicillin. Synergistic effect was found with

Erythromycin on *E. coli* in the presence of [G4, G5, G6 or G8] . Non of the G-compounds had synergy with Ampicillin or Erythromycin in one polyresistant clinical isolate of *E. coli*. The structure activity relationships of Verapamil analogues and their synergy with Ampicillin and Erythromycin will be discussed.

GVOZDYAK, R. I., L. V. KABASHNA, L. A. PASICHYK, E. A. MAKARCHUK

Interaction between endophytic bacteria of wheat seeds and pathogens

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Endophytic microflora of four varieties of wheat: Kharkivska 37, Khersonska 86, Albatros, Lutescens was investigated. Special attention was payed to the sterilization of seeds and removal of epiphytic microflora. Absence of epiphytic microflora on sterilized seeds was checked by washing them in a broth, with the subsequent incubation: absence of growth indicated the sterility of seeds. From free epiphyte seeds endophytic bacteria were isolated by plating their pestled mass on a nutrient agar and placement of the externally sterilized seeds on the surface of nutrient medium. Depending on the variety the quantity of wheat seeds containing the internal bacteria was different, but this did not exceed 60%. Among isolated bacteria the phytopathogens were not revealed. Certain endophytic isolates belong to *Pantoea agglomerans* and the genus *Bacillus*. Spraying of wheat seeds by these endophytes completely suppressed their overlaying by micromycetes, with in the control overlaying of seeds by micromycetes was 70-100%. Endophytic bacteria of wheat seeds were not antagonists to *Pseudomonas syringae* pv. *atrofaciens*, the dangerous disease agent of wheat. At the same time endophytes affect a pathogen aggressiveness. At artificial infection of plants at the time of vegetation with the mixture of cells of pathogenic and endophytic bacteria aggressiveness of *Pseudomonas syringae* pv. *atrofaciens* was significantly lowered. At some ratio of pathogen and endophyt, pathogen lost ability to cause diseases in plants.

HÁBER, K. J.¹, M. TAKÁCS², A. NAGY¹, A. SZENDRŐI², P. DEZSŐ¹, Gy. BERENCSI², J. MINÁROVITS¹

Cloning and sequencing of mycobacteriophage DNAs

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Two phages - differing from each other in their morphologies and DNAs – were separated by linear gradient equilibrium centrifugation from mycobacteriophage D29 in our laboratory. Phage D29A (of higher density) has isometric heads and long, noncontractile tails; phage D29F also has long, noncontractile tails but its heads are elongated. Comparing these phages to the sequence of mycobacteriophage D29 published in 1998 we found that phage D29A DNA shows some similarity to D29 DNA: (i) both DNAs have only one EcoRI cleavage site (ii) non of these DNAs are cleaved by SacI endonuclease. On the contrary, D29F DNA has several cleavage

sites of EcoRI and that of SacI enzyme. For the purpose of sequencing we began to clone D29A and D29F DNA fragments into pUC18 plasmid. A clone containing a ~1,2 kb KpnI-EcoRI fragment- and clones harbouring ~1,3-, 1,2- and 0,4 kb KpnI-EcoRI fragments were isolated from D29A and D29F DNA, respectively. Cleavage sites of SmaI-, SacI-, BamHI- and PstI enzymes were determined on the ~5,8kb EcoRI-EcoRI fragment of D29F-DNA inserted into the vector earlier. On the basis of this map subclones were also constructed. We also present a search of the GenBank database carried out with sequences determined from these clones.

HAJDÚ, Cs.

Involvement of mono- and polysporic cultures of wild *Pleurotus* spp. in the breeding work

Korona Spawn Plant and Research Laboratory, Demjén, Department of Plant Physiology, University of Horticulture and Food Industry, Budapest, Hungary

The oyster mushrooms (*Pleurotus sp.*) are cultivated in the largest quantity in the world after *Agaricus bisporus*. The cultivation of these mushrooms has been increased in some areas of the world, so their importance is undoubted.

In the breeding work in the Korona Spawn Plant and Research Laboratory, wild *P. ostreatus* and *P. pulmonarius* tribes with wide geographic distribution and significant genetic variability are used in breeding programs. The aim is to create hybrids, which can fruit at higher temperature, thus suitable for solving the problem of summer cultivation.

This paper details the process of making tissue, mono- and multispore cultures from wild specimens, the observation of these cultures' development and morphological features in laboratory conditions under optimal and extreme circumstances.

HALBRITTER, A.¹, V. KRISTUFEK²

Milestones of microbial ecology and the key personalities behind

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Microbial ecology emerged as an energetic and dynamic branch of science only in the early 1960's, and as such new and fast developing sciences, looks into the future. The history of microbial ecology is therefore not widely known even among microbial ecologists, although their subject's position among other branches, the research topics, and fundamentals cannot be fully understood, only in historical context. 'Ecologists' think only in macro-organisms, and the studies in the history of microbiology concentrated on fields like medical microbiology or microbial genetics. But many of the concepts and paradigms of ecology were born in the field of microbiology, enough to mention that even the term 'ecology' in its current use

was first defined by a protistologist, Ernst Haeckel.

As microbial ecology is getting more and more role in university education, we tried to look up material for this topic and build up a short introduction for students of biology. As a skeleton of the history of microbial ecology we have chosen some main ecologically important topics, namely the *existence, role, physiology* and *communities* of microbes and tried to follow the development of the knowledge on these problems. Among the hundreds of scientists who deserve salute we included only those, whose name can be connected to a main step of the elucidation of the problems. Some of them were not microbial ecologist *sensu stricto* but all they (even the earliest pioneers) shared an ecological approach to the problems.

HANCZÁR, T.¹, R. CSÁKI¹, L. BODROSSY^{1,2}, J. COLIN MURRELL³, K. L. KOVÁCS^{1,2}

Hydrogenases in methanotrophic bacteria

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The thermotolerant methanotroph *Methylococcus capsulatus* strain Bath was shown to have genes for the small and large subunits of a typical Type I (Ni-Fe) hydrogenase by Southern hybridisation with a heterologous probe from *Thiocapsa roseopersicina*. These genes have been cloned and the hydrogenase structural genes have been sequenced and compared with the sequences of known hydrogenase genes from other organisms. The hydrogenase genes have been used in marker exchange mutagenesis experiment in order to create hydrogenase- minus mutants of *Methylococcus capsulatus*. Hydrogenase activity has also been examined in whole cell assay with *Methylococcus* grown under a variety of different growth conditions. Preliminary biochemical characterisation of the hydrogenase enzyme has also been carried out. This hydrogenase shows considerable potential for bioremediation process involving soluble methane monooxygenase since it may supply valuable reductant for this enzyme in whole cells under certain growth conditions.

HEGEDŰS, A.¹, H. E. A. F. BAYOUMI HAMUDA², M. KECSKÉS²

Enhanced growth of gerbera plants in the presence of *Trichoderma* spp. inoculates

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HERPAY, M., É. CZIRÓK, I. GADÓ, H. MILCH

Laboratory strategy in diagnosis of Shiga toxin producing *Escherichia coli* in

Hungary

"B. Johan" National Center for Epidemiology, Budapest, Hungary

Alltogether 29 Shiga toxin-producing *Escherichia coli* (STEC) were isolated and characterized according to their serotypes, virulence markers, and association with human illness. For screening faecal samples were examined for free Shiga toxins (Stx(s)), additionally they were cultivated on sorbitol MacConkey (SMAC), cefixime and tellurite (CT)-SMAC and chromogenic plating media (BBL CHROMagar O157). Comparison of *E. coli* O157 detection was made between direct plating, immunomagnetic separation (IMS) and plating. Premier EHEC enzyme immunoassay was applied for the detection of Shiga toxins (Stx(s)). Specimens positive by Premier EHEC test and negative for *E. coli* O157 were serotyped. Our O157 STEC positive human faecal samples originated from bloody diarrhoea (13), non-bloody diarrhoea (2) and HUS (1). In 13 cases free Stx were detected. Two mixed cultures and 5 isolates (O157-2, O18ab-2, O19-1) of these cases were Stx-positive, too. In two cases STEC strains (O157-1, O76-1) could be isolated, however Stx(s) failed to be detected in their faecal samples. Mixed cultures of lateres were also Stx(s) positive. All faecal samples from patients with diarrhoea should be screened for the most frequent serogroup O157, or if this is not possible, at least those from patients with bloody diarrhoea. CT-SMAC and CHROMagar are selective for O157 STEC. Premier EHEC test is valuable as a routine method for the detection of non-O157 Stx-producing *E. coli*. Successful Stx diagnosis can be expected from different kinds of simultaneously performed methods.

HOLB, I. J.

Influence of acidity in structure development of *Monilinia fructigena*

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In pome fruit growing areas, *Monilinia fructigena* (Aderh. & Ruhl.) Honey is one of the main pathogens causing fruit rot. Together with *Monilinia laxa* (Aderh. & Ruhl.) Honey and *Monilinia fructicola* (Winter) Honey forms the group of brown rot fungi. Plant pathologists have studied the brown rot fungi very intensively to decrease fruit loss, and these investigations suggested that overwintered mummified fruits played an important role of the pathogen life cycle as a main source for primary inoculum, but the amount of primary inoculum depends on the quality of mummies. Many factors (internal quality of the fruit, abiotic conditions) influence the mummification process after infection. During this mummification process (i.e. hardening of the fruit tissue), stroma formation develops inside the fruit, which serves as overwintering structure. For rational control is important to know which conditions are favourable for the development of stroma inside the fruit and how this process reacts to changes in the environment. Although there is a lot known about this subject, for example many factors (nutrition, temperature, light, age of the culture) have already been examined but study about the influence of acidity on stroma

formation in *Monilinia fructigena* has not been described yet, although it is one of the main factors of the internal quality of the fruit and probably plays an important role of the development of stroma formation. Therefore physiological study were made. The aim was to evaluate the effect of different pH (acidity) ranges in the disease progress of *Monilinia fructigena* on agar plates (*in vitro*) and in apple fruits (*in vivo*). In our experiment, two isolates of *Monilinia fructigena* were used (JAP.2316 /Japanese/ and HU.D2 /Hungarian/) which represented the characteristic features of the two main *Monilinia fructigena* groups. For the agar experiment buffered PDA (Oxoid) media were prepared with a range of the initial pH from 2.5 to 6.5 (pH 2.5, 3.5, 4.5, 5.5 and 6.5). The dishes were inoculated with 5 mm plug of each *Monilinia fructigena* isolate. Incubation took place at 23 °C in darkness. The cultures were allowed to develop for three days and then daily the diameter of the colony in mm were measured until the colonies filled the dishes. When the stromata were considered mature, the stromatal plates were harvested. For fruit experiment cv. James Grieve apple fruits 2 weeks before fruit harvested were used in five course of time. Inoculation technique was the same as it is described in agar experiment. During stromata development in fruits, the pH changes of the fruit were detected according to the disease progress (after 7, 14, 28 and 35 days artificial inoculation). In the plates experiment the most intensive mycelial growth was at pH 4.5 and pH 3.5, resp. in Japanese and Hungarian isolate. After having appeared stroma formation on cultures, the pH of the cultures was finally stabilized by the fungus between 4.6 - 5.4 (without control) 20 and 50 days after incubation, resp. in Japanese and Hungarian isolate. Both isolates formed the highest amount of stromata at pH 5.5. The Japanese isolate produced about two or three times more stromata per petri dishes depending on the initial pH. Higher pH (> pH 4.0) was also more favourable for stroma formation in the fruit experiment. Presented pH changes, weight of the stromata and fruit assessment showed that a higher pH (pH 4.0-5.5) equals lower acidity was more favourable for stromata development than lower pH (pH 3.0 - 3.2) of healthy fruits. Consequently, low acid content fruits of apple cultivars or matured fruits are likely to be favourable for mummification of *Monilinia fructigena*.

HOLLAND, I. B.

The secretion of *E. coli* haemolysin by the type I, ABC-dependent mechanism

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Type I secretion from *E. coli* involves a transenvelope translocator composed of the inner membrane protein HlyB, and the inner membrane protein HlyD, which spans the periplasm to interact with the third protein TolC, an outer membrane pore. Haemolysin, HlyA, a 107 kDa toxin (forming Ca²⁺/K⁺ channels in target membranes), docks with the translocator using a 46 amino acid secretion signal, at the extreme C-terminus of the HlyA molecule. Secretion is extremely rapid and direct to the external surface, without any periplasmic intermediate. HlyA is apparently unfolded during translocation and the C-terminal secretion signal, which is not removed during transport, is involved in refolding the toxin before it leaves the surface. We have recently obtained evidence that HlyD may have two functions,

one to provide a pathway across the bacterial envelope, and also to provide a multimeric folding chamber, providing a chaperone-like function, perhaps in association with TolC. HlyB is the bacterial prototype of an ABC-(ATPase) transporter, a member of the super family of proteins, including Mdr (P-glycoprotein) Mrp and CFTR, which are important in human disease. HlyB provides energy for transport through its highly conserved, cytoplasmic ATPase domain. We are currently analysing the 3-dimensional structure of this domain and this structure and its possible function will be discussed.

HÖLZLE, L. E., M. M. WITTENBRINK

Molecular characterisation of chlamydiae from swine

Institute of Veterinary Bacteriology, University of Zürich, Switzerland

In our report lung and intestine of 49 pigs with respiratory diseases and endocervical swabs from 205 sows with reproductive disorders were investigated for *Chlamydia* infection by polymerase chain reaction. Samples from 49 healthy slaughter pigs and endocervical swabs from 30 fertile sows served as controls. PCR primers targeted DNA sequences flanking almost the entire *Chlamydia omp1* gene and sequences flanking a 590-bp fragment of the *Chlamydia omp2* gene. PCR amplicons of the expected size were generated from 49.0% of pigs with respiratory disease and 60.0% of sows with reproductive disorders. Corresponding values for the respiratory healthy controls were significantly lower (24.5%; $p < 0.05$). No PCR amplicons were obtained from endocervical swabs of fertile sows. By DNA hybridization of PCR amplicons a high prevalence of mixed infections with *C. psittaci* serotype 1 and *C. trachomatis* in the porcine lung and intestine was found and further confirmed by restriction fragment length polymorphism analysis and nucleotide analysis of the *omp1*-gene-PCR amplicons. 81.3% of the PCR amplicons from endocervical swabs were identified as *C. psittaci* serotype 1, indicating an association of the known genitopathogenic *C. psittaci* serotype 1 with reproductive disorders in sows. Nucleotide sequence analysis of *omp1* gene amplicons identified as porcine *C. trachomatis* shared maximum 82.7% homology with the reference strain S45.

HRYNIEWICZ, W., A. SKOCZYNSKA

Characteristics of major clinical pathogens responsible for meningitis in Poland

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Bacterial meningitis remains major cause of morbidity and mortality world-wide, especially in children. Beyond the perinatal period, there are three major causative agents of bacterial sepsis and meningitis in industrialised countries: *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. During 1997-

98 National Reference Centre for Bacterial Meningitis collected 220 strains isolated from cerebrospinal fluid from patient with meningitis. Among them the most common was *N. meningitidis* (n= 90, 40.9%), followed by *H. influenzae* (n=58, 26.4%) and *S. pneumoniae* (n=46, 20.9%). Most of *N. meningitidis* and *H. influenzae* strains were isolated from children below the age of five. *S. pneumoniae* was mainly isolated from adult patients. Out of meningococcal strains 88.9 % belonged to group B. Most of them were highly sensitive to penicillin, however nine (10 %) of them showed decreased susceptibility to penicillin with MIC higher than 0.06 mg/l. All *H. influenzae* belonged to serotype b and were susceptible to 3rd generation cephalosporins and chloramphenicol. Five strains (8.6%) produced β -lactamases. Two isolates were resistant and 10 exhibited intermediate susceptibility to cotrimoxazole. Eight isolates (13.8%) exhibited intermediate susceptibility to rifampin. Broad distribution of serotypes was found among pneumococcal strains of which the most common were serotypes 3, 8 and 22F. Penicillin nonsusceptible strains constituted 13 % of all pneumococcal isolates (4 resistant and 2 intermediate susceptible strains). Three of resistant strains belonged to 23F serotype. Among pneumococci 22 % were resistant to chloramphenicol and 8.8 % to cotrimoxazole. Molecular techniques such as PCR based methods and PFGE have been also developed for epidemiological typing as well as for identification.

IMZILN, B.

Incidence of mesophilic *Aeromonas* within aquatic environments in Marrakech, Morocco

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Under arid climate regions, water shortage is the main occupation of the population of these areas. Generally, in Marrakech, hurries did not exceed 250 mm per year, and drinking water is generally obtained from fresh waters (river waters, lakes, etc). During the past twenty years, mesophilic aeromonads have been frequently recognised as responsible for several diseases, both in humans and in animals. Indeed, aquatic environments were frequently considered as the major sources of infection by these bacteria. Furthermore, *Aeromonas* bacteria have been recognised able to grow fast in temperate area and when conditions are favourable. The incidence of mesophilic aeromonads in river waters, and sewage outfalls during 12 months in Marrakech, Morocco was determined. Water samples were collected from 8 sites in different geographic areas of the town. Samples of water were collected from fixed sites at three rivers (Oukaimeden, Ourika and Tensift). Samples from open sewers were taken from two representative sites in the spreading field of the town. Bacteriological analysis were done from samples, and *Aeromonas* isolates were identified by standard biochemical tests and tested for their ability to produce some virulence factors. Densities of *Aeromonas* spp. varied from 10^4 cfu per 100ml to 5.6×10^6 cfu/100ml. Higher concentrations were recorded in raw wastewater's and the lowest in waters from Oukaimeden river the proportion of *Aeromonas sobria* to other species increased considerably (especially in warm periods). Among the tested strains, the majority 98.4 and 96% of *Aeromonas sobria* and *Aeromonas*

hydrophila, respectively produced haemolysin. Among the *Aeromonas caviae* strains 53% were found to be haemolytic. Survival study demonstrated that *Aeromonas* strains were able to grow in river waters and quickly in river waters supplemented with sewage or organic matter. In conclusion, we think that in warm regions as Marrakech, the presence of mesophilic aeromonads with great concentrations in several aquatic ecosystems may be a serious risk of infection and consequently will be a reason behind the restriction of moult-uses of these waters. Further investigations on the fate and vulnerability of mesophilic aeromonads in aquatic environments, where the risk of direct infection to humans is high, are required.

ISMAIL, M. H.¹, A. A. ABOU-ZEID²

Studies on alfalfa mosaic virus of alfalfa in Egypt

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ISMAIL, M. H.¹, M. S. AHMED²

Properties of sweet potato feathery mottle virus (SPMFV)

¹Botany Department, Faculty of Science, ²Botany Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

IVÁN, J.¹, N. NAGY², I. KACSKOVICS³

Examination of B cell development in the bursa Fabricii after *in-ovo* injection of an infectious bursal disease immune complex vaccine

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The functional activity of the bursal B-lymphocytes was studied in a critical period in which gene conversion occurs, a known feature providing immune repertoire in chicken. To evaluate the influence of a newly developed immune complex vaccine against infectious bursal disease (IBD) on this stage, 18-day old SPF chicken embryos were vaccinated and the changes of chB1 gene expression were studied from one-day old chickens up to 48 days of age. ChB1 lectin has been recently described as a marker of bursal B-cells undergoing gene conversion. Northern blot analysis showed a dramatic decline in the expression levels 7 days after hatching. Detectable gene expression returned around day 35 with individual differences. Immunohistochemical studies detected vigorous B-cell depletion from the bursal follicles at day 7 after hatching and also showed follicles regeneration. These results coincide with the Northern blot suggesting a reversible character of this damage.

Based on our study this immune complex vaccine temporarily interrupted the normal development of the bursa Fabricii inducing a strong depletion of the bursal B-cells but after about a four-week regeneration phase the follicles were repopulated by functionally active B-cells.

IZADPANAHI, R.^{1,4}, M. BENKŐ², Á. DÁN^{2,3}, K. URSU^{2,3}, M. RUSVAI¹, B. HARRACH²

Genetic analysis of the fiber gene and the early region 4 of bovine adenovirus 2

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Bovine adenovirus 2 (BAdV-2) is a non-enveloped double stranded DNA virus which is a mild pathogen causing economic losses in cattle industry occasionally. Physical mapping of the BAdV-2 genome has been accomplished and many parts of it including two-third of the fiber gene and the right end of the genome have been sequenced. Now, we report on the sequencing and analysis of the region which is completing the fiber gene (knob) and the E4 region. We have used the *Sall* - *EcoRI* fragment located at 63-90.5 map units in transposon insertion based DNA sequencing. Based on the new sequence data, the size of the fiber gene could be specified to be 560 amino acid long of which the knob region is 176 amino acids. Our sequencing also revealed a new ORF in the E4 region. Homology comparison with human adenovirus 2 indicated that this region contains a gene homologous to the 17 kD protein. The re-analysis of the E4 region previously sequenced by Y. Haj-Ahmad's group shows slight differences in the ORFs compared to the published data. It is interesting that according to our results in BAdV-2, the ORF closest to the right ITR has a similarity to the dUTPase found in eukaryotic organisms, as well as in pox viruses and on the opposite end of the genome of fowl adenovirus 1 and 9 (published as "type 8").

JAKUCS, E.

***Thelephoraceae*-mycorrhizae in Hungarian *Populus*-forests**

Department of Plant Anatomy, Eötvös Loránd University, Budapest, Hungary

Besides *Boletaceae* and *Russulaceae*, *Thelephoraceae* are the most widespread ectomycorrhizal fungi in needle and deciduous forests playing an outstanding role in water and mineral supply of trees. However, up to now only few ectomycorrhizae of the family has been described comprehensively. *Thelephoraceae*-ectomycorrhizae isolated from soil samples taken in different *Populus alba* forests of the Hungarian Plain has been characterized using the morphological and anatomical methods, introduced by AGERER, including SEM, PhC and Nomarski microscopy and histochemical investigations. Three unknown ectomycorrhizae, belonging to the genus *Tomentella*, has been described from *Populus*-roots. Identification of the

species was carried out by comparing morphology of cystidia and ITS-DNA-sequences of ectomycorrhizae and fruitbodies.

JENEY, Cs., O. DOBAY, B. BANIZS, É. ÁDÁM, I. NÁSZ

In the absence of epsilon-cop the human adenovirus type 5 regurgitates into the extracellular space

Institute of Microbiology, Semmelweis Medical University, Budapest, Hungary

JEVCSÁK, I.¹, B. BIRÓ², H. E. A. F. BAYOUMI HAMUDA¹, M. KECSKÉS¹

PGPR effect and herbicide sensitivity of some pseudomonads depending on their origin and plant hosts

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The *in vitro* growth of some home-isolated PGPR pseudomonads were examined in liquid nutrient broth supplemented with glyphosate (N-phosphono-methyl-glycine) herbicide. Four concentrations (0.1, 1, 10 and 100 $\mu\text{g l}^{-1}$) were used in a micro-fermentor, where the cell number of the various strains was investigated after 14 hours of incubation starting from the 10^6 CFU ml^{-1} in liquid YEM media. Different isolates of the fluorescens-putida type *Pseudomonas* sp. were collected from various sites and hosts, such as crownvetch (*Coronilla varia*), sugarbeet (*Beta vulgaris*), potato (*Solanum tuberosum*), lupine (*Lupinus albus*), river Tisza etc, so as to select candidate isolates for inocula-production. Plant growth promoting (PGPR) effect of the various strains was also calculated from the *in vitro* siderophore production. Glyphosate herbicide was found to have only a slight influence of the *in vitro* growth rate of tested pseudomonads. No significant differences developed among the effect of various rates, except the 100 $\mu\text{g l}^{-1}$ dose in some cases. There was no correlation between the siderophore production or the herbicide sensitivity and also between the host-plants or the origin (rhizosphere or water) of the strains. Testing some physiological characteristics, such as the antagonistic ability against the soil-borne plant pathogens or the sensitivity to abiotic stress factors (pH, salinity, drought...etc.) proved to be especially important, however for *in vitro* selecting the candidate inocula strains for further *in vivo* applications.

Sponsored by the Hungarian Research Fund (OTKA): T 023543 and T 030941.

JUHÁSZ, Á.¹, Zs. HAMARI², B. TÓTH¹, L. FERENCZY^{1,2}, F. KEVEI¹

Interpretation of mtDNA recombination events among vegetative incompatible *Aspergillus japonicus* strains

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The mitochondrial genomes of strains of *A. japonicus* belonging to the imperfect black *Aspergilli* display highly variable RFLP patterns. Transmissions of mitochondria were carried out between oligomycin resistant mutant (oliR) strain as standard donor and sensitive recipient ones with different mtDNA RFLP patterns by using protoplast fusion technique. All intraspecific mitochondrial transfer experiments resulted in oliR progeny with recombinant and/or unchanged donor mtDNAs. In our previous work physical and functional maps of a recombinant' and its parent's mtDNAs were developed for interpretation of recombination processes. The results elucidated that the recombination process was based on movement of introns. The recombinant progenies inherit basically the resistant donor mtDNAs, which are modified by introns of recipient mtDNA. Four introns were determined, which are involved in formation of recombinant characters. These introns were used as DNA probes in hybridisation experiments to determine intronic relations among different recombinant progenies and their parents. In our recent work another recombinant and its parents were also investigated to find novel mobile introns, which may play a role in recombination events. Physical and functional maps of parental and recombinant mtDNAs were constructed and compared. Regions involved in recombination processes were studied at sequence level.

JUHÁSZ-ROMÁN, M.¹, E. SIMON², Á. TÓTH³

Special fermented milks with low protein- and phenylalanine content for phenylketonuric persons

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For phenylketonuric persons is very important a dietetic life-style.

Normal kefir cultura and intestinal Lactobacilli were used to produce the therapeutic kefir and probiotics from low protein- and phenylalanin- based milk.

The next chemical and microbiological parameters were examined in details:

- acidity
- aroma compounds
- viable cell count of Lactic acid bacteria
- antibiotic sensitivity of intestinal Lactobacilli

-phenylalanin content

The acidity was higher and the aroma profile was poor in case of probiotics then in normal kefir therefore we supplemented these products with fruit juices.

(This supplementation seems to be the best with heated ananas for the phenylketonuric children.)

KÁLMÁN, M.¹, E. SZÖLLŐSI¹, A. FÓNAGY¹, M. ZIMÁNYI¹, L. LACZKOVICS²

Examination of serum antibodies to *Campylobacter jejuni* ssp. *jejuni* and GM₁ antibodies in peripheral neuropathy

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The Guillain-Barré syndrome (GBS) is the most common cause of peripheral paralysis. Two-thirds of GBS patients develop the syndrome following various infections, the leading cause being campylobacter enteritis. Patients with GBS subsequent to *C. jej.* ssp. *jej.* enteritis frequently exhibit a high antibody titer to GM₁ ganglioside, probably through molecular mimicry between ganglioside and the LPS of *C. jej.* ssp. *jej.* isolated from GBS patients. We examined serum antibodies (IgGAM, IgG and IgM) to *C. jej.* ssp. *jej.* and to GM₁ ganglioside by ELISA. The serum samples were collected from patients with GBS, meningitis, encephalitis or acute flaccid paralysis, from healthy young adults, some working under conditions of risk (poultry-processing factory) and from *C. jej.* ssp. *jej.*-positive persons (with enteritis, but without GBS). Elevated GM₁ antibody titers were detected in 6 of 7 GBS patients demonstrating high IgGAM antibody titers to *C. jej.* ssp. *jej.* The serum of a patients with encephalitis displayed a high IgGAM titer to *C. jej.* ssp. *jej.* and to GM₁ ganglioside, but this patient had received DIPERTE vaccine 5 days earlier. 7 % of the healthy adults and 59 % of those working under conditions of risk demonstrated a high IgGAM titer to *C. jej.* ssp. *jej.*, but the sera of only a few of those working under conditions of risk exhibited a low antibody titer to GM₁ as well. The sera of *C. jej.* ssp. *jej.*-positive patients without GBS showed a high IgGAM titer, but no antibody to GM₁ ganglioside was detected.

KAMOTSAY, K., I. DUNAY, Zs. CSUKÁS, K. LATKÓCZY, K. GLATZ, Zs. BEREK, F. ROZGONYI

Distribution and antibiotic susceptibility of blood culture isolates

Institute of Microbiology, "Semmelweis" University Medical School, Budapest, Hungary

Between 1 January and 31 December 1998, 426 microbes were isolated from 2587 blood culture bottles. 65% of the bacteria belonged to the Gram-positive group and 35% of them belonged to the Gram-negative group. 0.02% of the positive results

were *Candida* spp. The most frequent microorganisms were *Staphylococcus aureus* (29%), followed by coagulase-negative *Staphylococcus* spp. (20%), *Enterobacter* spp. (13%), group-D *Streptococcus* (7%) and *Escherichia coli* (7%). The most potent drugs against *S. aureus* were vancomycin (100%), teicoplanin (92%), chloramphenicol (92%), netilmicin (74%), ciprofloxacin (62%), oxacillin (48%), and against coagulase-negative *Staphylococcus* spp. vancomycin (100%), teicoplanin (83%), chloramphenicol (90%), netilmicin (67%), ciprofloxacin (90%), oxacillin (60%). Strains of group-D *Streptococcus* were susceptible to vancomycin (100%), teicoplanin (80%), imipenem (87,5%), piperacillin (76,5%) and amoxicillin/ampicillin (67%). In case of *Enterobacter* spp. the most effective drugs were imipenem (94%), and ciprofloxacin (77%). Strains of *E. coli* were susceptible to imipenem (100%), cefuroxime (74%), ceftriaxone (88%), ceftazidime (88%), cefotaxim (66%). The most potent drugs against *Pseudomonas* spp. were netilmicin (73%), amikacin (65%) and ciprofloxacin (55%). In conclusion, bloodstream infections of inpatients seem to be caused by multiple resistant bacterial strains.

KANJO, A. H.

Our experience with Crystal identification system E/NF

Microbiology Laboratory, Árpád Hospital, Budapest, Hungary

Since more than two years we have been using several traditional and other commercial non-automated systems in our laboratory, for the identification of isolated bacteria. Some of the isolates were identified with Crystal Enteric/Non-Fermenter ID Kit (Crystal E/NF) system which is based on 30 enzymatic and biochemical substrates divided over three rows of 10 wells. The BBL CRYSTAL Identification (ID) System associated accessories include, light box or panel viewer, color reaction chart, report form and software package.

The aim of this work is to evaluate the accuracy of this system for the identification of different groups of bacteria isolated from several clinical specimens. Altogether 700 clinical isolates of fermentative and non-fermentative bacteria including the family Enterobacteriaceae, and genera *Stenotrophomonas*, *Pasteurella* and members of the most common nosocomial pathogens *Pseudomonas* and *Acinetobacter* spp., mostly isolated of Intensive Care Units (ICUs) and surgical wards, were investigated.

147 *Acinetobacter baumannii* strains were tested. 117 strains were identified with no unusual reaction. The identification score was above 99%. *Escherichia coli* strains (145) were correctly identified with this system, too, 78 were identified with no unusual reaction. Mostly melibiose substrate gave unusual results (for 49 strains). 93 *Enterobacter cloacae* strains were identified. For 84 strains the identification score was above 98%. Glycine and inositol substrates were evaluated as giving mostly unusual reactions.

It can generally also be concluded, that as the percentage of positive wells in a panel increases, the ratio of borderline reactions similarly increases. This in turn

influences the value of test score.

KANSOH, A. L., A. M-ALI, A. A. EL-GAMMAL

Xylanolytic activities of *Streptomyces* sp.

Microbial Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

Twenty-four different strains of *Streptomyces* sp. isolated from Egyptian soil were tested for their ability to produce extracellular xylanases. Of all these isolates, a *Streptomyces* sp. that had the highest potential for xylanolytic activity was chosen. From various morphological, physiological and antagonistic properties, this isolate was found to belong to *Streptomyces lividans*. Factors affecting xylanase production by this organism in a basal salt medium containing purified sugar-cane-bagasse xylan as a sole carbon source were examined. A noticeable increase in enzyme activity was observed in the presence of peptone or soyabean meal. However, a slight increase was noticed with ammonium sulfate. Optimum production for xylanase was achieved after five days incubation on a rotary shaker (180 r.p.m) at 30 C. The initial pH values were around neutrality. In addition, this organism has high potential for xylanolytic activity when grown on lignocellulosic wastes including corn cobs, wheat bran, peanut shells, sawdust, wheat straw and sugar-cane-bagasse. Partial Purification of the enzyme in the culture supernatant was achieved by salting out at 50-80% ammonium sulfate saturation with a purification of 9.03 fold and 57.9% recovery.

KANSOH, A. L., Y. M. H. ABU-AYANA, F. F. ABDEL-MOHSEN

Evaluation of encapsulated copper salts or copper complexes by various types of vinyl polymers as fungicides

Microbial Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

The biological activity of Cu-salts or Cu-complexes encapsulated by different kinds of polymers were studied against various types of fungal strains. The dose and rate of leaching of Cu-ions were significantly controlled by the type of polymer film used and their solubility in the medium. The different kinds of used polymers improved the tenacity of the fungicides on the leaf surfaces and also, improved the dispersion of Cu-salts suspension. The results provided laboratory support for the concept that the polymers containing chemically bound biocides were useful for controlling microorganisms growth. The effective concentrations of the biocides were (0.1-0.2mg/ml). In field application, encapsulation appears to be a feasible route to obtain both economic and environmental advantages that can be used in rainy and windy places. The kinetics of Cu-uptake by fungal strains were studied to determine the difference in their behaviour. The uptake strategy was examined by TEM. In addition, the histological studies on & in cucumber leaves showed a good entrance with high efficiency. The acute and subacute toxicity of these compounds were also studied.

KARAFFA, L., E. SÁNDOR, A. SZENTIRMAI

Chemostat – a powerful tool in basic research

Department of Microbiology and Biotechnology, Kossuth Lajos University, Debrecen, Hungary

Growth and product formation in batch culture is a process that always terminates after some finite and usually relatively short time interval. Altering the batch process by continuously supplying fresh nutrient medium to a well-stirred culture and simultaneously withdrawing the broth containing cells and products results in a culture called chemostat, in which growth can be maintained for prolonged periods. Furthermore, a steady-state can be maintained such that the cell concentration, specific growth rate and culture environment (e.g. nutrient and product concentrations) do not change with time. As a consequence, chemostat culture provides a unique tool for investigating the response of microorganisms to their environment and for elucidating the relations between an organism and its environment.

In this lecture we will focus on the changes in the morphology and differentiation of the fungus *Acremonium chrysogenum*, that can be characterized by dimorphism. In batch culture, a progressing fragmentation and also a significant vacuolation process can be observed, resulting in wide, swollen cells at the stationary phase in contrast with the filamentous morphology of the exponential phase. It was established, that the fragmentation process is due to changes in specific growth rate during cultivation. On the other hand, vacuolation depends on the sugar consumption rate rather than the growth rate. The importance of glucose-limited chemostat cultures in differentiating between the two phenomenon is discussed.

KARAFFA, L., K. VÁCZY, E. SÁNDOR, I. PÓCSI, A. SZENTIRMAI

Intracellular peroxide concentrations correlate with cyanide-resistant alternative respiration in *Acremonium chrysogenum*

Department of Microbiology and Biotechnology, Kossuth Lajos University, Debrecen, Hungary

Besides the cytochrome pathway, the filamentous fungus *A. chrysogenum* also exhibits a cyanide-resistant, salicylhidroxamic acid (SHAM) sensitive, non-phosphorylating alternative respiration. It has been reported, that upon addition of inhibitors of the cytochrome pathway, high rates of H₂O₂ production in mitochondria can be observed. On the other hand, inhibition of the cytochrome path normally results in enhanced cyanide-resistant respiration. A correlation between alternative respiration and the presence of peroxides in the culture medium has already been demonstrated in higher plants like *Petunia hybrida* and in the yeast *Hansenula anomala*. In this work we will show that enhancement of the intracellular peroxide concentration, either resulted from the direct addition of H₂O₂ or from a facilitated production due to the inhibition of the enzyme catalase by salicylic acid,

coincides with an increased cyanide-resistant alternative respiration in *A. chrysoenum*, and has a severe impact on the basic growth parameters (biomass production, substrate consumption, growth yield) of cells.

KARCH, H., W. BRUNDER, H. SCHMIDT

New discoveries in molecular biology of enterohaemorrhagic *Escherichia coli* O157

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Since 1982, enterohaemorrhagic *Escherichia coli* (EHEC) have been identified as a cause of diarrhoea and haemorrhagic colitis. The most serious complication of the infection is the haemolytic-uraemic syndrome (HUS) that develops in 5 to 10% of children with diarrhoea. Shiga toxins (Stx) are the most important virulence factors of EHEC known at present. After reaching the bloodstream, the toxins cause damage of endothelial cells but also of tubular cells in the kidneys and this results in the renal failure. In EHEC O157 isolates from patients we were able to identify seven different combinations of *stx* genes that occurred with different frequency. The genes encoding Stx are located in the genomes of prophages that are integrated in EHEC chromosomes. In addition, EHEC O157 strains possess a chromosomally located pathogenicity island termed LEE that contains numerous pathogenicity genes including the *eae* gene encoding intimin. Moreover, EHEC O157 strains harbor a 93-kb plasmid where are located the genes encoding the EHEC-hemolysin (EHEC-HlyA), a serin protease (EspP) that cleaves factor V and a protein called ToxB that shows homology with the toxin of *Clostridium difficile*. The EHEC O157 strains exist in two variants, namely non-sorbitol-fermenting O157:H7 and sorbitol-fermenting O157:H- strains that are evolutionary older. Our results obtained up to now demonstrate marked differences in epidemiology of the infection caused by the respective EHEC O157 variants. EHEC O157:H7 strains occur worldwide whereas sorbitol-fermenting strains have been found in Germany and recently also in the Czech Republic. While the EHEC O157:H7 strains occur mainly during warm months, the sorbitol-fermenting strains are more frequent during cold season of the year. In addition, differences exist with regard to the resistance to heavy metals, the plasmid structure, and the reservoir. We postulate the hypothesis that sorbitol-fermenting O157:H- strains occur only in human intestine, whereas non-sorbitol-fermenting O157:H7 strains got adapted to other hosts, such as cattle, which enables more rapid spread of the strains.

Kecskés, M. L.¹, I. Schröder², B. C. Ahohuendo³, M. Kuhlmann⁴, W. Dröge-Laser⁴, Z. Bozsó¹, G. P. Ott¹, Z. Klement¹, K. Rudolph²

A candidate factor in resistance mechanisms induced by phytopathogenic pseudomonads and xanthomonads: lipopolysaccharide

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KELEMEN, G. H., M. J. BUTTNER

Transcriptional switching during morphogenesis in *Streptomyces coelicolor* A3(2)

John Innes Centre, Norwich, United Kingdom

Two alternative sigma factors, encoded by *whiG* and *sigF*, play important roles in the transformation of the multigenomic aerial mycelium into chains of unigenomic spores in *Streptomyces coelicolor*. The initiation of sporulation is dependent on *whiG*, while *sigF* is involved in spore maturation after sporulation septa have been laid down. Transcription studies performed on both these genes in wild type and in several *whi* mutants showed *whiG* transcription to be independent of known sporulation genes, while *sigF* transcription is dependent on all six known early sporulation (*whi*) genes. However, *sigF* is not directly dependent on *whiG*, suggesting an involvement of other sigma factors in sporulation.

In a search for suitable candidates, a new group of genes homologous to *sigF* has been identified and three of them have been sequenced. The genes encode alternative sigma factors that belong to the group of sporulation specific and stress response sigma factors of *Bacillus subtilis*. Several of the new sigma factor genes are potentially part of polycistronic operons and they are preceded by sequences coding for putative anti-sigma factors. The biological functions of these new sigma factors and their activation are currently being investigated and their potential involvement in differentiation will be discussed.

KILANI, M. K.¹, A. H. KANJO²

Bacterial load of the air of Budapest

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In this study the total colony counts of heterotrophic-aerobic bacteria was investigated and the existence of several kinds of bacteria (at species level) in the air of Budapest at 15 different places was characterised.

This highest mean CFU counts were found at Gergely street and at Kálvin square. Isolated and purified strains proved, that Gram positive organisms absolutely dominated. The same is true for catalase positivity. Among cocci, members of the genus *Micrococcus* dominated, in leading position with *M. luteus*. Among rod shaped bacteria, members of the genus *Bacillus* dominated with *B. brevis*.

Coryneforms and nocardioforms were also present. Members of genera *Microbacterium*, *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Rhodococcus*, *Clavibacter* and *Gordona* were identified.

KIRSCHNER, A. K. T.¹, A. FARNLEITNER², A HERZIG³, G. KAVKA², S. KÖLBL⁴, B. VELIMIROV¹, T. ZECHMEISTER⁵

Determination of the potential toxic risk of botulism neurotoxin C1, responsible for the outbreak of avian botulism, and the relation to ecological variables of flat saltwater pools in the Austro-Hungarian National Park Neusiedler See – Seewinkel (Fertő Hanság)

¹Institut für Medizinische Biologie, Universität Wien, ²Institut für Wassergute des BAW, Wien, ³Biologische Station Neusiedler See, Illmitz, ⁴Institut für Virusseuchenbekämpfung, ⁵Institut für Allgemeine Biochemie, Vienna Biocenter, Wien, Austria

KISS, I.¹, S. KECSKEMÉTI¹, J. TANYI¹, S. B. KLINGEBORN², S. BELÁK²

Studies on the invasion and distribution of feline coronaviruses in naturally and experimentally infected cats

¹Department of Virology, Veterinary Institute of Debrecen, Hungary, ²National Veterinary Institute, Uppsala, Sweden

Two RT-PCR assays were developed for the amplification of the highly conservative nucleocapsid (N) and ORF7b genomic regions, respectively. The two assays were applied to study the shedding and distribution of the virus in naturally and experimentally infected cats. The infection remained subclinical, but the two PCR assays revealed that the majority of the animals shed the virus via feces throughout the experiment and the virus could be detected in the blood and in the large intestines. In addition, in a few cases sequences corresponding to the N region were amplified from the cortex, dura mater, pancreas, lungs, third eyelid, and the heart muscle. Interestingly, the ORF7b region was only detected in the pancreas and in the heart muscle. These data correlate with previously reported in vitro observations, indicating that the ORF7b region can be lost during virus replication.

The single strand conformational polymorphism (SSCP) analysis of the PCR products revealed that FCoV has quasispecies nature during in vivo replication. In the large intestines, both genomic regions showed continuous changes during the course of infection. In the other organs, the two examined regions were highly conserved, as it was revealed by SSCP and nucleotide sequencing.

KISS, K., J. ZALA, T. NAGY

Comparison of in vitro antifungal susceptibility tests

Department of Mycology, "Johan Béla" National Center for Epidemiology, Budapest, Hungary

Fungal infections have increased significantly over the past years. Besides the frequency of nosocomial fungaemia due to unusual yeasts has increased dramatically. Resistance of yeast human pathogens to antifungal agents used in hospitals has been rarely reported during 70's and 80's. It's only during the last decade that antifungal drug resistance has been recognised as a more significant problem. Therefore the in vitro antifungal susceptibility tests have important role in choosing the acceptable treatment and recognising the drug resistance.

In our study three methods for antifungal susceptibility test were taken. The E-test for Amphotericin B (on semi-synthetic agar medium), Itraconazole (on Casitone-agar medium), Fluconazole (Casitone-agar) were compared with Fungitest. The disc diffusion test for Ketoconazole and Miconazole (both semi-synthetic agar mediums) and 5-Fluoro-citazine (semi-synthetic agar) were compared with Fungitest again. Uptill now 61 *Candida* strains were examined for all three tests. The results with E-test and Fungitest with Amphotericin B were the same in 93%. The E-test for Itraconazole was according to Fungitest in 80%. Fluconazole gave the biggest difference the accord was 74%. The results were same with Ketoconazole, Miconazole, 5-Fluoro-citazine in 80% , 87% and 93%. The azoles especially Fluconazole were the least corresponding. The different mechanism of action (e.g. Fluconazole has fungistatic effect while Amphotericin B has fungicid effect) may be a reason for that.

KISS, R¹, E. NAGY PAPP, Zs. FARAGÓ

Experiences by the use of rapid methods for hygienic and epidemiological connections in the course of food microbiological supervision and epidemiological surveillance of *Salmonella*

¹"Fodor József" National Centre of Public Health, 2Public Health Laboratory Service, Budapest

Nowadays in our country salmonellae are the most important foodborne pathogens. The detected and registered enteric diseases in Hungary in 1998 fell into the following categories: Salmonellosis (18,108), Shigellosis (752), *E. coli* enteritis (272), Campylobacteriosis (7,941), Yersiniosis (144), Enteritis infectiosa (15,909). We do not know in how many cases did not contact physicians when having observed the symptoms and in the 15,909 enteritis cases isolations of the pathogenic agent did not succeed.

In our present work we report on the fact that during routine supervisions, examinations of the background of diseases and/or observation of hygienic deficiencies, when applying methods of relevant standards, in some case the detection of the causative agent, couldn't be performed from environmental samples. By the use of immunomagnetic separation IMS (DYNAL) and ELISA (LOCATE) methods, respectively, the detection of the concerned microorganisms was successful in the environment of the production of the food.

Two cases are discussed in detail: isolation of *Salmonella bareilly* on contrast to classical methods gave 100% sensitivity in a child's home outbreak. Detection of

Salmonella enteritidis by the use of valid standard did not succeed from environmental samples taken in the place of preparation of the foods in a school outbreak. Using IMS method, a hygienic sample taken from a washed colander, proved to be positive, thus it was obvious that hygienic deficiencies occurred in the kitchen. The efficacy of the disinfectant cleaning has to be improved.

Sensitive and rapid methods like IMS and ELISA can help to clear up the mistakes of the hygiene for the quality assurance, the performance of the improving steps and the implementation of good manufacturing practice.

KISS, R.¹, M. FÜZI², Cs. BOGNÁR³, M. FEDERIGHI⁴, C. MAGRAS⁴

Detection of viable but non-culturable microorganisms. Detection of *Campylobacter* by the use of acridine orange

¹"Fodor József" National Centre of Public Health, ²Public Health Laboratory Service, ³"Johan Béla" National Centre of Epidemiology, Budapest, Hungary, ⁴Veterinary University, Nantes, France

The public health network performs *Campylobacter* surveillance in the case of outbreaks or sporadic cases of enteric diseases since the eighties. The number of campylobacteriosis cases shows a continuous increase - as we reported several times - but the examination of the causative, *Campylobacter* contaminated food and environment is performed very rarely. Diseases were caused by contaminated milk, egg, poultry, pork meat, salad and water in Hungary.

As a routine, control of foods does not prescribe the involvement of *Campylobacter*. In the course of our investigations - in accordance with the international literature data - we found high rate of positivity when examining poultry, meat, Danube-water using pre-enrichment, enrichment, filtration and active migration method. From heat-treated and frozen foods in several cases the cultivation of stressed microorganisms did not succeed. As the cultivation of *Campylobacter* is a demanding method we compared in our examinations three methods:

1. CAT (Oxoid) supplemented Preston enrichment and CCDA (ISO 102720 standard),
2. methods prescribed by the 3640/24-1989 *Campylobacter jejuni* Hungarian standard
3. examinations using acridine orange.

Surface samples were taken by the use of TIMI cleanness sampler (Labsystem) from poultry meat and the environment of processing. Microscope slides were stained by acridine orange.

In the smear the corkscrew-shape or twisted forms of *Campylobacter* were to be seen. Higher positivity was reached by the use of ISO 10272 and CAT. By the rapid method of staining also those laboratories get information on *Campylobacter*

contamination, which do not possess facilities for cultivation. Laboratories performing *Campylobacter* cultivation, however, may receive rapid information indicating positivity, which fact may be verified by cultivation.

By the use of CAT, we isolated *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis* species according to ISO 10272 standard.

KLEFFLER, T., T. DEÁK, A. MARÁZ

Biodiversity of yeast biota in vineyard and wine fermentation

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In the oenological process from grapes to wine, yeasts are of primary importance. Numerous previous investigations show that the composition of this yeast varies in the different vineyards. The diversity of yeasts is generally high on the grapes and the beginning of the fermentation but during the middle and last phase *S. cerevisiae* strains takes over the leading role.

The places of our experiments were: Etyek vineyard, preprocessing equipments and the processing factory in Budafok (Hungarovin), who are using active dry *S. cerevisiae* strains as starter cultures.

We identified the strains, isolated from the samples at spices level with traditional simplified identification test (SIM) or with the commercially produced API 20C and API 30C diagnostic kits. The following molecular genotyping methods were also used: pulsed field gel electrophoresis and RAPD-PCR.

Our result showed that the number of yeast cells increased by about twenty times on grapes and leaves. We found several yeasts above the dominant *Aureobasidium pullulans*. The number of *S. cerevisiae* isolates was low. In the processing equipments *Kloeckera* and *Torulaspota* species became dominant but the *S. cerevisiae* strains also increased. The biomass of yeast population was growing by two order of magnitude. In the main fermentation of must *S. cerevisiae* took over the leading role.

There are some questions, what the current molecular genotyping experiments have to answer. For example:

-Were there other *S. cerevisiae* strains above the active dry yeasts in the last fermentation phase that came from the grapes and enriched in the equipments?

-Were such yeasts isolated that survived in the equipments and had an important role in the fermentation?

This work was supported by the Hungarian Ministry of Education, MKM Project No. 1121/1997

KOCH, S.

György and the viruses. Post-war Hungarian virology initiated by György Ivánovics

This is a deliberately subjective commemoration of my Master and paternal friend György, with whom I had the privilege of working from 1945 till 1953. He remained my first and sharpest critic and a reliable friend until his death in September 1980. For ever I shall remain obliged to him for all he taught me as a scientific leader, a "boss", and last but not least an experienced mentor in the affairs of life. His deep and comprehensive knowledge of microbiology as a whole, his persistent interest in Science, his indefatigable participation in the everyday laboratory work, and his great sense of humour made him an exemplary personality for all his students, followers and friends. His minor venial weaknesses made him even more human and brought him even closer to those able to appreciate his qualities. As a scientist, he was not only the founder of the post-war Hungarian school of microbiologists, but also a highly reputed personality both in Hungary and abroad, as illustrated by the numerous honours he was awarded.

KOCSIS, B., I. KUSTOS, Z. PÉTERFI

Isolation and characterisation of a *Shigella sonnei* absolute rough mutant

Department of Medical Microbiology and Immunology, University Medical School, Pécs, Hungary

KOVÁCS, G., M. NIKOLAUSZ, A. HALBRITTER, I. VILLÁNYI, H. M. RIFAAT, K. MÁRIALIGETI

Bacterial communities of cattail (*Typha angustifolia*) rhizoplane

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The bacterial communities inhabiting the rhizoplane of *Typha angustifolia*, a common macrophyton in floating mat ecosystems was investigated by using classical and molecular methods. At first 250 bacterial strains, derived from root samples were characterised by investigation of numerous phenotypic properties. Based on results of computer generated cluster analysis selected strains of each phenon were characterised and determined by BIOLOG metabolic fingerprint assay. Strains chosen from each phenon were investigated by 16S rDNA sequence analysis to determine their precise taxonomic position. The results demonstrate that the culturable members of rhizoplane bacterial community are dominated with Gram-positive bacteria, with *Bacillus pumilus* as main coloniser. Other Gram-positive bacteria like *Staphylococcus warneri*, *Kocuria* spp., *Microbacterium lactis*, *Arthrobacter agilis* and *Streptomyces* spp., *Micromonospora* spp. appeared to be less frequent. The Gram-negative isolates represent principally *Acinetobacter lwoffii*,

A. radioresistens, *Xanthobacter flavus*, *Agrobacterium tumefaciens*, *Rahnella aquatica*, *Erwinia sp.* and *Pseudomonas fluorescens*.

ARDRA analysis of 16S rDNA-clones obtained from direct DNA-extraction of root mass was carried out in order to detect a broader range of bacterial community. In contrast with the culturable members of the rhizosphere, a significant part of clones analysed has fallen into the group of δ -proteobacteria represented with numerous sulphate-reducers. Other clones were clustered together with *Frankia*, *Clostridium*, *Rhodospirillum rubrum*, *Rubrivivax*, *Janthinobacterium* and *Pseudomonas* spp.

KÖVES-PÉCHY, K.¹, B. BIRÓ¹, I. VÖRÖS¹, T. TAKÁCS¹, R. J. STRASSER²

Enhanced activity of inoculated associative and obligate nitrogen-fixers (*Azospirillum* and *Rhizobium* sp.) by AM fungi

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The effects of single or tripartite co-inoculations of some nitrogen-fixers (*Azospirillum*-S, *Rhizobium*-R) and the indigenous arbuscular mycorrhizal fungi (M) were examined on the growth and development of alfalfa (*Medicago sativa* L.) in two soils originating from different environmental conditions.

Original (*C* = normal rhizosphere), sterilised (γ -irradiated, *G* = no microbes), or sterile but bacterial-reinoculated (*GB* = no AMF) soil-treatments were used for inoculating the host by the micro-symbionts separately or as combinations in pot experiments. Beside the mass production, nutrient contents, nodulation activity, MPN counts of associative diazotrophs and the AMF colonisation measurements, the physiological stage of the hosts was also examined by a new chlorophyll fluorescence rise (*OJIP*) test.

All micro-symbionts alone (*S*, *R*, *M*) or combined (*RS*, *RM*, *RSM*) increased the physiological parameters (the plant fitness) of the hosts in the steril, γ -irradiated /*G*/ soils, suggesting the synergistic effect of these beneficial microbes in optimal cases. Competence of the indigenous microflora on the other hand had a great influence depending on the origin of the soil-types. Inoculation proved to be more effective on the old arable soil (with intensive agricultural usage), in comparison to the more species-rich and diverse soil from a natural woodland environment. AMF inoculation enhanced the abundance and activity of the nitrogen-fixers, and increased the stress resistance of the alfalfa (stress-buffer effect). Chlorophyll fluorescence measurement proved to be an adaptable technique for the early *in situ* selections of candidate inocula-strains in case of these beneficial microbes.

Sponsored by the Hungarian Research Fund (OTKA): T0 17647, 23543, by the Swiss National Foundation (31-46.860.96/31-52.541.97) and the Soc. Acad. Geneve to R.J.S.

KRAMARENKO, T., H. KARP, A. IVASK, T. ALAMÄE

Glucose phosphorylating enzymes and sugar repression in methylotrophic yeast *Hansenula polymorpha*

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Glucose phosphorylating enzymes are suggested to be implicated in glucose repression and glucose signalling in a number of organisms: yeasts, plants and humans. In *Saccharomyces cerevisiae* only one (hexokinase PII) of the three glucose phosphorylating enzymes is most probably involved in glucose repression. Hexokinase might generate a repressing regulatory metabolite from glucose, but other possibilities such as protein-protein interactions between hexokinase and other regulatory proteins are also possible.

Methylotrophic yeast *Hansenula polymorpha* is suggested to be a reliable alternative model for the study of glucose repression mechanisms in yeasts since it has a perfect glucose-repressed metabolic system (enzymes and organelles implicated in methanol utilization) that can be used as a model. In addition, we have characterized another well-defined glucose-repressed system in *H. polymorpha*- maltase that is implicated in utilization of disaccharides. Regulation of synthesis of maltase has widely been used for the study of glucose repression mechanisms in different yeasts. Therefore, it will be possible to compare data obtained by using *H. polymorpha* model with data concerning other yeasts.

We have described the spectrum and kinetic properties of glucose phosphorylating enzymes in a methylotrophic yeast *Hansenula polymorpha*. By using mutagenesis, selection and genetic crosses we have isolated strains of *H. polymorpha* that carry different combinations of glucose phosphorylating enzymes. These isolates were used to study glucose and fructose repression of maltase and methanol-oxidizing enzymes.

KREDICS, L.¹, Zs. ANTAL², L. MANCZINGER¹

Influence of water potential and temperature on growth, enzyme secretion and in vitro enzyme activities of *Trichoderma harzianum*

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Influence of water potential and temperature on linear mycelial growth, secretion and in vitro activities of β -glucosidase, cellobiohydrolase, β -xylosidase, exochitinase and chymotrypsin enzymes of *Trichoderma harzianum* was studied. Nearly linear correlation was found between water activity and colony growth rate at 25 °C and at 10 °C with higher growth rates at higher temperature and water potential. Secretion of the enzymes depended on the water potential of the liquid media and not on the type of the salt (NaCl or KCl) used as osmoticum. Different

water potential values were optimal for the secretion of the different enzymes. In vitro enzyme activities were significantly affected by water potential and temperature. All enzyme activities were lower at lower temperatures. Significant activities were measured for most of the enzymes even at the water potential value of -14.80 MPa, which is below the limit of mycelial growth (-11,54 MPa). These results suggest the possibility of using mutants with improved xerotolerance for biocontrol purposes in soils with lower water potential.

This work was supported financially by grant FKFP-0218/97 of the Hungarian Ministry of Education.

KUBICEK, C. P., J. STRAUSS

Carbon catabolite repression in *Aspergillus nidulans*

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For a vast array of microbes, carbon response mechanisms ensure that catabolism of glucose results in severe repression of genes encoding enzymes for the degradation of alternative carbon sources. Among eukaryotic microorganisms, carbon catabolite repression has mainly been studied in the yeast *Saccharomyces cerevisiae* and to a lesser extent in filamentous ascomycetes such as *Aspergillus nidulans*. In the latter organism, carbon catabolite repression participates in the regulation of the expression of genes required for the utilization of carbon sources such as starch, cellulose, hemicellulose or pectin, and is thus of industrial relevance. Although there is much more known on the respective events in *S. cerevisiae*, carbon catabolite repression in *A. nidulans* appears to differ in the latter in a number of important aspects, such as structure and function of the DNA-binding protein (Mig1p vs. CreA) and signalling to it, thus necessitating studies with this organism. This review will focus on our recent work on the mechanism of functional activation of CreA by glucose, and on genes/proteins involved in this process.

KUCERA, I., S. RADULOVIC, S. VELIMIROVIC, N. BULAJIC, M. TATIC, V. BAJOVIC

Finding of intestinal protozoa cysts in stool using three methods

Institute of Microbiology, Authority for Preventive Medicine, Military Medical Academy, Faculty of Biology, Belgrade

This paper presents quantitative and qualitative comparison of findings of cysts of intestinal protozoa by comparative using of three methods: formalin-ether concentration by Ritchie (FAC), 33% Zinc Sulfate concentration (FZS) and gradient centrifugation in ficoll (FGC). In 28 (65,12%) of 43 examined samples the cysts of 5 species of intestinal protozoa have been found: *E.coli* (FAC 15; FZS 14; FGC 9), *E.nana* (9;9;8), *G.lambliia* (5;5;4), *B.hominis* (2;3;4) and *J.bütschlii* (2;1;1). Analyzing the number of positive findings, mean number of the cysts per high

power field, appearance of the cysts and the removal efficiency of other fecal contents, it has been concluded that FAC is the most suitable for concentration of most cysts of intestinal protozoa and for the use in routine diagnosis, because it provides the finding of the highest number of cysts. Hypertonic solution of ZnSO₄ deforms the cysts, which was seen in most smears. FGC method provides the best removing of fecal contents, but the number of cysts is the smallest per high power field; however, it appears particularly suitable for concentration of *Blastocystis hominis* cysts, which retain their viability, because inoculated into nutritive media they have been developed to typical vacuolar forms.

KUSTOS, I.¹, V. TÓTH^{1,2}, F. KILÁR³, B. KOCSIS¹, L. EMŐDY¹

Investigations of outer membrane components of *Proteus penneri* strains by electrophoretic methods

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LASKAY, T., H. LAUFS, N. JAHNKE, W. SOLBACH

Leishmania prevent apoptosis of neutrophil granulocytes

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The protozoa of the genus *Leishmania* are intracellular parasites responsible for diseases of major relevance in tropical and subtropical areas of the world. In mammals, these parasites are strictly intracellular and replicate in the phagolysosome of macrophages. Extracellular parasites are rapidly killed by complement mediated lysis. Therefore, for the survival of parasites in mammals, it is essential to be taken up by phagocytic cells to avoid hostile serum factors. We have recently demonstrated that the accumulation of monocytes/macrophages at the site of infection takes at least 48 hours. The leukocytes that are recruited rapidly to the subcutaneous site of infection are neutrophil granulocytes. Therefore, in the absence of macrophages, granulocytes could provide an intracellular site for the parasites in the first hours/days of infection. In the present study we investigated the potential role of granulocytes in the uptake of *Leishmania*. *In vivo* we could demonstrate intracellular parasites in granulocytes in the early phase of experimental infection in mice. *In vitro* analysis of human granulocytes have revealed that neutrophil granulocytes are able to take up both opsonized and non-opsonized *Leishmania*. We found that the apoptotic death of neutrophil granulocytes is significantly delayed after co-incubation with non-opsonized *Leishmania*. These data suggest that granulocytes may provide an intracellular environment for the survival of *Leishmania* within the first days of infection.

LATKÓCZY, K., F. ROZGONYI

A comparative study on factors influencing the susceptibility of bacteria to trimethoprim/sulfamethoxazole combination

Institute of Microbiology, "Semmelweis" University Medical School, Budapest, Hungary

The aim of this study was to examine that the relatively high incidence of resistance to the combination trimethoprim/sulfamethoxazole in strains of the family *Enterobacteriaceae* reported from different Hungarian laboratories could be due to the own resistance of bacteria or to some technical errors. Susceptibility to trimethoprim/sulfamethoxazole of 100 strains belonging to the family *Enterobacteriaceae* was parallelly determined on agar plates prescribed by the HUMAN Ltd, Budapest, Hungary, and on Mueller-Hinton agar plates (bioMérieux) recommended by the NCCLS using both OXOID and HUMAN discs. Minimal inhibitory concentrations of trimethoprim/sulfamethoxazole for the strains were measured by the E-test on both media and the results were compared to each others and to those obtained with the disc diffusion method. There was no significant difference between the results obtained with the HUMAN and OXOID discs as well as the E-test. In contrast, 77% the strains showed sensitivity on bioMérieux Mueller-

Hinton agar, while only 70% exhibited sensitivity on the agar prescribed by the HUMAN in the Resistest sheet. In conclusion, the results of the sensitivity tests to trimethoprim/sulfamethoxazole combination may considerably depend on the composition and quality of media used in different laboratories. The correct laboratory measurements greatly influence the choice of antibiotics and the cost of the treatment. Omission of trimethoprim/sulfamethoxazole combination attributable to a misdetermination of resistance seems to be unfounded in many cases.

LAUKOVÁ, A.¹, P. JURIŠ², S. CZIKKORVÁ¹, Z. VASILKOVÁ², M. MAREKOVÁ³, I. KRUPICER²

Inhibitory effect of enterocins against hygienic important bacteria in the cattle and pig slurry environment

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The preferred intensive methods of cattle and pig production in Slovakia are associated with the consequence of large volumes of slurry requiring disposal. This has resulted in the pollution problems (pathogenic bacteria and odour) leading to increased pressure on e.g. farmers to find satisfactory methods to manage waste. Aerobic and/or anaerobic treatment can be very effective in dealing with a wide range of those problems, but it is often not popular due to the high energy costs implied. Therefore, one possible way to solve this problem could be to use the ability of some bacteria to produce antimicrobial substances. Bacteriocins are proteinaceous compounds with an antagonistic activity generally exerted on closely related bacteria as well as on hygienic important strains. Knowledge of some bacteriocins, especially those produced by lactic acid bacteria has expanded dramatically over the last decade. Therefore, the aim of our study was to report the results associated with the experimental application of our two bacteriocins produced by *Enterococcus faecium* CCM 4231 and *Ent. faecalis* V24 strains for slurry treatment. The slurry used in our experiments was collected from a farm located in Liptovská Teplá or Spišské Vlachy and from a pig farm in Figa (Slovakia). In all experiments, the slurry was filtered and boiled to kill bacteria. Then both, the experimental and control samples (ES, CS) were inoculated with indicator organisms: *Listeria monocytogenes*, *Yersinia enterocolitica* and *Enterobacter cloacae* (1% inoculum of 10^7 cfu ml⁻¹). Enterocin CCM 4231 was added to ES at the beginning of the experiment in conc. of 3200 AU ml⁻¹. Whilst enterocin - like V24 was added to ES in a logarithmic growth phase of indicators in conc. 800 - 1600 AU ml⁻¹. Samples with enterocin CCM 4231 were incubated for 2 weeks at 30° C - 32° C. On the other hand, samples with bacteriocin (bc) V24 were incubated only for 24 h in water incubator at the same temperatures. Stable, suppressing effect of enterocin CCM 4231 on the growth of listerial cells was noted reaching a significant difference with 2.59 log cfu ml⁻¹ between ES and CS in the end of experiment. When bc V24 was added to the growing cells of *L. monocytogenes*, the inhibitory effect was found already 1 h after bc addition. The highest inhibition was noted after 2 h with effectivity up to 24 h. When bc V24 was added to ES with *Ent. cloacae* inoculum, no effect was detected in the moment as

well as after 1 h of bc addition. There, the inhibitory effect started after 2 h of bc addition with increased effectivity up to 24 h. *Yersinia enterocolitica* was inhibited already 1 h after bc V24 addition with stable inhibitory activity up to 24 h. Although the other experiments provided directly in the waste treatment plant are requested, those mentioned here indicate the possible use of bacteriocin producing strains and/or their bacteriocins to decrease or eliminate contaminants in slurry.

LEBUHN, M., B. MOGGE, M. SCHLOTTER, M. STOFFELS, A. HARTMANN

Improved *in situ* tracking of *Sinorhizobium meliloti* by a multiple staining approach, and effects of inoculation with *S. meliloti* on the rhizoplane microflora of alfalfa

GSF – National Research Center for Environment and Health, Institute of Soil Ecology, Neuherberg, Germany

LEBUHN, M.^{1,2}, W. ACHOUAK², M. SCHLOTTER¹, O. BERGE², A. HARTMANN¹, T. HEULIN²

Geno- and phenotypic diversity of *Ochrobactrum* sp. isolates from soil and wheat roots

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LEBUHN, M.^{1,2}, W. ACHOUAK², O. BERGE¹, M. SCHLOTTER¹, A. HARTMANN¹, T. HEULIN²

Polyphasic taxonomy of *Ochrobactrum* spp. isolates from environmental samples

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MAJOROS, L., C. MISZTI, B. SZABÓ

Isolation and identification of *Candida albicans* and non-*albicans* *Candida* species from humans by traditional and new methods

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Our laboratory have isolated 553 *Candida* species from 28419 specimens in 1998. After evaluation of germ tube tests we tried to identify *Candida* species using CHROM agar culture method and by 32 biochemical reactions (ID 32 panel). The

most frequent isolate was *C. albicans* (62, 2%), whereas the non-albicans *Candida* species were found less frequently (*C. glabrata* 12,8 %, *C. tropicalis* 4,5 %, *C. krusei* 3,6 %). The frequency of other non-albicans *Candida* species was below 1 %.

The antimycotic sensitivity of our isolates was tested by determination of MIC values and by E-test. Results of MIC test showed that Amphotericin B and 5-flucytosine were the most effective drugs.

In order to analyze the karyotype of recurrent *Candida* infections we used pulse-field electrophoresis. The identical chromosome pattern served as a proof in identification the same *Candida* strain in a recurrent infection. The pulse-field method was also suitable for exact determination of *Candida* species in cases when traditional methods gave questionable results.

MAKK, J., É. ÁCS, B. BESZTERI, O. ORAVECZ, K. SZABÓ

Investigation of diatoms associated bacterial communities of the River Danube

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Epilithon microbial communities, which colonize and grow on gravel, pebbles and rocks are important in aquatic environments, responsible for nutrient cycling, degradation of xenobiotics. Among algae the diatoms are found to be dominant groups of the epilithon communities of the River Danube. According to our electron microscopic investigations the biofilms on illuminated surfaces contain many types of cells, including bacteria on surfaces of the diatoms, so one has to realise that the surfaces of them serve habitats for different bacteria.

One of our study sites was at the north-eastern part of Szentendre Island in the River Danube main arm, where gravel samples were collected from the northern water gaining area of bank wall filtered drinking water wells serving Budapest. At other study site artificial substrata were submerged in the river at 50-60 cm water depth at 1669 river km, Alsógöd. These were incubated for 6 days time. Both collected samples were plated onto algal media in order to isolate diatoms, and then associated bacteria. The representative strains were selected on the basis of cultural-morphological-features, certain biochemical investigation and ARDRA analysis. Selected groups were identified by the Biolog phenotypic fingerprint method and 16S rDNA sequencing.

Concerning the Gram-negative organisms of our identification work strains belong to genera typically isolated from aquatic environments such as *Pseudomonas*, *Caulobacter*, *Aeromonas*, *Mycoplana*, *Sinorhizobium*, *Flavimonas*, *Flavobacterium*, *Sphingomonas*, and facultative methylotrophic or/and H₂ autotrophic bacteria such as *Xanthobacter*, *Variovorax*, *Ancylobacter*, *Azospirillum*, *Methylobacterium*. Among the Gram-positive bacteria *Bacillus*, *Microbacterium*, *Streptomyces*, *Rhodococcus*, *Nocardia*, *Aureobacterium*, *Rathayibacter* species were found.

The bacteria isolated are able to utilise such sugars and amino acids as only carbon

sources, which have been proved to be among extracellular products of diatoms. Thus we can suppose that association between diatoms and bacteria is based also upon metabolic interactions.

MÁNDI, Y.¹, Gy. FARKAS², T. TAKÁCS³

Relevance of procalcitonin in the prediction of sepsis and infected pancreas necrosis

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Procalcitonin (PCT) is a diagnostic parameter of bacterial infections. PCT is only induced with systemic reactions of the host to the infection. Infected pancreatic necrosis is an absolute indication for surgical intervention, therefore an early and accurate laboratory diagnosis is necessary to confirm the infection. The aim of this study was to analyse the clinical value of procalcitonin (PCT) for the prediction of infected necrosis and sepsis in comparison with IL-6 and sICAM-1.

A total of 25 patients were investigated; 10 patients with sterile necrosis, 10 with infected necrosis, 5 with sepsis with different origin. The concentrations of PCT in the patients' sera were measured by immunoluminometric assay (BRAHMS PCT Lumitest), the IL-6 concentrations by bioassay, applying the B-9 cell line, and sICAM-1 by ELISA (R&D). PCT was found in relatively high concentrations (25–115 ng/ml) only in patients with sepsis and infected necrosis. Positive values (>1 ng/ml) preceded positive bacterial results from either blood or surgical samples. In contrast, IL-6 and sICAM-1 were overproduced in both types (infected and sterile) of necrosis (150–500 pg/ml and 750–1250 ng/ml, respectively), and their levels remained elevated for several days even after surgical elimination of the infected focus. We could not detect PCT in patient's leukocytes by immunoblotting.

Elevation of serum IL-6 and sICAM-1 level is characteristic in systemic inflammatory response syndrome either of infectious or noninfectious origin. On the contrary, the PCT level is an accurate and available parameter for early diagnosis of sepsis, and for the discrimination of infected pancreas necrosis, and a helpful marker for surgical intervention. Further studies are necessary to identify the PCT-producing cells.

MARÁZ, A., A. POMÁZI

Molecular genotyping as a tool in the study of population dynamics of yeasts during wine fermentation

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Contribution and role of different species of yeasts to the quality and character of

wine have not yet been elucidated. Selective pressure of the environmental factors, which affect growth, fermentation and metabolite production of yeasts, are under a continuous change during wine fermentation. Successive changes of different species but rasher that of the strains belonging to a given species, can be monitored and studied effectively by the application of molecular genotyping methods as plasmid profile analysis, electrophoretic karyotyping, RFLP analysis of rDNA or mitochondrial DNA and PCR amplification of random DNA sequences.

Successive changes of the wine fermenting *Saccharomyces cerevisiae* killer populations were monitored by electrophoretic profile analysis of viral RNAs and also by the molecular fingerprinting of their nuclear genotypes. Genetic determinants for the toxin production were found to be dsRNA plasmids in every killer strain, what differed in the size of M dsRNA molecules. Killer toxins of activity at pH 4 were associated with different M dsRNA molecules, but killer strains from the same fermentation always harboured M dsRNA of the same size.

Electrophoretic karyotyping showed high degree of heterogeneity when killer yeast strains from different fermentation were compared, while more similarity was found when strains from the same fermentation were analysed. Both similarity and dissimilarity in the PFGE karyograms of the isolates were reflected in the cluster analysis of the RAPD-PCR profiles but more detailed distinction of strains, which belonged to the same PFGE group, was achieved by the latter method.

We found less diverse *Saccharomyces cerevisiae* yeast population when non-killer yeast isolates were subjected to molecular genotyping. Selection and dominance of unique strains were observed during increase of the ethanol content in wine.

This work was supported by the Hungarian Ministry of Education, FKFP Project No. 96/1999

MAREKOVÁ, M.¹, A. LAUKOVÁ², I. F. NES³

Study of bacteriocin-like activity produced by environmental enterococcal strains

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Bacteriocins are antimicrobial proteins, protein aggregates or protein complexes produced by several bacteria with antagonistic activities against other, more or less closely related, bacterial strains. In the majority were described bacteriocin-like producing strains, which were isolated from human, food or other sources.

Fifteen strains of *Enterococcus* sp., which were isolated from wastewater, were obtained as bacteria producing bacteriocin-like substances. As indicator of antimicrobial activity 22 strains were used. All examined enterococci produced inhibitory agents which showed a wide range of inhibition against Gram - positive and Gram – negative indicator organism from different sources. Clear zones of

inhibition (diameter 2 - 21 mm) were observed. Most bacteriocin-like substances produced by the strains of enterococci were stable and no decrease in activity were detected after 3 month at 10°C, by freezing (-20°C) and long - term storage at 4°C and -20°C. The maximum activity was produced by over night cultures. The inhibitory activity was not reduced by heat treatment at 50°C, 80°C and 100°C for 30 min. Some from studied enterococcal strains contained a plasmid DNA, but the evidence for plasmid - associated bacteriocin production needs additional experiments.

MARILLEY, L.¹, U. A. HARTWIG², M. ARAGNO¹

Influence of an elevated atmospheric CO₂ content on rhizobacterial populations beneath *Lolium perenne* and *Trifolium repens*

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Due to human activities, the atmospheric CO₂ concentration is increasing. However, the possible consequence on soil ecosystem is far to be understood. The major influence is indirect because CO₂ concentration in soils is at least 10 fold greater than in atmosphere. Consequently, the influence on soil micro-organisms is thought to occur by the intermediate of plant roots.

The rhizospheres of clover and ryegrass were divided into three fractions: the bulk soil, the rhizospheric soil and the rhizoplane-endorhizosphere. Bacterial community structure was assessed after isolation of DNA, PCR amplification and construction of cloned 16S rDNA libraries. The cloned 16S rDNA were then partially sequenced and analysed by a phylogenetic approach.

Our data show a very high bacterial diversity in soil, which is dominated by clones related to yet-uncultivated micro-organisms. The phylogenetic diversity dramatically decrease in the root environment, leading to a dominance of pseudomonads and rhizobia in the clover rhizosphere and to a dominance of pseudomonads in the ryegrass rhizosphere. The selective effect of plant roots is increased under elevated CO₂. Under these conditions, pseudomonads are outcompeted by rhizobia in the clover rhizosphere and the dominance of pseudomonads is increased in the ryegrass rhizosphere. Carbon source utilization analysis at the community-level confirms the CO₂-induced changes in the structure of the bacterial community. This work provides evidence for CO₂-induced changes in the structure of the rhizosphere bacterial populations, suggesting a possible alteration of the plant-growth-promoting-rhizobacterial effect and a root-mediated adjustment of bacterial populations to a CO₂-induced increase in plant nitrogen requirement.

MARITS, R.^{1,2}, D. FLEGO², A. ERIKSSON², V. KÖIV^{1,2}, M-B. KARLSSON², E. T. PALVA³

A two-component regulatory system *pehRpehS*, controls

endopolygalacturonase production and virulence in the plant pathogen *Erwinia carotovora* subsp. *carotovora*.

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Bacteria have evolved several mechanisms to sense environmental changes and to regulate its own gene expression accordingly. The pathogenicity of the major plant pathogenic enterobacterium *Erwinia carotovora* subsp. *carotovora* (*E. c.* subsp. *carotovora*) is correlated with its ability to produce and secrete several extracellular enzymes that can attack components of the plant cell wall. The synthesis of the corresponding extracellular enzymes is regulated by complex regulatory network involving both global and enzyme-specific factors and is also responsive to several environmental stimuli. Virulence in the *E. c.* subsp. *carotovora* has been shown to be globally regulated by a small diffusible signal molecule called the autoinducer. In addition we have isolated, sequenced and characterized the two-component regulatory system (*pehR pehS*) from *E. c.* subsp. *carotovora*, which is involved in specific positive regulation of PehA (endopolygalacturonase) production. The mutations in either, *pehR* or *pehS*, genes caused reduced virulence capability on the tobacco seedlings. The *pehR* (regulatory component) and *pehS* (sensor component) genes are similarly organised in an operon like *phoP-phoQ* regulatory systems of *Escherichia coli* and *Salmonella typhimurium*. The amino acid sequence of PehRS and PhoPQ are highly conserved except the regions which may correspond to the periplasmic loop. Functional similarity of *pehR pehS* and *phoP phoQ* has been shown by genetic complementation.

MASSAOUD, M. K., A.KOVÁCS, B. BLAHA, A. FODOR

Morphological characterization of phase variants of *Photobacterium luminescens*

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Primary and secondary phase variants of several *P. luminescens* strains were compared on conventional microbiological media and indicator plates. Electromicroscopic analysis were also made. A new secondary phase variant of strain *P. mol* were isolated and characterized from *H. bacteriophora* nematode. In an attempt to isolate phase variation mutant we used transposon mutagenesis and found several putative mutants. One of them called "Hyper" has also been characterized.

MÁTÉ, J.¹, I. RIMÓCZI², I. LENTI¹

Mycorrhizal fungus relationships in the oak forests of the Bátorliget primordial marsh

¹Agricultural College Department, Gödöllő University of Agricultural Sciences, Nyíregyháza,

MATOUG, A. A.¹, K. S. GHENGESH¹, R. KISS²

Characterisation of aeromonads isolated from different sources in Hungary and Libya

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In humans *Aeromonas* species are known to cause food-borne and water-borne infections, like gastroenteritis, septicaemia. Recently a number of food-poisoning outbreaks have been associated with these organisms. It is believed that some members of the genus *Aeromonas* are more virulent (e.g. *Aeromonas hydrophila* and *Aeromonas sobria*) than others (e.g. *Aeromonas caviae*). Therefore, exact identification of aeromonads isolated from different sources has a prime importance in understanding the epidemiology of these organisms and their role in diseases they cause in man.

In our work pathogens were searched in faeces, food, haemocultures, environmental, and wound samples. For the isolation of *Aeromonas* Blood agar and gsp (SIGMA) agar were used. From 87 *Aeromonas* strains 27 were isolated in Hungary and 60 strains in Libya. The strains originated from water (44.4%), food (14.8%; fish, raw milk, chicken sausage, salad), and clinical specimens (40.8%; faecal samples, haemoculture, wound). The identification of the strains was performed by BBL Crystal Rapid Stool/Enteric system.

13% of the Hungarian isolates were *Aeromonas hydrophila*, 48% *Aeromonas sobria*, 9% *Aeromonas caviae* and 30% of the isolates had atypical reactions. From the *Aeromonas sobria* strains, 64% were isolated from water, 9% from food, and 27% from clinical specimens.

Of the Libyan strains 40% were *Aeromonas hydrophila*, 35% *Aeromonas caviae*, 18% *Aeromonas sobria* and 7% were atypical *Aeromonas* species. Of the 24 *Aeromonas hydrophila* from Libya 54% were from water, 25% from food and 21% from clinical specimens.

In conclusion, water appears to be a major source of the pathogenic *Aeromonas* in Hungary and Libya. In some outbreaks when enteropathogens were not isolated *Aeromonas* caused enteritis. Furthermore, the presence of atypical *Aeromonas* species among the strains studied warrants the use of more sophisticated techniques (in our work Biolog GN microplate system was used) for the speciation of these organisms.

MATSUOKA, M.¹, K. ENDOU¹, H. KOBAYASHI¹, M. INOUE², Y. NAKAJIMA¹

A plasmid that encodes three genes for resistance to macrolide antibiotics in *Staphylococcus aureus*

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MAYER, Zs.^{1,2}, T. MÁTRAI¹, Zs. KÓKAI¹, I. SALAMON¹

***Aspergillus* invertase (AI) test for the rapid detection of feed spoilage**

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From hygienical point of view *Aspergillus* spp., nearer the members of the *A. flavus* group (*A. flavus*, *A. parasiticus*, *A. oryzae*, *A. nomius*) are the most important moulds:

- a.) They start earliest to deteriorate feed at a low a_w ,
- b.) generate moisture for further moulds growing at higher a_w ,
- c.) produce important mycotoxines, like aflatoxines and ochratoxines.

The principle of the *Aspergillus* invertase (AI) test is based on three research experiences:

- 1.) A technique has been developed, where mycelia of primarily *Aspergillus*, but also of *Penicillium* and *Poecilomyces* spp. could be grown rapidly in a 1 - 4 mm deep liquid medium at 37° C.
- 2.) Fungi common in feed can grow on Czapek-Dox medium, whose substrate is sucrose, must be able to produce invertase necessarily.
- 3.) Very small mycelial growth, invisible for naked eye can be detected through invertase activity in the submerged mycelium growing system by detecting the apparition of reducing sugar.

Procedure, aspecific interactions are described, sensitivity and practical use discussed.

MAYER, Zs.^{1,2}, T. MÁTRAI¹, Zs. KÓKAI¹, I. SALAMON¹

Growth kinetics of common feed moulds monitored in constant a_w moisture chamber

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A simple model for constant a_w moisture chambers is proposed, based on the standard relative humidity over saturated aqueous solutions of NH_4NO_3 (0.62), NaCl (0.75), KCl (0.84) and KH_2PO_4 (0.95). The chambers of two litre could be closed air-tight, the atmosphere got to an equilibrium at the 8th hours. 2 gms of sterilized ground feed samples of particle size less than 0.2 mm or whole grains were placed on aluminium trays of recorded tare, inoculated by dry dilution and incubated at 25 °C for 26 days.

Samples were taken and mould counts were determined according to ISO 7954.

Inoculated by *A. parasiticus* at a level of log 6.5, at $a_w = 0.95$, mould count started to grow rapidly, by approx. 0.5 log/day, attaining 8-9 log at the 19th day. At $a_w = 0.84$, mould count started to decrease till the 6th day, by 0.15 log/day, then started to grow by 0.2 log/day and attained the same magnitude at the 19th day. At $a_w = 0.75$, slight decrease until the 6th day could be also observed, then mould count levelled up to the initial level. At $a_w = 0.62$, initial mould count stagnated, without any decrease.

A. flavus and *A. parasiticus* and a *Penicillium* strain combined inoculations at $a_w = 0.95$ and $a_w = 0.84$ showed the similar propagation kinetics, except the decreases during the first 6 days. At higher a_w values *Penicillium* dominated rapidly, while at lower a_w its propagation started after the 6th day only.

MÉCS, I.

Biological effects of interferons

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The antiviral mode of action of interferon was pursued in interferon-pretreated and Semliki forest virus-infected chick cells. The progeny virus was inhibited in a dose-response relation, while several viral intermediates exhibited the following decreasing sequence of sensitivity parent-like single-stranded (+) RNA, viral RNA polymerase and the replicative form double-stranded viral RNA. The data suggest that the antiviral action of interferon may be related to the integrity of parent-like viral (+) RNA.

Interferon inducers or interferon treatment decrease the inflammatory responses in carrageenin paw edema of mice, in a dose-response manner, which can be suspended with anti-interferon immune sera. Human leukocyte interferon-alpha (Egiferon^R) also inhibits the inflammatory responses in carrageenin-treated mice, the extent of this inhibition being subtype-dependent. The data suggest that, apart from its antiviral action, interferon has several roles in the primary defensory

mechanisms, including the inhibition of inflammations.

MEGYERI, K., Y. MÁNDI, I. ROSZTÓCZY

Induction of cytokine production by different *Staphylococcus* strains

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The aim of our studies was to compare the cytokine inducing activities of different *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* strains. Our results demonstrated that *S. aureus* strains induced a transient interferon (IFN) production with highest yield between 12 and 20 h after induction. The IFN activity ranged from 300-2500 IU/ml. Serologic characterization of *S. aureus*-induced IFN indicated that alpha-IFN was the predominant type in these preparations. *S. epidermidis* strains induced low-titre (10-20 IU/ml) IFN production. No IFN was detected in supernatants of human mononuclear cells (MNC) stimulated with *S. saprophyticus*. Thus, the higher IFN-inducing activity of *S. aureus* may be characteristic of this species. Our RT-PCR assay demonstrated *S. aureus*-specific increase in IFNA mRNA. Transcription of IFNA gene was transient reaching a maximum between 4 and 8 h after induction and decreasing to baseline level at 24 h. A very weak expression was detectable in MNC stimulated with *S. epidermidis* at 4 h postinduction. We were unable to identify *S. saprophyticus*-specific increase in IFNA mRNA. Restriction analysis with Hinc II endonuclease which is specific for IFNA2 suggests the presence of this subtype in *S. aureus*-induced MNC. IL-6 production was also measured. The *S. aureus*, *S. epidermidis* and *S. saprophyticus* species stimulated high-titre IL-6 production. Only slight differences were observed in the IL-6 inducing activities of the *Staphylococcus* strains tested. A multiprobe RNase protection assay was used to assess gamma-IFN, IL-1 alpha, IL-1 beta, IL-1 Ra, IL-6, IL-10, IL-12 p35 and p40 mRNA levels. A high increase in IL-1 alpha, IL-1 beta, IL-1 Ra, IL-6 and IL-12 p40 transcription was detected in MNC stimulated with *Staphylococci* for 8 h, and all of the tested *Staphylococcus* strains proved to be very efficient in mediating induction of these genes. Production of IL-1, IL-6, IL-12 and IFN-alpha might play an important role in the pathogenic mechanisms of diseases caused by coagulase-positive as well as coagulase-negative *Staphylococcus* strains.

This study was supported by grant of the Hungarian Scientific Research Fund OTKA/T6057 (to R.I.).

MERBACH, W.¹, S. RUPPEL²

Influence of different N compounds on the dinitrogen fixation of *Azospirillum* and *Pantoea*

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METHNER, U., A. BERNDT, G. STEINBACH

Combination of vaccination and competitive exclusion to prevent *Salmonella* infection in chickens

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Vaccination and competitive exclusion (CE) represent accepted prophylactic measures to control *Salmonella* infections in chickens. To use the advantages of both CE technique and vaccination with live *Salmonella* vaccines the combination of these methods was studied.

It has been the purpose of our experiments to study competitive exclusion, inhibitory and immunological effects after combined use of CE and vaccination with both attenuated live *Salmonella* vaccine or non-attenuated *Salmonella* wild-type strains against *Salmonella* infection in chickens of different ages.

In experiments, SPF chickens were pretreated using combined or unique administration of CE and vaccination with live *Salmonella* Typhimurium strains on days 1 and 2 of life and challenged with a *Salmonella* Typhimurium strain on days 3, 15 or 40 of life. The caecal colonization of both the vaccine and the challenge strain and the antibody response after infection were examined to evaluate the protective effects of the different combinations.

The exclusion effect of the CE culture against *Salmonella* infection could be seen in very young chicks and was still considerable on day 40 of life of the birds. The combined administration of competitive exclusion and immunization resulted in a considerable additional protective effect above the level of the respective exclusive application of these prophylactic measures. Administration of the *Salmonella* vaccine strain prior to or simultaneously with the CE culture produced the best protective effect because such combinations ensure an adequate persistence of the vaccine strain as prerequisite for the expression of colonization inhibition effects and a strong immune response. The full exploitation of this potential using attenuated live *Salmonella* vaccines will require the presence of high inhibitory and immunogenic properties of the vaccine strain after attenuation of a selected parent strain.

MEZEI, M.¹, K. BALOG¹, M. TAKÁCS², Á. GYURIS¹, J. SEGESDI¹, Á. BAKOS¹, D. VÖDRÖS¹, D. BÁNHEGYI³, Gy. BERENCSEI², J. MINÁROVITS¹

Genetic subtypes of HIV-1 in Hungary

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HIV-1 shows remarkable genetic variation, which is the result of a high mutation

rate, rapid viral turnover and recombination of viral genomes. Genetic variation is most pronounced in the viral envelope gene. Phylogenetic analyses of diverse envelope sequences have shown that HIV-1 can be classified into multiple genetic clades or subtypes. Thus, ten genetic subtypes named A through J in group M (major) and a group of other subtype (group O, outlying) have been identified. Amino acid sequence variation within a subtype generally ranges from 5 to 20% , and between subtypes from 25 to 35%. All genetic subtypes have been found in Central African countries, whereas subtype B predominates in Europe and the USA.

HIV-1 genetic subtypes were determined in 20 adults from Hungary. Peripheral blood mononuclear cells (PBMCs) of HIV seropositive individuals and AIDS patients of the St. László Hospital, Budapest were cocultivated with PBMCs of HIV seronegative blood donors and PCR amplified *env* regions of HIV-1 proviral DNAs were analysed by heteroduplex mobility assay (HMA) and genomic sequencing. HMA results revealed that HIV-1 gp120 sequences from most patients were of subtype B. DNA sequencing confirmed the HMA results.

This study shows that subtype B is the predominant HIV-1 clade at present in Hungary.

MICSINAI, A., A. HORVÁTH, A. K. BORSODI

The bacterial communities inhabiting the rhizomes of healthy and degrading reed stands

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Rhizome samples from healthy and degrading reed (*Phragmites australis* /Cav./ Trin et Steudel) stands were taken in November 1998. Endophytic and outer surface attached bacteria were isolated using the serial dilution and plating method. The obtained 246 bacterial strains were subjected to phenotypic testing, numerical analysis and BIOLOG identification.

The results have shown that there is a marked difference in the bacterial community structure of the healthy and degrading reed rhizomes. The outer surfaces of healthy rhizomes are characterised by *Aeromonas media*, *Enterobacter* sp. and fluorescent pigment producing *Pseudomonas* sp. *Aeromonas media* and *Pseudomonas* sp. can also be found on the inner surfaces, together with a group of unidentified Gram positive, facultatively fermentative bacteria.

Rhizomes from degrading reed stands, however, contained *Aeromonas veronii/sobria*, *Erwinia* sp., a group of unidentified, facultatively slow fermenting bacteria and another group of unidentified, pigmented bacteria exhibiting cell cycle. Bacteria isolated from the inner surfaces of the degrading reed rhizomes belonged mainly to the latter group.

The differences detected in the community structure might explain some aspects of the reed decline. The presence of fluorescent pseudomonads in the healthy stands

might contribute to the resistance of the plants. Their disappearance in the degrading reed and the occurrence of several facultatively fermentative bacteria can either be an indication or a consequence of the premature rotting of rhizomes.

MICZÁK, A.^{1,2}, K. HÖNER zu BENTROP¹, D. G. RUSSELL¹

Lipid catabolism and intracellular survival of mycobacteria

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Pathogenic mycobacteria survive and replicate in macrophages. Phagosomes containing these bacteria do not fuse with lysosomes and the vesicles are only mildly acidified. In the search for the mechanisms by which pathogenic mycobacteria survive in the host macrophages, several intracellularly expressed genes have been identified. One of these genes codes for isocitrate lyase (Icl). The *Mycobacterium tuberculosis* CSU93 *icl* gene was cloned, expressed in *E. coli* and purified. Icl plays a key role in the glyoxylate cycle and is essential as an anapleurotic enzyme for growth on acetate and certain fatty acids as carbon sources. Its production and activity are enhanced under minimal growth conditions when supplemented with acetate or palmitate. In addition to western blots and enzyme assays, its expression in *Mycobacterium tuberculosis* under different growth conditions was also monitored by fusing the protein with green fluorescent protein under regulation of its own promoter.

To elucidate the glyoxylate bypass in more detail, we cloned the gene for malate synthase, too. Its expression in *E. coli* results in an active enzyme. In contrast with Icl, it appears to be expressed constitutively.

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Features of hepatitis C infection in personnel involved directly in health care

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Serum samples of 477 hospital workers (HWs) at our were tested with hepatitis C virus (HCV) EIA. HCV-RNA were measured of the HCV seropositive samples (Amplicor). The genotypes were determined by a line-hybridisation assay (Innogenetics) or by a serotyping ELISA (Murex). A total of 15 (3.1%) of the HWs proved HCV antibody positive. The seroprevalence was 0.7%, 4.5%, 9.2% and 9.5% in the age groups 21-30, 31-40, 41-50 and above 50 years of age, respectively. Eleven workers had HCV-RNA in their sera (73.3%). Genotype distribution of HCVs was as follows: six were 1b, two were 1, one was 3a, two were 4 and three were not typeable. Six of the workers developed chronic C hepatitis, out of them

three nurses were infected by needlestick injury. The treatment was interferon-alfa alone or plus ribavirin.

Conclusions: 1. The risk of HCV infection increases with age but it is lower than that of hepatitis B infection. 2. Needlestick injury is associated with an increased risk for acquiring HCV infection. 3. The carrier state of HCV and the titre of HCV genome-copies is changing during the years, independently of the therapy. 4. The HCV genotype distribution in HWs differs significantly from that of the donor population in Hungary. 5. None of the chronic "C" hepatitis HWs responded with complete and/or sustained remission to the treatment with interferon-alfa. 6. The HWs must be alert to the danger of occupational infections and pay vigorous attention to strict infection control procedures.

MIKLÓS, I.¹, M. SIPICZKY^{1,2}

Characterisation of a *Schizosaccharomyces pombe* mutant strain, defective in cytokinesis

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MISKOLCI, Cs.¹, I. LABÁDI², T. KURIHARA³, N. MOTOHASHI⁴, J. MOLNÁR¹

G-C rich regions of plasmid DNA can be the target in antiplasmid effect of phenothiazines

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Phenothiazines have been shown to inhibit the growth of *E. coli* bacteria and promote the elimination of plasmids. It has been proposed that some of these effects may be correlated to the binding of the phenothiazine to DNA by intercalation. Heterocyclic compounds such as caffeine and indole intercalate weakly with DNA and therefore may promote the stacking of phenothiazines. To test this hypothesis, we have studied the plasmid elimination effect of the phenothiazine promethazine in the presence of xanthine analogs caffeine, guanosine monophosphate and indole which themselves have no effect on the growth of *E. coli*.

The order of suppression of plasmid elimination produced by promethazine combined with different agents reflected the polarizing power of each agent. There might be a competition between the phenothiazine and GMP for intercalation sites of the DNA, by the binding of the phenothiazine to the G-C rich region or by molecular stacking of promethazine into the G-C rich region of plasmid DNA promoted by the xanthine analogs.

MOHÁCSI-FARKAS, Cs.¹, G. KISKÓ¹, J. FARKAS², T. SÁRAY²

Investigations on application of antimicrobial agents from plants for food preservation

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In the frame of an INCO-COPERNICUS EU-RTD cooperation project, the feasibility in laboratory scale of application of various essential oils produced from spices and herbs by the Department of Food Technology, Kaunas University of Technology, Kaunas, Lithuania, has been assessed as biopreservatives. Antimicrobial effects of volatiles from dill seed, thyme or oregano on the microflora of shredded cabbage and carrot cubes aerobically packaged in polyethylene pouches and stored, at various refrigeration temperatures, were tested. Antimicrobial activity of essential oils of thyme, dill weed and oregano added to pizza cream, mayonnaise and tomato juice, respectively, was also studied. Both the useful antimicrobial and the unwanted plant-physiological or sensory effects were strongly influenced by the concentration and the mode of application (vapour phase, spray or dipping solution) of essential oils. The very intensive flavour of essential oils limits their use as general food biopreservatives and the assessment of their utility in food preservation requires an item-by-item approach.

MOKHTAR, I. S., E. TÓTH, K. MÁRIALIGETI

Changes in the anaerobic bacterial community structure of skin in ewes due to *Wohlfahrtia myiasis*

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Wohlfahrtia magnifica is considered to be one of the most important myiasis causing species of livestock, poultry and humans in several countries in the World. In Hungary up to 40% of animals in sheep flocks may become infested during the summer. Former studies on the microbiology of wound myiasis caused by *Wohlfahrtia magnifica* have clearly demonstrated, that alterations in the skin bacterial communities, and the resulting production of the attractant volatiles can be responsible for the initiation of disease.

Healthy skin and myiatic lesion anaerobic heterotrophic bacterial communities were investigated and compared by numerical taxonomic methods, moreover the volatile and non-volatile fermentation end products of the isolates were investigated. An important shift in the species composition is evident, the amount of strictly anaerobic bacteria usually found in abscesses, etc. is higher in the lesion than in the healthy skin. The strains isolated from the myiatic lesions have a broader potential for volatile acid production than their healthy skin originating counterparts.

MOLNÁR, J., Gy. GUNICS, Cs. MISKOLCI

Models for reversal of resistance in bacteria and fungi

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Antibiotic resistant pathogens are emerging in the population. Many of the potent antibiotics and antitumor drugs have lost their effectiveness in the last decades. Antibiotic resistant bacteria are known to be responsible for more than 50% of hospital acquired infections. The multiple resistance means that patients with infections are ill for a longer time and they are at a greater risk of dying. At the same time too few drugs are developed to replace those which have lost their effectiveness. Therefore new combinations of compounds are needed to overcome drug resistance.

There is an urgent need to analyze the nature of interaction between chemotherapeutics and resistance reversing compounds. In model experiments the extrachromosomal genetic code of prokaryotes and eukaryotes was eliminated from bacteria and yeast by some heterocyclic compounds. Heterocyclics can affect DNA and membrane functions as well. As a consequence, some representative compounds were able to synergize the effects of a few antibiotics. The resistance reversing effect was dependent on the chemical structure, and stereospecificity of the resistance modifiers. The mechanisms of synergy between chemotherapeutics and resistance modifiers will be discussed.

MUCSI, I.¹, J. MOLNÁR¹, N. MOTOHASHI²

Combined effects of benzo(α)phenothiazines and acyclovir against herpes simplex virus in cell culture

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The combined antiviral effects of some benzo[*a*]phenothiazine derivatives and acycloguanosine (acyclovir, ACV) were studied on multiplication of herpes simplex virus type 2 (HSV-2) in Vero cells. Previously it was demonstrated that some phenothiazine and benzo[*a*]phenothiazine derivatives exhibited antiviral activity against HSV-2. Several anti-herpesvirus drugs are available but ACV is currently one of the most effective agent for the treatment of herpes simplex virus infections. Resistance to ACV may emerge particularly in severely immunocompromised patients. However, the combination of antiviral agents should have additive or synergic activity and should delay the development of drug resistance. In present studies the simultaneous application of benzo[*a*]phenothiazine derivatives and ACV during the serial passages of an plaque-purified ACV sensitive HSV-2 strain resulted in decrease or disappearance of the infective virus population. The antiviral effect of ACV on a wild strain of HSV-2 was enhanced in the presence of 5-oxo-5H-benzo[*a*]phenothiazine in yield reduction test. A mathematical formula was

used to interpret the drug interaction and the combination exhibited synergy. The results suggested that a special combination of some antiviral drugs with benzo[*a*]phenothiazines can increase the antiviral action probably due to reduction of the mutagenic rate in the virus population.

MUELLER, M.¹, U. BEHRENDT¹, P. LENTZSCH¹, J. KIESEL²

Characterisation of bacterial communities from the phyllosphere of crop plants

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Whereas the diversity of plants and animals in agroecosystems receives great attention, the diversity of micro-organisms is often neglected in the description of the biological diversity in a natural ecosystem. However, several methods have been suggested for genetic and functional analyses of microbial communities in the phyllosphere of crop plants. Moreover, there is an increasing demand for information about the influence of landscape structures, climate factors and land management practices on their composition. The present study was carried out to assess the possibility to use four different biological parameters concerning the phylloplane micro-organisms to characterise these microbial communities:

1. Total number of enterobacteria, a frequently occurring and important group on the surface of crop plants;
2. Part of the individual species of *Pantoea agglomerans* within the population of the enterobacteria;
3. Intraspecific genetic diversity of this species determined by PCR fingerprint using repetitive DNA sequences;
4. Enzyme activities (α - and β -glucosidase, acid and alkaline phosphatase) regarded as a term for the general metabolic activity of the whole microbial community.

Spatial and temporal variations of these biological parameters were presented along a transect in North-Eastern Germany about 200 kilometres in length with 40 sampling places in various distances. The study area comprises, as a result of its glacial origin in Young Pleistocene, a wide variety and a complex arrangement of land forms and landscape elements. We demonstrated the distribution of the biological parameters depending on several types of Young Pleistocene landscapes, on regions of different rainfalls and on the kind of crop plants.

MÜLLER, Th.¹, E.-M. OTT²

Occurrence and possible ecological function of enterococci on forage grass

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Enterococci are frequently found in grassland independent of utilisation for pasture farming. Their main reservoir is the litter layer. They colonise the rhizosphere as well as the above ground parts of grass, where they live in a truly epiphytic relationship with the plants, i.e. they are able to reproduce there. As saprophytic bacteria they are part of a naturally-occurring biological control of pathogens. Bacteriocin production in this group is very common, and enterocins are known to show activity against a broad spectrum of accompanying bacteria. This could possibly be of particular importance with respect to the elevated numbers of micro-organisms in extensively used grassland for suppression of species potentially pathogenic to animals or plants. Plant samples were taken from five meadows in a fen land area in NE-Germany in the course of a growing season. Enterococci could be detected at all investigation sites and on each sampling occasion in numbers of 10^1 - 10^4 CFU/g of grass. From each site 30 isolates per sampling occasion were identified (whole-cell protein pattern on SDS-PAGE and restriction analysis of PCR-amplified 16S rDNA) and tested for their ability to produce bacteriocins. About 20 % of the isolates were antagonistically active against other Gram-positive species. This activity was also detected when the enterococcal strains grew on "phylloplane agar", a medium which simulates the low level nutrient conditions found on the leaf surface, in a temperature range between 4 and 37 °C.

NAÁR, Z.¹, M. KECSKÉS²

Co-existence of different species of *Trichoderma* genus in various soil types

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Trichoderma species have very similar environmental requirements, however they can establish and maintain at the same time and in the virtually same habitat of soil. This co-existence of different *Trichoderma* species in various soil types of Hungary was studied (granted by OTKA F025924) to reveal physico-chemical parameters rendering their co-occurrence. The species composition of *Trichoderma* genus was recorded in 24 soil samples collected from A-horizons of various soil types at different parts of Hungary. The decreasing frequency order of total 13 species was as follows: *T.harzianum*, *T. virens*, *T. viride*, *T. atroviride*, *T. tomentosum*, *T. minutisporum*, *T. spirale*, *T. hamatum*, *T. koningii*, *T.longipilis*, *T. polysporum*, *T. strictipilis*, *T.strigosum*. 5 out of 24 soil samples contained only one *Trichoderma* species, thus their co-occurrence seemed to be a common phenomenon. The number of co-existing species ranged between 1-5, which allowed further analyses. Co-existence matrix was constructed which demonstrates that from how many soil samples have been isolated two particular species. The list of species in decreasing order of number of co-existing other species was as follows: with 11 species: *T. viride*, with 10 species: *T. harzianum*, with 9 species: *T. tomentosum*, *T.virens*, with 6 species: *T. atroviride*, *T.spirale*, with 4 species: *T. hamatum*, *T. koningii*, *T.longipilis*, *T. minutisporum*, *T. strictipilis*, with 3 species: *T. polysporum*, with 2 species: *T.strigosum*. 26 physico-chemical soil parameters and colonizing ability by *Trichoderma* were determined for each soil samples and used for building mathematical models to reveal a group of factors influencing the co-existence of *Trichoderma* fungi.

NAGY, E., J. SÓKI, E. FODOR, E. URBÁN, I. SZÓKE

Antimicrobial resistance in anaerobic bacteria

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NAGY, E.¹, I. SZÓKE¹, L. TÖRÖK²

Role of anaerobic bacteria in chronic prostatitis and male infertility

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The accurate diagnosis of chronic prostatitis syndromes poses a major challenge to physicians and clinical microbiologists. About half of all men suffer from symptoms

of prostatitis during some part of their life. In acute bacterial prostatitis, Gram-negative bacteria are the most common pathogens. The roles of Gram-positive bacteria in chronic bacterial prostatitis and of *Chlamydia trachomatis* in "non-bacterial" prostatitis are debated. Empirical antibiotic therapy is often used for the treatment of chronic prostatitis.

During this study, the urethral discharges and the prostatic fluids after prostate massage of 80 patients with therapy-resistant chronic prostatitis were cultured in parallel in aerobic and anaerobic environments. Thirty-seven patients exhibited infertility problems, with a 100 % decrease in the motility of their sperms. The samples of all patients were screened for the presence of *C. trachomatis*, *M. hominis* and *U. ureolyticum*. Thirty of the 80 patients gave negative culturing results for all pathogens screened, but 43 % of the patients harbored $>10^5$ CFU/ml anaerobic bacteria alone or together with aerobic bacteria. The most frequently isolated anaerobic bacteria were *B. ureolyticus*, *Prevotella spp*, *Porphyromonas spp* and *Peptostreptococcus*. *In vitro* tests after incubation for 2 to 18 hours demonstrated the negative effects of the anaerobic bacteria isolated from these patients on the motility of healthy sperms. In patients with massive anaerobic infection, long-term antibiotic therapy active against anaerobic bacteria led to elimination of the complaints, and the symptoms of chronic prostatitis. The use of molecular diagnostic methods will be discussed.

NAGY, K., B. KEMÉNY, A. HORVÁTH

CCR5 Δ 32 deletion affects course of disease in HHV-8 infected asymptomatic HIV patients

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HHV-8 has a role in the pathogenesis of Kaposi's sarcoma (KS). It is more frequent in HIV infection, and its presence may predict AIDS associated KS. Δ 32 mutation of CCR5 chemokine receptor gene results in partial resistance to macrophage tropic HIV-1 infection *in vitro*, and a slower disease progression *in vivo*. Interaction of Δ 32 mutation of CCR5 gene and prevalence and role of HHV-8 infection in the course of HIV disease was analysed in asymptomatic HIV positive patients.

Methods: HHV-8 antibodies to LANA were detected by IF, and verified by ORF26 PCR. CCR5 genotype was determined in 192 cases by PCR using purified DNA from PBMCs of 93 asymptomatic HIV carriers (including long term non progressors /LTNP/), 20 HIV negative sexual contacts and 79 healthy individuals.

Results and conclusion: i.) a Δ 32 allele frequency of 0.086 was found in HIV positive patients which was significantly lower than that of in the healthy group: 0.132, ii.) Δ 32 was most frequent in the LTNP group: 0.200, iii.) HHV-8 prevalence in HIV infected group was 25%, similar to that of Western Europe and US, iv.) in the HIV positive group with HHV-8 coinfection Δ 32 heterozygotism was 16.7 %, while without HHV-8 infection, this was 29.4%. This suggests that people with CCR5 Δ 32 deletion has an immune system more resistant to HHV-8 infection.

NAGY, T., K. KISS, J. ZALA

Theoretical comparative study of yeast identification with some test kits

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The fungal infections are increasing and there is a demand of correct identification of the species. The selection of strains of yeast species on Chromagar (BBL) is very useful, however, identification of yeast species with it is not verified.

Funid program developed in our department for identification was used to evaluate usefulness of test kits for yeast identification.

There have been several problems both in the scientific literature and with the test kits for yeast identification (Auxacolor, Mycotube, API20CAUX, API32ID).

Our preliminary results have shown good correspondence among Auxacolor, Mycotube, API 32ID and further comparisons have been intended to be done.

There are some advantages of test kits:

Standard composition

Standard method

The main problems with test kits are:

The names of some yeast species are incorrect.

Some yeast species are no longer exist.

The available codes are insufficient and/or unreliable for correct identification (c.f. kit documentation).

The spectrum of clinically important yeast species seems to be wider than the test kits can distinguish them.

The results between two certain test kits are almost incomparable because of the different fungal spectra and the names of species.

Proposals:

The accepted changes in scientific literature should be involved in test kits.

The species that are most important for clinically important yeast identification should be declared.

The names of the yeast species should be standardized.

NAGY, Z.¹, A. SZENTIRMAI¹, S. BIRÓ²

Purification and properties of β -galactosidase from *Penicillium chrysogenum*

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β -galactosidase is a well-known enzyme that hydrolyzes lactose. It is widely distributed in nature and many studies have been reported on the physiology and regulation of the enzyme from different sources, including bacteria, yeasts, moulds, plants and animals.

In *P. chrysogenum* the synthesis of the enzyme is induced by lactose and repressed by glucose. We studied the mechanism of induction/repression of the enzyme and found an interesting correlation with cAMP level in this species.

We also describe a procedure for the rapid purification and some molecular properties of an intracellular β -galactosidase of *P. chrysogenum*.

When *P. chrysogenum* is grown on lactose as carbon source it synthesizes one active form of β -galactosidase. The enzyme was purified by ammonium sulphate precipitation and substrate affinity chromatography. Its homogeneity was confirmed by SDS/PAGE. β -galactosidase of *P. chrysogenum* is a stable enzyme with an optimum pH value of 4.0 and an optimum temperature of 30°C.

NEER, Zs., I. PFEIFFER, J. KUCSERA

Examination of killer phenotype attributed to dsRNA viruses in *Cryptococcus hungaricus*

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The most researched, and thus best known, species within the *Cryptococcus* genus is *C. neoformans*, due to its pathogenicity. Our research was conducted on *Cryptococcus hungaricus*, a less known species of the genus. The species was first described by János Zsolt, under the name *Dioszegia hungarica*. Later (1970) Pfaff and Fell observed the same vegetative multiplication, that was thought to be unique to *D. hungarica*, in other *Cryptococcaceae* species, and therefore considered the *Dioszegia* genus redundant.

Double-stranded RNA viruses have been isolated from the CBS 6569 strain of *C. hungaricus*, and this strain shows killer phenotype. In our study we tried to prove that the killer attribute is attached to virus particles by deleting the virus particles from the cells, using cycloheximide and acridine-orange treatments, and then

examining whether the obtained strains still exhibit the killer phenotype.

We tested the mechanism, through which the toxin protein exercises its effect by fluorescent staining. Using various media and incubation temperatures the activity of the toxin. Furthermore, we examined the stability of the protein by exposing it to pH ranges differing from the optimum, prior to incubation. Our aim is to establish the effective mechanism and spectrum of the toxin, as well as trying to find sequence homology with the genomes of viruses found in ascosporous, and related basidiosporous fungi.

This research was supported by OTKA F 020870 and OTKA T 025849.

NEMCOVÁ, R., A. BOMBA, S. GANCARCIKOVÁ, R. HERICH, P. GUBA

Effect of administration of lactobacilli and fructooligosaccharides on the fecal microflora in weaning piglets

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The influence of administration of *Lactobacillus casei* alone and mixture of *Lactobacillus casei* and fructooligosaccharide (FOS) on faecal bacteria counts in the weanling pigs was investigated. In faeces of experimental animals receiving the mixture of *Lactobacillus casei* and FOS, significantly higher *Lactobacillus* ($p < 0.01$), *Bifidobacterium* ($p < 0.05$), total anaerobes ($p < 0.05$), and total aerobes ($p < 0.05$) counts have been found as compared to the control and significantly higher anaerobes ($p < 0.05$), total aerobes ($p < 0.05$), *Bifidobacterium* ($p < 0.05$), and *Lactobacillus* ($p < 0.05$) counts compared to *Lactobacillus casei* group. Compared to the control, significant decrease in *Clostridium* ($p < 0.05$), *Enterobacteria* ($p < 0.01$) counts was observed as well as an insignificant decrease in *Coliform* counts by 1 log. *Enterococcus* counts were significantly reduced ($p < 0.001$) compared to both control group and *Lactobacillus casei* receiving group. In faeces of experimental animals receiving *Lactobacillus casei*, significant decrease in *Clostridium* ($p < 0.05$) and *Enterobacteria* ($p < 0.05$) counts as compared to the control was recorded. *Coliform* counts were by 0.5 log lower compared to control. This difference, however, was insignificant similarly like with *Coliform* in previous experimental group due to the great variance of values in individual groups. *Lactobacillus*, *Enterococcus* and total anaerobes counts were identical in the both groups. An insignificant increase in total aerobes in favour of experimental group was recorded and vice versa, there was an insignificant decrease in *Bifidobacterium* as compared to the control group. The results obtained point out to a synergic effect of the combination of *Lactobacillus casei* and fructooligosaccharide on numbers of bacterial populations observed in the faeces of the weanling pigs. The combination of probiotics and non-digestible carbohydrates may be a way of stabilization and/or potentiation of the effect of probiotics. Such potentiated probiotics indicate a realistic way of using biological preparations in the prevention of gastrointestinal diseases in weaned pigs.

NIKOLAUSZ, M., K. MÁRIALIGETI, O. ORAVECZ, Cs. ROMSICS

Changes in the composition of eel gill bacterial communities as a function of eel nematode infection

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The cause of the mass destruction of eel in lake Balaton is presumably a result of joint influence of different factors (pesticide poisoning, effect of animal parasite, lack of oxygen, etc.), but it is certain that bacterial infections may play important role as predisposing factors. The most important target point of fish pathogens is the gill, particularly if floating and breathing of the fish is injured by an eel-nematode (*Anguillicola crassus*) parasitism.

In order to get a more detailed insight into the composition of the gill bacterial community of healthy and infected eels a polyphasic approach has been chosen. Homogenised gill tissue samples were used for obtaining pure cultures moreover for direct DNA extraction. Pure cultures were described by some phenotypic characters, grouped by ARDRA (Amplified Ribosomal DNA Restriction Analysis) and identified by partial sequencing of PCR amplified 16S rDNA.

The DNA isolated directly served as a template for amplifying 16S rDNA of the gill bacterial community. A second nested PCR resulted sufficient product for cloning. A clone library was generated by blunt-end cloning and it was screened using ARDRA method. Representative members of different band pattern groups were sequenced partially. The sequences were aligned and analysed by the ARB phylogenetic software package and compared to rDNA databases for taxonomic purpose.

The most dominant species from pure cultures proved to *Aeromonas veronii* in healthy eels, but its dominance is forced back by a more diverse community dominated by *Pseudomonas putida* and *Pseudomonas stutzeri* in eel nematode infected, sick eels. *Dietzia maris*, *Shewanella putrefaciens*, *Acinetobacter spp.*, *Plesiomonas shigelloides*, *Comamonas sp.* also were found in the gill tissues of infected eels. They are facultative pathogens in mammals but their pathogenicity in the case of fish is not always clear and must be examined and proved later.

The clone library can be described with the dominance of *Aeromonas veronii* in healthy eels. Other dominant species are *Arthrobacter oxydans*, *Acinetobacter johnsonii*, *Nocardioides simplex*. The evaluation of data is in progress, but they support the results based on the culturing methods, that in healthy eels the *Aeromonas* dominance is changed to a more diverse community where potential fish pathogens may appear, in the nematode infected animals.

OLÁH-ZSUPOSNÉ, Á.¹, J. KÁTAI², M. BESSENYEI²

The effect of amelioration on the quantity of soil bacteria and microbial activity of soil

About 80% of soils used for cultivation are exposed to physical, chemical and biological loading. Furthermore, more than 51% of Hungarian soils have unfavourable properties, which require improvement. In order to prevent of soil pollution and to improve soils having unfavourable properties, it is very important to do microbiological research in this field.

Field trials were conducted on meadow chernozem soil in the Research Institute Karcag, in 1997. The spot experimental site was set up with 7 different plant cultures and by applying two different limes - limestone powder and lime ooze, originating from a sugar factory - this procedure was repeated 4 times. This paper deals with the effect of soil improving materials on the microbiological processes in a meadow chernozem soil. In the course of laboratory analyses, the total number of bacteria, the amount of aerobic N₂-fixing, aerobic nitrifying and cellulose decomposing bacteria, as well as some important soil enzymes' activities- phosphatase, catalase, urease, and invertase were determined from the soil of two plant cultures - maize and sugar beet. Soil samples were taken 3 times during the growing season.

In soils on which sugar beet was cultivated, the favourable effect of limestone effected the quantity of total bacterium number and nitrifying bacteria. The advantageous effect of lime ooze was measured in case of aerobic N₂ - fixing and cellulose decomposing bacteria.

In the soil samples taken from maize fields the effect of lime ooze was more favourable on the quantity change of soil bacteria than the limestone – except for total bacteria count.

On the basis of the average result of similar treatments performed on both plant cultures, the effect of lime ooze is more favourable on the life - activity of the soil bacteria, than the lime powder.

Both of the two soil improving materials proved very effective in their results on the activities of soil enzymes, and CO₂ production increased in a similar level.

In conclusion, it can be stated that the effect of two soil improving materials - lime ooze and limestone powder were favourable on the life activity of soil bacteria, their number increased, as compared with the control treatment.

The number of aerobic N₂ - fixing and nitrifying bacteria increased in the case of lime ooze treatment. The activities of soil enzymes and the production of CO₂ were influenced positively by both two liming.

OLÁH-ZSUPOSNÉ, Á.¹, J. KÁTAI², M. BESSENYEI²

The effect of cultivation on the amount of soil bacteria and some enzyme

activity

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The most common/universal antropogenic effect on cultivated soil is soil cultivation and soil utilisation. This is the reason why it is very important to research the soil microbiological consequences of the different cultivation methods.

Soil cultivation and utilisation field trials were set up and conducted on the experimental site of the Karcag Research Institute on meadow chernozem soil, in 1997. In the experimental field two types of soil cultivation methods were compared - traditional ploughing and conservation tillage - with 7 different plant cultures, as well as according to the size of small and large plots, repeated 4 times.

The effect of cultivation methods on the quantity changes of some soil bacteria - total number of bacteria; aerobic N₂ - fixing; nitrifying; aerobic decomposing bacteria - as well as the activities of some soil enzymes – catalase, phosphatase; urease; invertase - and CO₂ production were examined and evaluated. During the growing season, soil samples were taken three times, in spring, summer and autumn. According to the results originating from the spring soil sampling, the bacterium number increased 7-42% in the case of conservation tillage, in the average of four physiological groups of bacteria.

In autumn the opposite was experienced. In traditional ploughing treatments the number of soil bacteria increased except in the case of nitrifying. The differences between the two cultivation methods were between 19-62% depending on the different physiological groups of bacteria. It is probable that by the effect of long-term-rainy weather, the soil conditions for aerobic soil bacteria were decreased in the cultivated layer of conservation tillage soil.

As for the results related to enzymes activities, can be stated that both in spring and autumn the treatment of conservation tillage increased the activities of different soil enzymes from 2-25%. The most favourable effect can be experienced in the case of catalase. CO₂ production increased by 6,6 % in spring and, by 20 % in autumn, in the case of conservation tillage.

In conclusion it can be stated that the number of bacteria examined - total number of bacteria; aerobic N₂ - fixing; aerobic cellulose decomposing bacterium - was higher in the treatments using traditional cultivation - except for nitrification. The activities of soil enzymes - phosphatase; urease ; catalase sacharase - as well as CO₂ production increased as a result of conservation tillage.

ONGRÁDI, J.^{1,2}, A. AHMAD², A. HORVÁTH¹, J. MENEZES²

Induction of key cytokines in PBMC by human herpesvirus 7

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Altered cytokine profile induced by viruses may contribute to the pathomechanism of acute and chronic infections. Human herpesvirus-7 (HHV-7) isolated recently is one of the causative agents of exanthem subitum and pityriasis rosea, and its latent infections are frequently activated in immunosuppressed patients. Pathomechanism of neither HHV-7 nor these clinical entities has been revealed yet. Therefore, the production of some key immunomodulatory cytokines by peripheral blood mononuclear cells (PBMC) was studied upon primary and secondary infections in vitro. After combined treatments with live or inactivated viral preparations and/or mitogens (PHA, LPS, anti-CD3 monoclonal antibodies) some of the cytokines (IL-1 β , -2, -4, -6, 10, IFN- γ , TNF- α) were quantitated by sandwich ELISA kits, and TGF- β was bioassayed on mink lung epithelial cells. It was established that individual cytokines were produced at maximal output at different time periods after infection; and their levels depended on primary or secondary HHV-7 infections. Inactivated viral preparations also induced cytokine release. Production of IL-2 and IFN- γ after mitogenic stimulation was augmented in primary, but was diminished in secondary infections. Release of TNF- α was parallel to that of IL-1 β , but the combined effects of HHV-7 with mitogens increased the level of IL-1 β . Induction of IL-4 and IL-6 was not affected, that of TGF- β was augmented by HHV-7. HHV-7 induced IL-10 production, which latter is known to inhibit cytokine release from helper T cells and consequently might play a role in those inflammatory skin diseases mentioned above. In contrast to the closely related HHV-6 found in severe immunocompromised conditions, the effect of HHV-7 on the cytokine balance seems to be mild. HHV-7 induced skin disorders and immunosuppression show a tendency of spontaneous recovery.

Supported by grants from OTKA, NAB (HU), MRC (CA).

OROS, Gy.

Acquired tolerance to benomyl modifies developmental stage response of *Botrytis cinerea* Pers. to various chemicals

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OTT, E. M.¹, Th. MÜLLER²

Bacteriocins of plant-associated enterococci

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Six bacteriocin-producing strains of plant-associated enterococci (*E. faecalis* P 7644, P 8041, P 8403, *E. faecium* P 8079, *E. mundtii* P 7826, P 8382) obtained in an ecological study on extensive managed meadows were used for comparative studies of their antagonistic substances. Tests were carried out with bacteriocin crude extracts produced by ammonium sulphate precipitation, dialysis and freeze-drying. The antibacterial activity of all extracts was completely destroyed by treatment with proteinase K, pronase E and papain, while α -amylase treatment showed no effect on activity. Pepsin, α -chymotrypsin and trypsin inactivated antagonistic compounds produced by *E. faecium* and *E. mundtii* strains while they reduced activity of those produced by strains of *E. faecalis*. All bacteriocins were pH- and heat-stable except on E 8382 which loses activity when boiled for more than 10 min.

To test antimicrobial activity spectra of crude extracts, a total of 109 bacterial strains was used as indicator in the Agar-Diffusion-Assay. The antibacterial substances affected a wide spectrum of lactic acid bacteria, especially enterococci. All enterocins strongly inhibited *L. monocytogenes* and showed activity against clostridia. Additionally, extracts E 7644 and E 8041 (both produced by an *E. faecalis*) caused weak inhibition of a *Bacillus* strain and several strains of *Clavibacter* and *Curtobacterium*. Gram-negative indicators were not affected. Cluster analysis of the results grouped the enterocins corresponding to the species the producing strains belong to. This finding suggests that strains of the same enterococcal species produce bacteriocins of similar characteristics.

PÁL, T.

Experiences obtained from the microbiological examinations of activated sludge sewage treatment plants of Hungary

BIOTOP Bt. Budapest

The author deals with saprobiological problems of the biological sewage treatment plants since 1965. In the latest years in Hungary several biological sewage treatment plants have been built mainly on the base of activated sludge technology.

Beside the technical problems of the construction and operation of these plants a few efforts have been paid so far for the microbiological problems of them. However, according to the experiences gained from the microscopical sludge investigation a poor quality final effluent is very often caused directly by the less than optimum condition of the sludge flocs in the plant. The standard investigative procedure used most often nowadays for checking the course of the treatment process and providing information for adjustment if necessary, involves mainly chemical and physical analysis give little direct information to plant operators about the actual quality of the activated sludge floc in the aeration tank. As a result it is often not possible to indicate the cause of disturbance in the treatment process and, consequently, to initiate corrective action in order to improve plant operation.

Microscopic investigations of activated sludge is a simple analysis which gives information about the form and structure of flocs, the presence of filamentous microorganisms, the number of protozoa etc.

The author as an independent expert has made several microscopical investigations during the last years in different communal and industrial sewage treatment plants in Hungary.

In this lecture his experiences will be summarised which can contribute to a better insight into the composition and structure of flocs and through this into the functioning of the activated sludge process.

A digitalized video film enregistered by the author will be shown demonstrating eg. the problem of bulking sludge, etc.

PALKOVICS, L.¹, N. KARAMOVA², D. PRIBÉK³, E. BALÁZS¹

Changes on the 5' noncoding region of plum pox virus in connection with symptom development

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Plum pox virus is a member of the Potyviridae, the largest family of plant viruses. The genome of PPV is a messenger-polarity RNA molecule of 9786 nucleotides (nt) in length (PPV-SK68 Acc.No.: M92280) with a VPg protein at the 5' end and a poly(A) tail at the 3' end. The genomic RNA has a single open reading frame which is translated into a large polyprotein.

The role of the 5' noncoding region (NCR) in virus replication and translation initiation has been analysed in detail, but limited information is available on the contribution of this region to disease symptom development. We have identified two nucleotide changes in the 5' NCR which induce clearly different and considerably milder symptoms than those induced by the wild-type PPV-SK68 on *Nicotiana benthamiana*. Both of nt position (G₉₄ and C₁₁₇) are important in symptom expression, and both of the mutation alone had quite similar effects on symptom phenotype. The amount of viral RNA in the systemic infected leaves was not modified by the mutations. The only difference between the parental and the mutated strains could be detected at the coat protein level. These results suggest that the reduced protein levels can be responsible for the mild symptoms.

PAMJAV, H.¹, D. TRIGA¹, E. SZÁLLÁS¹, A. FODOR¹, Zs. BUZÁS²

PhastSystem PAGE PCR-RFLP analysis: new molecular technique for identification and phylogenetic analysis of entomopathogenic nematode – symbiotic bacteria, *Photorhabdus* and *Xenorhabdus*

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A recently newly developed PhastSystem polyacrylamide Electrophoresis method was adopted to analyze the RFLP pattern of the PCR-amplified spacer region of the 16S - 23S rDNA operon of *Photorhabdus luminescens* and several *Xenorhabdus* spp. Three enzymes (*AluI*, *HinfI*, *MseI*), provided highly reproducible patterns. On the basis of comparative pattern analysis DSMZ types strains of *X. nematophilus*, *X. bovienii*, *X. poinarii*, *X. beddingii* and *P. luminescens* could be identified unambiguously. Natural isolates and laboratory strains of the *Xenorhabdus* species hardly differed from the respective type strain. Symbionts isolated from different *Steinernema* (nematode host) species provide characteristic pattern, indicating that the cospeciation is characteristic for the symbiotic pattern of *Steinernema* / *Xenorhabdus* relations. *Photorhabdus* strains belonging to different 16S rDNA subclusters also provided characteristic pattern, but there were significant differences between some strains belonging to the same 16S rDNA groups. Considering that the PhastSystem PAGE technique proved also suitable for molecular identification of the nematode symbionts on the basis of PCR - RFLP analysis of the internal transcribed spacer (ITS) region of the 18S - 5.8S - 26S rRNA operon, there is a molecular tool for studying coevolution of the nematode - bacterium symbiotic complexes available.

PAPP, J.

Microbiota on leaves of oats grown in lead and zinc mine spoils

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Microflora of oats leaves was studied. The oat plants were grown in lead and zinc mine spoils submitted to remediation procedures under laboratory conditions. The colony-forming microorganisms that could be cultivated on malt extract agar used as nutrient medium belong to six groups: rod-shaped Gram-negative bacteria, nonsporogenous Gram-positive bacteria, endospore-forming Gram-positive bacteria, Gram-positive cocci, yeasts and filamentous fungi. Gram-negative bacteria were the most frequently found microorganisms in the phyllosphere of oats, followed by Gram-positive cocci and filamentous fungi. The nutrient medium made it possible to record a more frequent occurrence of aerobic rod-shaped Gram-positive bacteria and Gram-positive cocci than that expectable based on literature data.

PAPP, T., Á. NAGY, Zs. PALÁGYI, M. VASTAG, Cs. VÁGVÖLGYI

Genetic studies on sexual processes of *Gilbertella persicaria*

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Gilbertella persicaria (Eddy) Hesseltine is an agriculturally important postharvest pathogen. Similarly to the majority of the organisms belonging in the Zygomycetes,

this species is heterothallic. The sexual cycle of most of these fungi is rather long, which makes genetic studies difficult. In contrast, after the mating of the partner strains and zygospore formation, *G. persicaria* requires a short period of dormancy (5-6 days) for germination of the zygospore, and it might therefore be used as an excellent experimental object in genetic studies.

Little is known about the genetic background of the inheritance of the Zygomycetes. Previous studies with an other species (*Mucor hiemalis*) suggested non-Mendelian distribution of the alleles among the offspring. The present work involved a study of the presence of the two mating types (+/-) among the descendants and also the distribution of RAPD (random amplified polymorphic DNA) markers in the meiotic products derived from isolated zygospores. The results suggested that the inheritance of both the mating types and the mating type-specific RAPD markers is non-Mendelian; more than 90% of the progenies were found to be of (-) mating type. The distribution of other RAPD markers and the appearance of new (recombinant) RAPD patterns were also analysed in detail.

This research was supported by Hungarian Research Fund (OTKA) grant F/4 017677 and Soros Foundation grant 230/1/676.

PAPP, T.¹, Cs. FEKETE², M. VASTAG¹, Á. NAGY¹, Cs. VÁGVÖLGYI¹

Presence of double-stranded RNA molecules and virus-like particles in *Rhizopus* strains

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Double-stranded ribonucleic acid (dsRNA) molecules have been found in a wide variety of phylogenetically-diverse fungi. However, the screening of fungal species belonging in the Zygomycetes from this aspect has been neglected. dsRNA molecules have so far been observed only in three genera: *Entomophaga*, *Mucor* and *Mortierella*.

In the present study, 26 strains representing 5 *Rhizopus* species were examined for the incidence of double-stranded RNA elements. These genetic elements were found to be present in 5 strains: 1 *R. oryzae*, 1 *R. microsporus* and 3 *R. stolonifer* strains. Electrophoretic separation of the nucleic acids revealed 5 different RNA patterns, with 1 to 5 discrete dsRNA bands. The molecular weights of these dsRNA bands ranged from 2.7 to 9.1 kbp. The presence of virus-like particles was also investigated by electronmicroscopy; all 5 strains were found to harbour virus-like particles. This is the first description of a mycovirus in the *Rhizopus* genus.

This research was supported by Hungarian Research Fund (OTKA) grant F/4 017677 and Soros Foundation grant 230/1/676.

PASICHNYK, L. A., R. I. GVOZDYAK, S., F. KHODOS

Bacterial microflora of seeds and growing wheat plants

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Bacterial microflora healthy seeds and growing wheat plants of different varieties was investigated. Epiphytic bacteria were isolated by washing of seeds, leaves and ears by steril tap water with subsequent plating of dilution on nutritious media. In 4 - 5 days the colonies were picked up and analysed.

It is established, that main microflora of wheat seeds is presented by *Pantoea agglomerans* (50 - 90 % depending on the varieties). Total quantity of bacteria on a surface of seeds also depends on the varieties. Absolutely another picture is observed on the growing wheat plants. In a phase of plantlet *P.agglomerans* are absent on the plants, and in a phase of booting they are detected very seldom. But in process of seeds maturing their quantity is increasing, and gaining maximum in a milk-dough stage. It is necessary to note, that with maturing of seeds the total quantity of bacteria on leaves and ears is increasing. By morphological, physiological and biochemical properties bacteria isolated from healthy wheat plants were identified as *Pseudomonas* spp., *Erwinia* spp. and *Bacillus* spp. The main epiphytic bacteria of the genus *Pseudomonas* belong to saprophytic bacteria *P.fluorescens*. Besides we isolated the agent *P. syringae* pv. *atrofaciens*, which does not differ in biological properties from *P. s. pv.atrofaciens*, isolated from the affected tissue of wheat. Epiphytic strains of *P.s. pv. atrofaciens* at the artificial infection are pathogenic for wheat.

Thus epiphytic microflora of seeds and growing wheat plants includes *P. agglomerans*, *P. fluorescens*, *P.s. pv. atrofaciens*, and also bacteria of the genera *Erwinia* and *Bacillus*.

PÁSZTI, J.¹, I. GADÓ¹, V. G. LÁSZLÓ², B. NAGY³, J. KIRÁLY¹, P. KOPPÁNY¹, L. ORBÁN¹, H. MILCH¹

Occurrence and spread of multiple resistant *Salmonella enterica* sv. *Typhimurium* strains in Hungary

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The number of publications reporting the occurrence of strains resistant to one, two, three or more antibiotics increased from the middle of the nineties. The majority of the reports on the rising tendency of multiple resistance concerned mainly the strains of animal origin. It was observed, that most of these strains belonged to DT104. The Hungarian National Salmonella Reference Laboratory collected the data on the sensitivity to antibiotics and the distribution of phage types (PT) of *S. typhimurium* of human origin, since 1960. According to our data multiple resistance occurred

more frequently from 1996 onwards, the increase continued in 1997 and it reached 55%, in 1998. The distribution of phage types was also examined using the method of Felix-Callow. On the basis of statistical analysis a change was found in the distribution of phage types. Parallel with the higher incidence of multiple resistance the ratio of PT-s 2 and 2c increased. There was no consequent changes in the level of PT 35 and PT 4 and Nt (not typable) decreased. Phage types of 45 strains were compared using Felix-Callow's and Anderson's methods. All of the strains of PT 2 and 2c belonged to DT 104, famous in the literature. There was no such between other Felix-Callow phage types and DT 104.

PATTERSON, M. F.^{1,2}, M. LINTON¹, J. M. J. McCLEMENTS¹

High pressure processing of foods for microbiological safety and quality

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Consumers are demanding foods that are "natural", of good nutritional and sensory quality, free from chemical preservatives, microbiologically safe and with extended shelf-life. High pressure processing can, potentially, meet these criteria. Recent advances in equipment design now allow foods to be processed up to 900 MegaPascals (130,000 psi). However, further work is required to more fully understand the factors that can affect the response of micro-organisms, including pathogens, to pressure so that treatments can be optimised and microbiological safety can be assured. This presentation will describe how the pressure resistance of micro-organisms can vary depending on factors such as species, strain, stage of growth and food composition. Strategies for overcoming the problem of pressure resistance will be discussed, for example the use of pressure cycling and the combination of pressure with mild heat. The current commercial uses of high pressure to preserve foods will be reported and potential applications will also be discussed.

PENYIGE, A.¹, Gy. BARABÁS²

The involvement of GTP-binding proteins in triggering morphological differentiation and antibiotic production in *Streptomyces griseus*

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Previously we have shown that *Streptomyces* strains possess GTP-binding proteins (GBPs). Stimulation of GBPs lead to restoration of sporulation and antibiotic production in a bald mutant of *S. griseus*. In a search to identify possible signal transduction pathways triggered by stimulated GBPs, we have found two possible targets in the bald mutant.

(i). The activity and substrate specificity of the ADP-ribosyltransferase (ADPRTase) enzyme was altered as a consequence of stimulation of GBPs. ADPRTase is responsible for ADP-ribosylation - a posttranslational modification - of proteins. Previously this enzyme was shown to be involved in the regulation of sporulation in *S. griseus*.

(ii). Stimulation of GBPs causes an immediate depolarization of the membrane potential in the bald mutant. This electric signal might regulate voltage-gated ion channels in the cellular membrane of cells. We have found that the flux of Ca^{2+} ions was affected by the state of membrane potential. It is well known that the intracellular Ca^{2+} concentration is an important factor affecting the differentiation of *Streptomyces* species. Therefore we suggest, that modulation of the membrane potential serves as a regulatory signal during the morphological differentiation process.

Interestingly, we have also found that A-factor potentiates the effect of GBPs.

PÉTERFI, Z., B. KOCSIS

Optimisation of ELISA test used for detection of serological cross-reaction between lipopolysaccharides

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Enzyme-linked immunosorbent assay (ELISA) is probably the most frequently used method for estimation of antibodies. In case of lipopolysaccharide (LPS) antigens their poor coating to microplate is a problem. Takahashi et al. described a good method for coating LPS antigens using poly-L-lysine for pre-coating. Another problem of the ELISA method is the non-specific binding of antibodies to the plastic wells. To reduce this disadvantageous phenomenon, blocking agent, such as bovine serum albumin, casein is commonly used. We have to choose the blocking agent carefully because unfortunately LPS can bind proteins aspecifically. This process can inhibit LPS-specific antibody activity and diminish the sensitivity of ELISA test. In this poster we present an ELISA for LPS in which normal goat serum is used for blocking instead of bovine serum albumin and casein. Goat serum gave statistically significantly lower OD value for negative control and statistically significantly higher OD values for positive control. Our study on *Shigella sonnei*, *Escherichia coli* and *Proteus morgani* lipopolysaccharides demonstrates that this type of ELISA using goat serum for blocking is the best method not only for detection of LPS and anti-LPS antibody reaction, but in crossreaction study too.

PICEK, T.¹, R. TYKVA², H. Š ANTRUČKOVÁ¹, M. SIMEK¹, B. PAVLU²

Glucose decomposition in soil after change of aeration status

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Soil was pre-incubated in oxic and/or anoxic conditions, respectively, for 10 days. Then ^{14}C -labeled glucose was added, aeration status was changed from oxic to anoxic and *vice versa* and soil was incubated for next 3 days. Soils incubated in permanently oxic and in permanently anoxic conditions were used as controls. Oxic incubation was performed in continuous flow system in a flow of CO_2 -free air and anoxic incubation was carried out in static conditions in O_2 -free N_2 . Glucose consumption, $0.5\text{ M K}_2\text{SO}_4$ extractable C (C_{EXTR} ; $^{14}\text{C}_{\text{EXTR}}$), C in microbial cells (C_{MIC} ; $^{14}\text{C}_{\text{MIC}}$), ^{14}C incorporated into polymeric compounds ($^{14}\text{C}_{\text{POLY}}$), CO_2 and $^{14}\text{CO}_2$ were measured 0, 8, 16, 24, 48 and 72 hours after glucose addition. If soil was incubated in **oxic conditions** all glucose was consumed within 72 hours and no effect of oxic and/or anoxic pre-incubation on ^{14}C distribution was observed. Thus at the end of experiment about 25 % of ^{14}C consumed were found in $^{14}\text{CO}_2$, 1.5 % in $^{14}\text{C}_{\text{EXTR}}$, 16 % in $^{14}\text{C}_{\text{MIC}}$ and about 56 % in $^{14}\text{C}_{\text{POLY}}$ fractions. However, mineralization of soil organic C and its assimilation into microbial biomass were enhanced when anoxic conditions were changed to oxic as compared to permanently oxic incubation. If soil was incubated in **anoxic conditions**, glucose consumption was strongly inhibited by oxic preincubation, as only 35.8 % of added glucose were consumed within 72 hours; 51.4 % of consumed glucose C were evolved as $^{14}\text{CO}_2$, 4.5 % were found in $^{14}\text{C}_{\text{EXTR}}$, 23.2 % in $^{14}\text{C}_{\text{MIC}}$ and 20.9 % in $^{14}\text{C}_{\text{POLY}}$ fractions. If soil was incubated in permanently anoxic conditions, all glucose was consumed within 72 hours; 30 % of consumed glucose C were found in $^{14}\text{CO}_2$, 27.5 % in $^{14}\text{C}_{\text{EXTR}}$, 8.4 % in $^{14}\text{C}_{\text{MIC}}$ and 34.1 % in $^{14}\text{C}_{\text{POLY}}$ fractions. No significant effect of pre-incubation on mineralization and assimilation of soil organic C in anoxic conditions was observed.

The work was funded by the Grant Agency of the Academy of Sciences of the Czech Republic (Project No. A6066901) and by Ministry of Education (Project No. 136/99).

POSTA, K.¹, G. BAKONYI², I. KISS², M. FÁBIÁN², P. NAGY

Density dependent regulation of arbuscular mycorrhizal fungi by fungivorous collembolan

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Arbuscular mycorrhizal (AM) fungi increase the plant nutrient uptake under different circumstances. Enhanced phosphorus uptake by AM plants compared to that of non-AM plants is one of the best known among related phenomena. Soil fauna can affect AM fungi directly by grazing. This influence could result in positive or negative effects on plant nutrient uptake.

One reason of these results may be the density dependence of this interaction. A field experiment was set up to test whether collembolan density can have a direct impact on the number of spore, hyphal length and colonization of *Glomus mosseae*

on maize plant.

In addition, collembolan influence on the number of fungi and *Trichoderma* spp. was studied to see whether fungivorous collembolan predominantly graze on AM hyphae or other fungal hyphae.

A clear and strong density dependence was found regarding the number of spore and colonization of AM. Collembolans in low density enhanced the number of spore and colonization of mycorrhizal fungi, while in greater numbers decreased these parameters. No such correlation was observed with respect to hyphal length. *Trichoderma* spp. density showed opposite trend. Lowest values were found at moderate collembolan density.

PRILLINGER, H., W. SCHWEIGKOFER, K. LOPANDIC

Systematics of Asco- and Basidiomycota based on cell wall sugars, 18S rDNA sequences and urease activity

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Different from the morphological systematics, where commonly bipartite systems (e. g. Basidiomycota: Hetero- and Homobasidiomycetes; Ascomycota: Hemi- and Euascomycetes) prevail, tripartite systems dominate, when molecular characteristics like the qualitative and quantitative monosaccharide pattern of purified cell walls or partial or complete sequences of ribosomal DNAs are used. Based on cell wall sugars and complete 18S ribosomal DNA sequences the Basidiomycota are divided into three classes: the Urediniomycetes, the Ustilaginomycetes and the Hymenomycetes. Similarly three classes are found in the Ascomycota: the Hemiascomycetes, the Protomycetes and the Euascomycetes. Within the Ascomycota cell wall sugars can only be used to show that the Protomycetes are a sister group of the Euascomycetes. The presence of urease activity and the ultrastructure of septal pores are additional characters which suggest a sister group relationship between the Protomycetes and the Euascomycetes. The Hemiascomycetes occupy a basal position to this sister group. Morphological and ultrastructural data of *Mixia osmundae* (Nishida et al., Can. J. Bot. 73 (suppl.1): S660-S666), cell wall sugars of *Taphrina vestergrenii* (Prillinger et al. Z. Mykol. 56: 219-250, 1990) and 5S ribosomal DNA data from Gottschalk & Blanz (Z. Mykol 51: 205-243, 1985) and Walker (System. Appl. Microbiol. 6: 48-53, 1985) suggest the Protomycetes to be ancestral to the Euascomycetes and the Urediniomycetes of the Basidiomycota. Although yeasts predominate within the Hemiascomycetes, yeasts or yeast stages occur within all classes of the Ascomycota and the Basidiomycota. Within the Basidiomycota a polyphyletic origin of the smut fungi, the non-gilled Hymenomycetes, the gilled mushrooms and the "Gasteromycetes" becomes obvious. Within the Ascomycota the "Plectomycetes" as indicated by the Erysiphales, the bitunicate Ascomycota ("Loculoascomycetes", black yeasts) as indicated by the Chaetothyriales, and the Ophiostomatales appear heterogenous. Based on sequence information of the ribosomal DNA there is no need for the artificial group of the Deuteromycetes.

RAINEY, F. A.

Ionising radiation: a selective enrichment tool in culturable diversity assessment

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Our limited knowledge of the diversity of members of the domain Bacteria has been demonstrated using the culture-independent approach. Soon more taxa will exist in the form of inserts in a plasmid and as 16S rRNA gene sequences in a database than as pure cultures in culture collections. Investigation of the characteristics and function of such environtaxa is not possible. The microbial diversity of various environments have been studied to different degrees, some extensively, others poorly. Arid and hyper-arid soils are an example of environments about which little is known of the microbial components and their function. With the aim of expanding our knowledge of the culturable diversity of arid soils, and further investigate the link between ionizing radiation and desiccation resistance, we exposed soils to various doses of gamma ionizing radiation, ranging from 0.1 and 3.0 MRad.

Plating of these soils on routine culture media allows us to determine the numbers and the phylogenetic diversity of the survivors obtained at all doses. Using this approach we have been able to add novel species to already existing genera and to discover new genera. This selective enrichment technique has added at least eight additional species, to the classical ionizing radiation resistant genus *Deinococcus*, six of these coming from a single soil sample. Many of the survivors belong to the single species genus *Geodermatophilus* and their isolation has expanded this genus, at both the species and strain level. Strains representing novel genera within the cytophaga group and alpha-proteobacteria lineages have also been isolated. The fact that ionizing radiation eliminates fast-growing and potentially inhibitory competitors within the microbial community, allowing the novel taxa to proliferate has made this isolation strategy successful. This study has provided novel strains and species of existing genera, and demonstrated that ionizing radiation resistance may be more prevalent across the domain Bacteria than was previously thought.

RAJČÁNI, J., I. VOJVODOVÁ, I. ORAVCOVÁ, M. KÚDELOVÁ, J. KOŠOVSKÝ, J. MATIS

Characterisation of strain HSZP of herpes simplex virus type 1 (HSV 1)

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RAJNAVÖLGYI, É.¹, A. HORVÁTH¹, N. NAGY², Á. SIMON¹, I. K. FALK², I. ERNBERG², É. KLEIN²

The role of Cd4+ T-lymphocytes in viral infections

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Major histocompatibility complex (MHC) class II-restricted CD4+ T-lymphocytes are major regulators of immune responses. Besides delivering cognate help for antigen-specific B-lymphocytes they are able to condition dendritic cells for efficient antigen presentation and support the generation, differentiation and survival of CD8+ cytolytic T-lymphocytes (CTL). CD4+ T-cells may exert cytotoxic activity mediated by the Fas/FasL or by the tumor necrosis factor (TNF) receptor/TNF interaction. Their other effector functions are related to the secretion of a wide array of cytokines. These mechanisms are involved in the down regulation of the virus-specific immune response when the pathogen is eliminated and also in the maintenance of sustained antibody and CTL memory.

In our studies the protective role of CD4+ T-lymphocytes, directed against a subdominant influenza hemagglutinin (HA) epitope not affected by antigenic drift, was demonstrated in BALB/c mice. Repeated injections with linear or branched synthetic peptides, comprising the CD4+ epitope and an inscribed in B-cell determinant, resulted in the activation of peptide-specific CD4+ T-cells as well as in the production of peptide-specific antibodies. Peptide preimmunization elicited a subtype cross-reactive immune response and conferred enhanced protection against lethal oral reinfection. Our results demonstrate that activation of functionally relevant CD4+ T-lymphocytes can generate efficient anti-viral memory.

In the human system promiscuous, MHC class II-restricted CD4+ T-cell epitopes were identified in a repetitive region of the Epstein-Barr virus (EBV) nuclear antigen-6 (EBNA6). The majority of EBV carriers produce antibodies to this region which encompasses multiple overlapping core regions with binding capacity to a group of related HLA-DR molecules. Peptide-specific CD4+ T-cells, isolated from EBV seropositive individuals, could also recognize B-lymphocytes which expressed EBNA6 provided they were MHC-matched. Opposing the strict MHC class I-restricted recognition of EBV-infected cells by CD8+ T-lymphocytes these results suggest that memory CD4+ T cells are focused to such regions of latent viral antigens which can be recognized and maintain a sustained CTL and antibody memory in many individuals. These CD4+ T-cells may also be important for the development of CD8+ CTL which recognize latently infected EBNA6-expressing B-cells which have the capacity to proliferate and are controlled by the cellular immune response.

RASPOR, P.

The influence of chromium compounds on yeast physiology

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Successful, ionic nutrition is related to the metals biosorption and bioaccumulation which are important and essential for all living creatures. Selective advantage are

confirmed for those species which are capable of accumulating and retaining those components which are present in limited, trace amounts in surroundings. Uptake of metal ions from the environment is mediated by biosorption and bioaccumulation mechanisms. The uptake starts with fast metal biosorption on the cell wall and then continues with slower bioaccumulation. Bio-accumulation is generally defined as active mode of metal accumulation by living cells. Tolerance of particular organism to particular metal ion may reflect the ability of an organism to survive in an environment with a high concentration of metals, or to store it. This can be due to an ability to control the intracellular concentrations of the ion in the range which is not toxic to its metabolic processes. Effect of chromium on yeasts growth and the problem on metal ions toxicity and/or resistance to it is discussed through new model for evaluation. For first screening the agar diffusion test is shown to be essential followed by advanced procedure for precise measurement of yeast viability or mortality. Values for pH, temperature, a metal's biological availability, etc. are considered as one of the most important environmental parameters in the mechanism of metal ion translocation. The metal ion's uptake is essentially a biphasic process consisting of a metabolism-independent and metabolism-dependent step. The initial biosorption step for metal ions is rapid. In the case of chromium we found at different starting pH values in yeast *S. cerevisiae* correlation between pH and chromium (III) accumulation in the pH range from 2 to 6. Under the experimental conditions applied, the cell surface deposition capacity for chromium increased with the pH. During cultivation in batch operation mode, the high inhibitory effect of supplemented chromium (III) in the media on yeast growth was observed during prolonged lag phase and consequently reduction of biomass yield. The toxic effect of chromium ions was found during continuous cultivation in biomass, protein and total cell RNA reduction. During the cultivation of *C. intermedia* in combined batch/fed-batch mode favourable chromium ratio in biomass was achieved, as well as higher yeast cell capacity in transformation of accumulated chromium into organic fraction. On the contrary, higher biomass accumulation in batch cultivation mode supported in higher total chromium accumulation capacity. The high concentration of chromium in the environment always causes a reduction of RNA and protein concentrations on the cellular level. On the macro scale, chromium causes a reduction of growth rate and biomass production, and an extension of lag phase. In *S. cerevisiae* organic compounds with a molecular weight from 100,000 to 10,000 showed the highest intracellular chromium binding capacity. The diversity of intracellular organelles and biomolecules provides a wide range of potential binding sites. When a higher concentration of chromium was present in the environment, a 47 times higher amount was found in the yeast biomass. The distribution was found in favour of organically bound chromium. Speciation of organically bound chromium by a molecular sieve showed that the organic substances with a molecular weight of between 100,000 and 10,000 expressed a high binding capacity to a chromium ion. Similar results were obtained in yeast biomass *C. intermedia* isolated as intracellular low-molecular-weight chromium-binding polymers.

RAZAVILAR, V., S. SHEKARFOROUSH

Factorial growth of *Clostridium perfringens* as affected by temperature, salt, pH, acid type and storage time

REES CED, CER DODD, P.J. HILL, W. M. WAITES

Development and use of techniques for studying microorganisms in situ in natural and man-made environments

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Most studies of microorganisms have been of growth of single organisms in suspension in liquid media in laboratory conditions. However, in both man-made and natural environments, microorganisms grow in mixed culture and are often not planktonic but sessile. There is now a large amount of evidence which shows that microorganisms growing attached to surfaces form biofilms, where, compared with planktonic cells, cells are more resistant to a number of external forces including drying, antibiotics, chemical disinfectants, bacterial viruses and even heat. In order to study such organisms, techniques which allow their numbers and their physiology and biochemistry to be examined *in situ* without perturbation need to be developed.

Our studies have used the green fluorescent protein from *Aequorea victoria* and the *lux* gene system from *Photobacterium luminescens* as marker and reporter genes, as well as 16S rRNA-directed oligonucleotide and antibody-linked probes to differentiate between different species and between their surface components. Using such methods, coupled to molecular biology and image analysis techniques, we have demonstrated that, compared with the flagellated wild type, a mutant of *Listeria monocytogenes*, which does not produce flagella, shows a much reduced ability to attach to stainless steel surfaces. We have also been able to detect the sites of growth of *Salmonella in situ* within whole eukaryotic organisms including plants and mice.

These and other examples of such innovative techniques will be discussed in order to demonstrate that the methods are now available which will allow mixed cultures of bacteria to be studied *in situ* in both natural and man-made environments.

RESKONÉ, M. N.¹, A. BORSODI²

Long-term monitoring of sulphur cycle associated bacteria in Lake Velencei

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Lake Velencei is a shallow lake which has eutrophicated in a natural way. The most studied period of the lake was in the past one and a half decades. The shallow swampy lake has changed considerably due to the basin recreation. The once reed-covered lake remained undisturbed only on one third of its area after 1983. As a

result of the basin recreation the formerly unique diversity of the natural habitats has changed or vanished. Today the lake consists of two, well-separable regions where the reed-covered area with small water surfaces has conserved the sulphuretum characteristics of the lake.

The dry period beginning in the late 80's has triggered off several limnological problems still unsolved. The low water level after 1991 caused a shift in the ecological balance of the lake and considerable change was observed in the water quality (concentration of salts, oxygen-, temperature-, light conditions, etc.).

The major part of the organic materials arriving with the water supply is being decomposed here.

The microbial processes of sulphur cycle associated bacteria in connection with sulphate cycling were studied between 1993 and 1998 in order to interpret the ecological modifications. We have compared and according to chemical characteristics valued the sulphuretum-like bacteriological results obtained from cultivating these bacteria from the water and sediment.

Tracking the changes in the distribution of anaerobic bacteria in the water and sediment it was found that their numbers decreased and became uniform in the water of the lake, whereas in the sediment no such significant changes were observed.

With the elevation of the water level the examined representatives of aerobic and anaerobic groups have reappeared. Different regions can be characterised and compared by the ratio of these two groups. The investigation of the quantitative changes of typical sulphur based microbial communities a suitable method was attained for the long term monitoring of the ecological state of the lake.

RIFAAT, H. M., K. MÁRIALIGETI, G. KOVÁCS

Comparative analysis of actinomycete communities of cattail and papyrus rhizoplane

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Wetlands play an especially important role in diverting plant material towards fossilisation. These habitats act as sinks for the atmospheric carbon, since mineralisation of organic materials is inhibited by the effects of the specific local N and S cycles, the anaerobic environment, by low pH value, etc. Therefore it is not surprising that microbial activity was detected to be highest in the rhizosphere of wetland plants.

Cattail samples from a floating mat in the Soroksár Arm of the River Danube and papyrus samples from a floating mat in the River Nile were collected. The "root-hair" regions of the samples were cut off, washed with aseptically prepared solutions and plated. Using the plate-count technique with three different media suited for the

cultivation of actinomycetes, in the case of cattail, an average of 10^3 CFU/g was detected whereas papyrus germ count values were 10^4 CFU/g. All actinomycete colonies were isolated, subjected to purification and differential diagnostic analysis (phenotypical test and partial 16 S rDNA sequencing). Samples taken from the cattail rhizoplane of a floating mat community at Soroksár arm contained approximately equal "amount" of monosporic and polysporic "actinomycetes", whereas monosporic actinomycetes were mostly absent from the papyrus samples. In the former case the dominant actinomycetes were *Str. anulatus*, *Str. albidoflavus*, *Str. rochei*, *Micromonospora chalcea* and *M. carbonacea*. In the papyrus rhizoplane *Streptomyces anulatus* and *M. carbonacea* dominated.

The ecological tolerance abilities of the members of dominant groups revealed during laboratory investigation indicate that these bacteria might be active in the rhizosphere and can be present there in their vegetative forms.

RIGÓ, K.¹, J.TÉREN², J. VARGA¹

Ochratoxin contamination and decomposition caused by *Aspergillus* species

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ROMANO, P.

Selection of starter cultures for winemaking

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Collections of *Saccharomyces* wine strains from natural fermentations have demonstrated a strong strain diversity and the different strain specific patterns can be presumed as typical for each fermentation. In recent years data have been accumulated which indicate that the characteristics of the yeast strains used in wine-making play an important part in wine quality. Therefore, the use of the same strain for fermenting different types may be non-appropriate, due to a potential uniformity of aromatic characteristics in the final products. In order to typify each product for the varietal and geographic characteristics, it becomes useful to isolate natural autochthonous strains, which, in addition to the desirable technological characteristics, can exhibit a metabolic profile corresponding to each wine.

Bearing this in mind, we have developed a methodological approach for selecting strains which are more appropriate to the standard individual characteristics of each wine. This approach is based on the characterisation of indigenous wine *S.cerevisiae* strains for technological traits and the results for each strain are correlated to quality and individual determinants of the wine. Successively, the selected cultures should be tested for the genetic segregation of technological traits in order to identify strains completely homozygous for the characteristics considered.

In this report thirty strains of *S.cerevisiae*, isolated from different Aglianico grape musts, were tested for the production of secondary compounds. A considerable phenotypic variation was found, confirming the environmental role of natural yeasts and the importance of individuating the main and desirable traits, that the selected culture must possess to preserve the individual characteristics of that specific product.

Only 4 strains, exhibiting a metabolic profile corresponding to the individual Aglianico wine characteristics, were selected and underwent to genetic analysis. All the strains were homozygous for the homothallism gene, whereas most single spore cultures, tested for the production of secondary compounds, were heterozygous from one to more metabolic characteristics.

The application of this strain selection approach allows to obtain a final product characterised by the desired aromatic profile, consistent with flavour-determinants which are typical of each wine.

ROS, C., S. BELÁK

Bovine herpesvirus types 1 and 5, caprine, cervine and rangiferine herpesviruses types 1: studies of genetic relationship and improved molecular methods for detection and identification

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The glycoprotein B and D genes have been partially sequenced from five ruminant alphaherpesviruses, bovine herpesvirus 1 (BHV-1), bovine herpesvirus 5 (BHV-5), caprine herpesvirus 1 (CapHV-1), cervine herpesvirus 1 (CerHV-1) and rangiferine herpesvirus 1 (RanHV-1). The nucleotide sequence alignments revealed a highly conserved gB gene, with homologies ranging between 87.2% to 99.6%, and a more variable gD gene, with homologies ranging between 71.3% to 98.9%. The phylogenetic analysis of the gB and gD nucleotide and deduced amino acid sequences revealed that BHV-5 is the most closely related virus to the BHV-1.1/BHV-1.2 cluster and CapHV-1 is the most distant. The phylogenetic data showed a close relationship of all the studied viruses with suid herpesvirus 1.

Based on the sequence data from the gB gene, a nested PCR combined with restriction enzyme analysis (REA) of the PCR products has been developed for the simultaneous detection and identification of the studied viruses. Nested primers have been selected from highly conserved sequence stretches in order to amplify a region of 294-bp in all the five viruses, and a subsequent REA of the PCR products allowed the specific identification. A mimic molecule has been constructed to serve as internal standard of the amplification efficiency. The practical diagnostic applicability of the assay has been evaluated on clinical samples consisting of semen and organ specimens from experimentally infected animals.

ROSENGARTEN, R., C. CITTI

Host-pathogen interactions in *Mycoplasma* pathogenesis: immune evasion and

exploitation of host cells

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The genus *Mycoplasma* belongs to a group of prokaryotes which are the smallest and simplest self-replicating organisms, of which many species are known etiologic agents of disease in man and animals. They are characterized by (i) their lack of a cell wall, therefore emphasizing membrane surface proteins as the key components for a variety of functions involving host interactions and immune avoidance; (ii) their small genome with a limited coding capacity that makes them "minimal cells" which are dependent on the supply of nutrients from their in-host environments; and (iii) the apparent paucity of recognizable components that regulate gene expression in response to environmental changes. All of these features have recently been confirmed by the complete genome sequence of two mycoplasma species, namely the human pathogens *Mycoplasma genitalium* and *Mycoplasma pneumoniae* (1). From an evolutionary point of view this enormous reduction of genetic information that precludes several conventional metabolic pathways must have led to the obligate parasitic mode of life of these organisms. The parasitic lifestyle must have forced the mycoplasmas to maintain a high number of genes devoted to attachment and to exploit diverse mechanisms of genetic and phenotypic variation as a strategy for survival and adaptation to the microenvironmental changes encountered in the host, including those through adaptive immunity. In fact, the molecular characterization of genetic mechanisms directed towards evasion of the host immune system is one of the "hot" subjects of current mycoplasma research. Several recent studies in this area indicate that high-frequency, reversible mutations affecting both the structure (size) and the expression of abundant membrane surface proteins may be widespread among different pathogenic mycoplasma species, which underscores their importance as a means of governing key functional aspects of these organisms. Although the functional consequences of this mutation-based structural variation, phase variation or antigenic variation are still poorly understood, the impact of these variations imposed by multiple types of mutations associated with individual genes may be considerable and may contribute in many ways to the survival, propagation, and virulence potential of a pathogenic mycoplasma species. Recent interesting findings that some species of pathogenic mycoplasmas are capable of active invasion into non-phagocytic host cells will open new ways for defining the role of genetic variation in mycoplasma-host cell interactions and will provide new insights into the molecular events of mycoplasma pathogenesis.

ROZGONYI, F., Á. GHIDÁN

The effect of iodine polyvinylpyrrolidone (Betadine®) on multiple antibiotic resistant bacteria and *Candida albicans*

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The bactericidal and fungicidal effects of Betadine at different concentrations were examined on 10^5 and 10^9 microorganisms/ml using a plate count method and a biophotometric measurement. Betadine was bactericidal and fungicidal even in 100-fold dilution, i.e. 0.1% concentration on methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*, extended spectrum beta-lactamase producing *Klebsiella pneumoniae* and fluconazole-resistant *Candida albicans* strains. A 500-fold and a 1000-fold dilutions suspended the bactericidal and fungicidal effects, however, a bacteriostatic and fungostatic effects still existed for a longer period of time. The results indicate that using Betadine at proper concentrations to disinfect skin and mucosal surfaces prevents colonisation of multiple-resistant bacteria and *Candida*. Betadine at lower concentrations may delay the multiplication of such microorganisms consequently the infections may not take place.

RYCHLIK, I.¹, L. CARDOVA¹, A. SVESTKOVA¹, G. MARTIN², U. METHNER²

Interactions of stationary cultures of *Salmonella typhimurium* F98 and its defined mutants

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Currently used live attenuated vaccine strains of *Salmonella typhimurium* in poultry are less microbiologically competitive and does not protect very young chicken against infection till protective immunity 10 to 14 days post immunization is raised. Therefore we screened more than 3000 Tn mutants of *Salmonella typhimurium* to identify genes involved in microbiological competition which occurs soon after reaching stationary phase of growth. Mutants identified belonged to i) general regulators (*oxrA*, *oxrG*, *crp*, *cya*, *arcA*), ii) respiration (*nuoG*, *cydA*, *uncH*), and iii) chemotaxis (*fliD*, *fliM*). Further mutations occurred in *ompC*, *ompD*, *tdcC*, *aroA* and *aroD*. All the mutants were characterised by their biochemical activities, flow cytometry and their ability to restore the wild type phenotype by the addition of homoserine lactone and related amino acids. Finally, defined mutations (*ompC*, *asd*) and *luxAB* fusions (*rpoS*, *sdiA*, *gyrA*) in selected genes were created to assess the events in *Salmonella* cells entering stationary phase of growth. Most of the interactions can be explained by differences in nutrient uptake and utilization, however cell-to-cell signalling cannot be excluded as well. All the findings can be utilised in new vaccine development.

This work has been supported by the grants from the Grant Agency of the Czech Republic no. 524/98/1089 and the Czech Ministry of Education no. ME077.

TALESKI, V.¹, T. L. HADFIELD², S. STOJKOVSKI¹

Human brucellosis in the Republic of Macedonia and current diagnostic possibilities

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Brucellosis is a typical zoonoses, caused by bacteria of genus *Brucella* that belongs to risk group III of laboratory hazards and present a potential biological weapon.

The epidemic and epizootic of brucellosis in the Republic of Macedonia started in 1980. A total of 7337 cases of human brucellosis were documented until the end of 1998, with average morbidity of 18,3/ 100 000 (highest rate of 44,2/100 000 in 1992). The disease is markedly seasonal with lowest morbidity in December and highest in May and June. According to the questionnaires 34 % of patients were infected by direct contact, 23 % by alimentary way and 43 % buy both, including aerosols. The patients were male in 65 %, female in 35 %, citizens in 20 % and peasants in 80%. The highest percentage of disease in animals was reported in 1992, when out of 1 163 000 examined herds of sheep and goats (source of infection), 6890 (0,6%) were infected. *Brucella melitensis* biotype-2 was recognized as the etiological agent.

The diagnosis of human brucellosis is mainly based on the clinical features of the disease and the classical serological tests: Rose Bengal - Slide Agglutination Test, Wright - Serum Agglutination Test in Tubes, Coombs – Antihuman Globulin Test, CFT- Complement Fixation Test and 2-Mercaptoethanol Test. Our two comparative studies pointed out the significant superior sensitivity and specificity of competition ELISA (c-ELISA - CVL, New Haw, Addlestone, UK) and ELISA (NOVUM-Diagnostica) than the classical serologic tests, especial in diagnosis of chronic brucellosis. The bacteriological isolation and identification is not implemented, since no adequate laboratories exist in Republic of Macedonia. Primary isolation and identification of *Brucella spp.* is difficult, since all species are slow growing and fastidious. Molecular diagnostic techniques, based on PCR, allow to overcome bacterial isolation and identification, by in vitro amplification of the DNA- target, and therefore has the potential to result in a quicker (one day only) and more reliable diagnosis of *Brucella* infection. The first PCR results of our collaborative study with AFIP, in diagnosis of human brucellosis by PCR from blood, were four positive in ten. Mononuclear cells were separated by Becton Dickinson's VACUTAINER CPT Cell Preparation Tubes with sodium citrate. PCR reactions were designed around the insertion sequence IS711 and upstream genes for the *Brucella* species. The testing was done on a Perkin Elmer DNA sequence detector model 7700. Thermal cycling master mix consisted of 200 uM dNTP's, UNG, 50 uM Mg⁺⁺, Taq Gold, forward primer and reverse primer and probe. Thermal cycling conditions were 10 minutes at 94°C, followed by 40 cycles of 94°C for 20 seconds and 60°C for 60 seconds (2 step PCR).

TARJÁN, V., E. UJHELYI

HIV, hepatitis B and C status of Hungarian drug addicts

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TIGYI, Z., T. PÁL

Relationship between the sensitivity to *Shigella sonnei* colicin type 7 and the presence of the invasion plasmid in enteroinvasive *Escherichia coli*

Department of Medical Microbiology and Immunology, University Medical School, Pécs, Hungary

TIMOSHOK, N. O., N. I. GRABCHENKO, N. Ya. SPIVAK

Synergetic action of recombinant interferon - γ and tumour necrosis factor - α on the process of experimental *Staphylococcus* infection

Zabolothy Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kiev, Ukraine

It is known that cytokines (IL-1, IL-6, Interferon, TNF- α etc.) play an important role in an organism defence under bacterial infection. Antibacterial efficiency of these preparations is displayed under intensifying of microbe elimination out of organism, rising of phagocyte activeness and oxidizing metabolise of phagocytes. It is mentioned in a number of works that IFN themselves or TNF as the only stimule are not enough for activation of antibacterial microphages functions. As a result of it has been studied the combined action of recombinant preparation IFN- γ and TNF- α on staphylococcus infection process. It is marked the sinergenic action in suboptimal dozes of r-TNF- α and r-IFN- γ on mice process infection.

The momentary aspect introduction of preparations led to reducing of a number of persisting staphylococci in animals kidneys. At the same time the double introduction of these preparations with the interval of 48 hours between the introduction of it, increased considerably microbes elimination in comparission with controlled animals. Reinforcement of staphylococci elimination out of organism is correlated with raising of macrophage activity.

TÓTH, B.¹, Zs. HAMARI², Zs. BEER¹, F. KEVEI¹

Detailed physical and functional maps of mitochondrial DNAs of an *Aspergillus niger* and an *A. tubingensis* strain and their interspecific recombinants

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Mitochondrial DNA (mtDNA) polymorphisms are frequently observed among imperfect black *Aspergilli*. Strains of the *Aspergillus niger* species aggregate can be divided into three groups based on their nuclear and mitochondrial DNA RFLPs. MtDNA RFLP types 1, 2 and 3 correspond to ribosomal DNA types I, II and III. These groups equal three different species, *A. niger*, *A. tubingensis* and *A. brasiliensis*. MtDNA types 1 and 2 consist of several subgroups (1a-1e, and 2a-2f, respectively). Successful mitochondrial transfers were carried out between incompatible strains possessing different mtDNA patterns by selecting for the oligomycin resistance carried by the mtDNA of the donor parent. Intraspecific transfers resulted in a single type of recombinant RFLP profile, while interspecific transfers resulted in recombinant progenies with highly variable mtDNA RFLP patterns. The 1a → 2b interspecific transfer gave most variable recombinant mtDNA patterns. Sizes of the observed recombinant mtDNAs also varied, some of them were smaller than the parental mtDNAs, while others harboured larger mtDNAs than the parental strains.

Physical and functional maps of the parental strains and intraspecific recombinants were constructed, and the homologous fragments which exhibited size differences between the parental strains and the recombinants were cloned and sequenced in order to identify the region where the recombination event took place.

TÓTH, E., K. MÁRIALIGETI, L. HAVASI, I. S. MOKHTAR

Comparative studies on the bacteriology of wound myiasis of sheep caused by *Wohlfahrtia magnifica*

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In the last years the bacteriology of traumatic wohlfahrtiosis of sheep was studied in detail (the isolated bacteria were identified by using pheno-, geno and chemotaxonomical methods). Comparing the healthy and myiatic regions of sheep it could be demonstrated in general that

1. the number of pyogenic cocci (e. g. *Staphylococcus* sp.) decreased during the development of the maggot containing lesions, later they totally disappeared from the wound
2. the number of gram positive aerobic cocci (*Micrococcus* sp., *Arthrobacter* sp.) also decreased in the wound during the fly development
3. bacteria belonging to the family Enterobacteriaceae could be detected just from the healthy regions.

In the bacterial communities of different developmental stages of the fly there are also characteristic differences:

1. during pupation bacteria belonging to the genus *Proteus* almost totally disappear

2. the number of a characteristic but so far unknown microorganism (going to be a new γ -proteobacterium) also decreased during the metamorphosis of the fly
3. *Bacillus* spp., *Corynebacterium* sp. *Micrococcus* sp., *Arthrobacter* sp. were present in all stages.

Later the biotic interaction among these microorganisms using antibacterial assays as well as some aspects of the significance of these bacteria in the development of *Wohlfahrtia magnifica* were tested. The new group of bacteria together with the *Arthrobacter-Micrococcus* group presumably have an important role in the metamorphosis of the fly as they have strong chitinase activity. It could be also proven that the shifts in the bacterial communities usually were results of collective function of several microbial interactions:

1. the authentic *Staphylococcus* strains as well as those originating from the sheep and the test organisms from the family Enterobacteriaceae were sensitive for compounds produced by *P. mirabilis*, *E. coli*, *Serratia* sp., *A. johnsonii*, *B. diminuta*, *P. stuartii*
2. almost all tested bacteria were sensitive for compounds produced by *Enterococcus faecalis*
3. the new (presumably symbiotic) bacteria and the *Proteus vulgaris* strains were extremely sensitive for the metabolic compounds of lot of other bacterial strains
4. the low number of aerobic cocci in the wound containing third stage fly larvae can be connected mainly to the recurrently appearing anaerobic conditions and possibly not the direct function of other bacteria.

TÓTH, I.¹, Zs. RUZSICS², V. KARCAGI³, B. NAGY¹

Rifampicin – resistance associated mutation in *fliC* flagellar gene of *E. coli* O157:H7

¹Veterinary Medical Research Institute, Hungarian Academy of Sciences, 2"B. Johan" National Centre for Epidemiology,

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E. coli O157:H7 prototype strains 7785 and EDL933 and their spontaneous rifampicin resistant (Rif^r) mutants with reduced motility and non-motile (NM) phenotype were compared by PCR using a primer-pair specific for the variable region and the C terminus of the *fliC* flagellin gene. DNA was amplified from the wild type *E. coli* O157:H7 strains and from K-12 strains, but no amplicon was detected from the corresponding Rif^r mutants analysed by agarose electrophoresis. In Southern blot analysis no polymorphism could be seen among these wildtype and mutant *E. coli* O157 strains when genomic DNA samples of 7785, EDL933 their Rif^r mutants and an *E. coli* O157:NM were digested with either *Bam*HI or *Hin*FI and were probed with the *E. coli* K-12 -specific amplicon. *fliC* -specific amplicon hybridised to a different sized *Bam*HI fragment in *E. coli* K-12 and O157 strains, but to the same sized *Hin*FI fragment as in *E. coli* K-12.

TÓTH, R., I. PFEIFFER, J. KUCSERA

A comparative analysis of the mitochondrial genomes of *Saccharomyces dairensis* strains CBS 421 and CBS 4309

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Saccharomyces dairensis is a member of the *sensu lato* group of the *Saccharomyces* genus, but its exact taxonomic position is debated. The type strain of the species, CBS 421, was isolated by Naganishi, in 1917. The CBS 4309 strain was first described as a separate species, *Saccharomyces castelli*, by Capriotti, in 1967. Later taxonomic studies listed both strains as *S. dairensis*, since distinction was unreliable with conventional methods.

Electrophoretic karyotyping and nuclear DNA hybridization have also been carried out within the *Saccharomyces* genus. In cases of other species, a study of the RFLP patterns of the mitochondrial genomes proved to be a useful tool for determining taxonomic position.

In our study, we compared the RFLP patterns of the type strain of *S. dairensis* (CBS 421) and the CBS 4309 strain with the use of various restriction endonucleases. We measured the size of the mitochondrial genomes, and carried out DNA-DNA hybridization, using heterologous gene probes from *Aspergillus nidulans*, and *Candida parapsilosis*, to determine the location of individual genes.

Examination of mitochondrial mutants is another practical approach in the study of mitochondrial genome organization. Some yeasts remain viable with impaired respiratory functions, and form smaller, 'petite', colonies on solid medium, versus the normal, 'grand' phenotype. Petite phenotype may also result from nuclear mutations.

We successfully isolated petite mutants of both strains using ethidium-bromide induction. According to literature, ethidium-bromide treatment produces mutations mainly on the mtDNA. Deciding whether the isolated petite mutants are cytoplasmic, or nuclear is one of our research aims.

TÓVÁRI, J.¹, I. NÉMETH¹, S. H. M. JEURISSEN², J. M. SHARMA³, T. F. DAVISON⁴, Cs. N. DRÉN¹

Comparative assay of chicken and geese immunoglobulins and lymphocytes

¹Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary, ²Institute for Animal Science and Health, The Netherlands, ³University of Minnesota, College of Veterinary Medicine, USA, ⁴Institute for Animal Health, Compton, UK

TÖMÖRY, Gy.¹, Zs. SZÉNÁSI¹, J. SZABÓ², M. JESZENSZKY³, Zs. OZSVÁR⁴, E. VESZELOVSZKY¹, E. NAGY¹

Analysis of the serological results of *Toxoplasma* screening of pregnant women in Szeged

¹Department of Clinical Microbiology, ²Department of Obstetrics and Gynaecology, ³Department of Infectious Diseases, Municipal Hospital of Szeged, Szeged, ⁴Department of Infectology, Szent György Hospital, Székesfehérvár, Hungary

TÖRŐ, K.¹, Zs. CSUKÁS², L. TÓTH¹, F. ROZGONYI²

Combined effects of pre/postnatal risk factors and microbiological agents in sudden infant death

¹Institute of Forensic Medicine, ²Institute of Microbiology, "Semmelweis" University Medical School, Budapest, Hungary

Objectives: Many pre- and postnatal factors have an important role in the pathomechanisms of sudden infant death. We investigated whether the combined effect of pre/ postnatal effects and bacterial colonization in throat further increase the risk for SIDS.

Methods: Questionnaire-based screening for SIDS risk factors based on family interview and taking samples for microbiological examination was performed among healthy symptom-free infants. The outcome was compared to the history data of SIDS cases. This case-control study included 17 SIDS and 74 controls and was analysed by conditional logistic regression. **Results:** The outcome of our survey showed the infants in favourable social and economic environment / living in crowded housing, infants with low birth weight or twin, and infants exposed to drugs, smoking or narcotics during gestation, having young unmarried mother/ are at risk for SIDS and microbiological agents. The information feedback to the concerned family doctors and health visitors will be the basis for an increased attention paid to the future care of these infants.

Conclusion: Identification of SIDS risk factors in healthy infants could be highly important in the prevention of sudden infant death.

TURNBOUGH, C. L. JR.

Gene regulation by reiterative transcription and transcriptional start site switching

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Recent studies of gene regulation from this lab have elucidated a number of *E. coli* control mechanisms that employ two unusual reactions catalyzed by RNA polymerase: reiterative transcription and transcriptional start site switching. Reiterative transcription is the repetitive addition of a nucleotide (UMP in this case)

caused by slippage between a homopolymeric stretch of nascent transcript and a stretch of (≥ 3) complementary nucleotides in the DNA template. Transcriptional start site switching is the regulated selection of alternative transcript start sites at a particular promoter. One or both of these reactions has been shown to play key roles in pyrimidine-mediated regulation of a number of operons involved in pyrimidine nucleotide biosynthesis and salvage, including the *pyrBI*, *pyrC*, *carAB*, *codBA*, and *upp* operons. Details of these regulatory mechanisms will be described, and the use of these reactions in global gene regulation will be discussed.

UJHELYI, E.¹, E. KORCHMA², V. TARJÁN¹, J. SZABÓ¹, P. VÁGÓ²

HIV, hepatitis B, and C epidemiology in a stomatology department in Hungary

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VARBANETS, L. D., N. V. MOSKALENKO

Interferoninducing activity of *Ralstonia solanacearum* lipopolysaccharides

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Ralstonia solanacearum is a most destructive bacterial pathogen affecting plants in more than thirtythree genera. These bacteria are transmitted via food crops (tomato, potato, sweet pepper and others) to the warm -blooded macroorganisms this way affecting their immune system. So far as lipopolysaccharides (LPS) of gram-negative bacteria are known as classical immunomodulators, interferoninducing activity of *R. solanacearum* ICMP 5712 and 7859 LPS and its structural components were studied. O-specific polysaccharide (O-PS) of *R. solanacearum* ICMP 5712 contained two types of structurally different oligosaccharide repeating units: branched pentasaccharide (70%) and linear tetrasaccharide (30%) with the following structures:

→ 3)-β -D-GlcpNAc-(1→ 3)- α -L-Rhap-(1→ 2)-α -L-Rhap-(1→ 3)-α -L-Rhap-(1→

↑ 4

| 1 1

β -L-Xylp

→ 3)-β -D-GlpNAc-(1→ 3)-α -L-Rhap-(1→ 2)-α -L-Rhap-(1→ 3)-α -L-Rhap-(1→ 2

while O-PS of *R. solanacearum* ICMP 7859 was characterized the structure 3:

→ 3)- α -D-GlcpNAc-(1→ 2)- α -L-Rhap-(1→ 2)- α -L-Rhap-(1→ 3)- α -L-Rhap-(1→ 3)

As the main structural components of the core-oligosaccharide (CO) were observed heptose, 2-keto-3-deoxy-mannooctonic acid, rhamnose and glucose. The chemical identification of lipid A indicate that C_{12:0} (2.5%), C_{14:0} (22.0%), 3-OH-C_{14:0} (35.9%), C_{16:0} (5.9%), 3-OH-C_{16:0} (5.9%), C_{18:0} (1.6%), 3-OH-C_{18:0} (7.1%) were the predominant fatty acids.

The studies indicate that *R. solanacearum* LPS effectively induce the γ -interferon (IN) production (530 U/50/ml) comparable with activity of classical immunomodulator - *Escherichia coli* LPS ("Sigma"). The high levels of γ -IN production were observed in response to lipid A (1070 U/50/ml) and CO stimulation of mice peritoneal macrophages. The O-PS have been shown to be low in interferoninducing activity (320 U/50/ml). In order to estimate the chemical groups which are responsible for interoninducing activity, coordination compounds of the *R. solanacearum* ICMP 7859 LPS and lipid A with germanium were obtained. LPS preserved interferoninducing activity while lipid A modification have led to complete loss of activity. The results of IR-spectroscopy of modified preparations and and interferoninducing activity of a number of synthesized compounds give possibility to suppose the following: phosphate at C4¹ GlcN II can be responsible for interferoninducing activity, while carboxylic groups of CO or O-specific polysaccharides are responsible for that in native LPS molecule.

VARGA, A.¹, W. SOTOKOSTKA-KÖHLER², W. PRESBER², V. von BAEHR³, R. von BAEHR³, R. LUCIUS¹, D. VOLK³, J. MOLNÁR⁵

Interaction between protozoan parasites and cancer cells: *Toxoplasma* infection is able to reverse multidrug resistance of mouse lymphoma and human gastric cancer cells *in vitro*

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Most intracellular parasites weaken their host cell but do not kill it. Our aim was to examine the effect of parasite infection on a multidrug resistant (mdr) tumor cell.

Here we report that an infection of cancer cells with *T. gondii* could reduce the multidrug resistance of the tumor cells against the cytostatic drugs.

Two mouse lymphoma cell lines (Mdr L 5718 and Par 5718) were infected with

Toxoplasma gondii in vitro and the reduction of efflux pump activity of the cells was measured. The drug accumulation (Rhodamine-123) was increased in the infected mdr cell lines compared with non-infected mdr and parental cells, and no effect was shown by infection in the parental cell line. The mdr-1-gene expression was reduced in the infected cell lines 48 hours after the infection. A co-cultivation of *Toxoplasma gondii* with mdr-cell lines separated by a microfilter from tumor cells was performed, but this co-cultivation didn't change the mdr efflux activity.

The effect *Toxoplasma gondii* infection on the efflux pump activity and mdr-1 gene expression was also examined in the human gastric cancer cells. A sensitization of resistance gastric cancer cells was also achieved by parasite infection.

This phenomenon is an evidence that a reduction of resistance in tumor cells *in vitro* can be achieved by a parasite infection. It is yet unclear whether an active infection or infection or another substance of *T. gondii* is responsible for this phenomenon.

VARGA, E.¹, A. MARÁZ², K. CSEDŐ¹

Effect of microelement enrichment to the vitamin content of yeast

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Yeast cells enriched in microelements are frequently used as paramedicinal drugs. Yeasts are able to take up certain microelements and to built into organic molecules which are more favorable to human and animal organisms.

In the course of our experiments we determined the microelement (Zn, Fe, Cr, Se) content of yeasts after 24 hour fermentation.

We prepared yeasts enriched in zinc-iron-selenium and chromium microelements by fermentation in 2 L volume. From this biomass we determined concentration of microelements with ICP-AES method. The microelement content was as follows: Cr=4µg/g d.w., Fe=104µg/g d.w., Se=2159µg/g d.w. and Zn=1075µg/g d.w.

Also we determined the B₁ and B₂ vitamin content of the yeast biomass with a microbiological method in viable cells and after thermal inactivation.

We found 50-70 % loss of the original vitamin content during inactivation of cells.

We prepared yeast biomass enriched with chromium and selenium microelements in 20 L fermentor. The microelement content of this biomass was as follows: Se=1841µg/g d.w. and Cr=9µg/g d.w. We are using this biomass to animal diet after thermal inactivation. Experiments are in progress.

VARGA, I. M., A. P. BATTEN

Fulminant cerebral listeriosis

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Listeria monocytogenes (LM) infection in adults usually involves the central nervous system (CNS). In Canada, about 90 % of the cases have one or more known predisposing factors, somehow facilitating LM invasion.

We present a fulminant case of cerebral listeriosis which caused the death of the patient before the proper diagnosis was established.

Listerial cerebritis simulating a cerebrovascular accident is known to occur, but is not widely recognized or described. Listerial CNS infections are usually subacute, but this case demonstrates that a fulminant course is also possible. Establishment of the correct diagnosis depends on the bacterial culturing of blood or spinal fluid. In the climate of cost-cutting, these cultures may not even be considered and a false diagnosis may be readily accepted. The blood cultures in the moribund state of this patient were actually ordered to exclude infection, as no infective signs and symptoms appeared until the last hours of the patient's life.

Only speculations may be made as concerns the numbers of false diagnoses of similar patients, as there may be more of them than are recognized. With appropriate clinical suspicion and bacterial cultures, the lives of these patients may be saved.

VARGHA, J., É. KEVEI, K. RIGÓ, B. TÓTH

Phylogenetic analysis of the toxigenic *Aspergillus ochraceus* species

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The genetic variability of *Aspergillus ochraceus* was examined using genotypic methods. Based on the *Hae*III-*Bgl*II generated mitochondrial DNA restriction profiles, most isolates could be classified into two distinct groups. These two groups could also be distinguished by the random amplified polymorphic DNA technique, and by using telomeric or IGS-specific sequences as primers to amplify fungal DNA in the PCR reactions. None of the isolates exhibiting type 2 mtDNA profiles produce ochratoxins. Some strains (e.g. *A. ochraceus* ICMP 939) displayed strain-specific mitochondrial DNA patterns, and their amplified DNA profiles were also different from all other *A. ochraceus* strains examined. Phylogenetic analysis of sequences of the intergenic transcribed spacer region of some of the strains resulted in a dendrogram of the same topology as that based on mitochondrial DNA and amplified DNA data. Based on these results,

A. ochraceus ICMP 939 possibly represents a new ochratoxigenic species within *Aspergillus* section *Circumdati*.

VARGHA, M., G. SZABÓ, K. MÁRIALIGETI

Investigation of the decomposition of atrazine in a bank-wall filtered well model system

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Atrazine is one of the most abundant herbicides in the world. Due to its widespread agricultural usage, it can be detected in soil, subsurface and surface water-flows. Its constant concentration is 1-2 ppb in Danube river. The drinking water of many cities in Hungary, including Budapest, is obtained from the Danube through bank-wall filtered wells. In well water, not even trace amount of atrazine is detectable, therefore it is presumed, that the compound is degraded in the gravelbed. However, the investigation of biotransformation and other filtering processes is difficult on site. In this study, a laboratory model system containing natural sediment sample was constructed in order to examine biodegradation. Validity of the model system was tested by chemical and hygienic measurements, the change of parameters having importance in drinking water quality was followed throughout the system. The results (e.g. significant decrease of nitrite and ammonium ion concentration) show high correlation with the data from previous on site study. Microbial communities of the gravelbed sample were investigated by the characterisation and partial identification of micro-organisms isolated from the model. Dominant Gram negative strains belong to *Pseudomonas* genus (mainly RNA group I.), and include several facultative H₂ autotrophic strains. Gram positive strains are mainly coryneforms or *Bacillus* species. In general, species composition is similar to natural river sediment communities. In conclusion, the model represents well the gravelbed in every respect.

Atrazine degradation was examined in laboratory and field experiments as well. However, our study can unite the advantages of both methods, since circumstances can be standardised, but the complexity of communities and their metabolism is approaches that of natural systems. Micro-organism capable of atrazine mineralization were only recently isolated, and are usually *Pseudomonas* spp. In the model, biodegradation of supplied atrazine was detected after induction. Major metabolite was hydroxi-atrazine. Four strains isolated from the sediment sample were found capable of atrazine degradation. Metabolism of the most efficient atrazine degrading strain (identified as *Tsukamurella paurometabolum*) was investigated in details, and it presumably differs from previously known pathways.

VASTAG, M., G. KRISZTINA, T. PAPP, K. ÁCS, Cs. VÁGVÖLGYI

The antifungal activity of lovastatin against *Rhizomucor* strains

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The members of the genus *Rhizomucor* (Zygomycetes) are distinct from *Mucor* by

their thermophilic nature. These fungi are of value in both theoretical and applied microbiology. They are good producers of different extracellular enzymes, while in other cases they may be the agents of frequently fatal opportunistic mycotic diseases.

Lovastatin (mevinolin) was discovered as a cholesterol-lowering fungal metabolite in an *Aspergillus terreus* culture. Its action is connected with the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which catalyses an early, rate-limiting step in the biosynthesis of sterols and isoprenes. In the present study, the antifungal activity of lovastatin against 24 *Rhizomucor* strains representing three species (*R. pusillus*, *R. miehei* and *R. tauricus*) was investigated. The inhibitory effect of lovastatin on colony radial extension was different under different culturing conditions: it was less effective at lower pH and on complex media. A substantial strain-to-strain variability in the sensitivity was also detected, however, in all the conditions tested, the *R. pusillus* strains revealed greater sensitivity to lovastatin than the investigated *R. miehei* strains.

VIDÁCS, I.¹, G. KISKÓ²

DSC measurements of sporeforming bacterium *Bacillus cereus* T

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In our study we have tried to detect the changes occurring in *Bacillus cereus* T spores due to irradiation with the help of the DSC instrument. Suspension of 10^{10} spore/cm³ was irradiated with 3kGy with a ⁶⁰Co source. The DSC results were compared with results obtained with the measurement of untreated control spores. The heat resistance of the irradiated spores has decreased due to the treatment, which is shown by the shifting of the endothermic processes to a lower temperature range.

The other aim of the experiment was to determine if the curve obtained by an isotherm DSC measurement could be interpreted as a growth curve, and if so under what circumstances.

The suspension of 10^5 spore/cm³ *Bacillus cereus* T spores was placed into the DSC cell. The cultivation was carried out at 37° C and the heat-current changes were monitored continuously. Parallel to this measurement the growth curve of the microbe was also determined with traditional plating method under the same circumstances. Based on the incubation time we have compared the two curves and we have found the following: The detection limit of the DSC instrument is above 10^6 microbe/cm³, correlation between the measured heat-current changes and the real growth can be found between 10^7 - 10^9 microbe/cm³. This means that only at very high concentration of the microbes can the growth parameters (growth rate and lag-phase) determined and calculated from the DSC isotherm measurements.

VÖDRÖS, D.^{1,3}, R. THORSSTENSSON², G. BIBERFELD², E. M. FENYŐ¹

Co-receptor usage of sequential simian immunodeficiency virus (SIVsm) isolates from cynomolgus monkeys with progressive disease

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Sequential virus isolates from seven cynomolgus monkeys experimentally infected with SIVsm (sooty mangabey origin) were studied for co-receptor usage in human osteosarcoma cell lines, GHOST(3), engineered to stably express CD4 and each of the receptors CCR3, CCR5, CXCR4, Bonzo and BOB. The cell lines also contained the green fluorescent protein (GFP) gene driven by the HIV-2 LTR. Activation of GFP expression following infection with SIVsm was observed in fluorescent microscope and quantitated by flow cytometry. Antigen production was measured by HIV-2 antigen ELISA.

Details of the progressive immunodeficiency and the antigenic changes of the virus isolates, has been described earlier (Zhang et al. *Virology*, 197:609, 1993). In the present work, we infected the GHOST(3) cell lines with different SIVsm inoculum viruses and two or three reisolates each from seven monkeys. All virus isolates used CCR5 and BOB, and with the exception of one also used Bonzo as co-receptor for cell entry. CCR5 usage was very efficient and stable in all of the monkeys studied. While uniform at the beginning, Bonzo and BOB usage of later reisolates varied in efficiency. Also, CXCR4 usage was evident with early reisolates, but disappeared gradually over time.

The results show multiple changes in virus populations of monkeys undergoing progressive immunodeficiency.

VÖLGYI, A.¹, T. FARKAS², A. FODOR¹, S. FORST³

Phospholipid membrane fluidity and fatty acid profile in natural phase variants, transposon-induced, deletion and complemented phase-variant mutant of the entomopathogenic bacterium *Xenorhabdus nematophilus*

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Phase variation is one of the most peculiar feature of entomopathogenic nematode symbiont bacteria, *Xenorhabdus* and *Photorhabdus*. In *X. nematophilus*, the shift from the phase 1 cells to phase 2 occurs spontaneously and is of very low reversibility. A several gene active in Phase one switch off, while the lipase switched off in Phase 1 switches on at phase shift. By mutating Phase 1 cells by using a *Tn10* minitransposon, conjugation and selection, several mutants of phase 2 phenotypes were isolated. By analyzing one of one of them, finally the wild type allele of novel gene (*var-1*), capable of complement the transposon tagged and

deletion mutant was isolated, cloned and sequence. One of the pleiotropic phenotypes of the secondary (phase 2) cells is more rigid nature of the membranes, which could be detected both biochemical and biophysical methods. We found, that both the Tn10 induced, and the deletion mutant for *var-1* showed the phase 2 phenotype while the complemented ones showed the phase 1 phenotype. We hope we did manage to isolate one of the most important regulatory gene of the gene-cascade system behind the phase variation of *X. nematophilus*.

VÖRÖS, G., L. BAJNOK

Effect of *Bacillus toyoi* (ToyoCerin®) on the performance, mortality and aerob caecal flora of young rabbits kept in a large scale farm

PO-RA-VET Research and Development Ltd., Gödöllő, Hungary

An eleven week long trial with ToyoCerin^R (50, 100, 200 ppm in feed) with control group was carried out at a rabbit farm of 800 does. Does involved this experiment started to consume ToyoCerin treated feed from 5 days before delivering. Litters of 70 does were equalized to 9 after kindling and the same feed including 50, 100, 200 ppm ToyoCerin was consumed by suckling and weaned rabbits. Every ToyoCerin dose not significantly improved the weight gain of weaned rabbit caged either individually or small groups. Regarding to the whole post weaning period feed conversion ratio of ToyoCerin treated groups were not significantly better than that of the control group. Mortality of suckling rabbits of ToyoCerin treated groups were far more lower than that of control group. Especially losses of 100 ppm ToyoCerin treated group was very low (5.9 %). Mortality of weaned and treated rabbits caused by enteric diseases was also lower immediately after weaning than mortality of control group. *Bacillus toyoi* depending on the ToyoCerin dose and treating time composed 28-70 % of aerob flora of caeca of ToyoCerin treated rabbits. Comparing the effect of different dose 100 ppm ToyoCerin achieved the best performance and mortality figures in this trial so we recomend to use this dose in practice.

WERNICKI, A., A. PUCHALSKI, R. URBAN-CHMIEL

Antimicrobial susceptibilities and plasmid DNA profiles of *Pasteurella haemolytica* strains

Department of General Prophylaxis and Bird Diseases, Faculty of Veterinary Medicine, University of Agriculture, Lublin, Poland

Pasteurella haemolytica is an important agent causing shipping fever or pneumonic pasteurellosis in cattle. In most population of agents antimicrobial resistance is often found, so the purpose of this study was to examine and compare antibiotic - resistance and plasmid profiles *P. haemolytica* strains isolated from cattle with shipping fever or pneumonic pasteurellosis. We report on plasmid profiles characterisation and antibiotic resistance *P. haemolytica* strains from several sites in Poland. The pathogens were isolated from nasal swabs and trachea (live animals) or

pulmons (died animals). Bacterias were identified by using standard criteria:

- Growth on 5% sheep blood agar plates
- Antimicrobial susceptibilities were determined, using a disk – diffusion assay on Mueller Hinton agar plates with 5% sheep blood.
- The DNA plasmid preparation were made using a modification of the lysis procedure according to Sambrook et al. (1989). Plasmid DNA preparations from 18 - hour cultures in BHI were electrophoresed in horisontal agarose gels in TBE buffer. Gels were stained with ethidium bromide and photographed over UV light.

In examined isolates *P.haemolytica* strains was observed multiple antimicrobial resistance. Besides there is no correlation of plasmid content and antimicrobial resistance in strains.

The presence and absence of plasmids were observed with isolates that have multiple resistance to antimicrobials.

YOUSSEF, M. S.

Mycopathological studies of *Tinea* diseases in Sohag Governorate, Egypt

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Eighty cases of dermatomycoses were recorded in patients from Sohag Dermatology hospitals during November 1997 - April 1998. Cases of tinea versicolor, tinea capitis, tinea corporis, tinea cruris, tinea pedis and tinea manuum were diagnosed. Tinea versicolor, tinea capitis and tinea corporis were the most dominant dermatophytic diseases in Sohag Governorate. Generally, males were more susceptible to dermatomycoses than females (77.5% versus 22.5% of examined cases). All dermatophytic specimens were examined directly by microscope and the results were positive in 66 cases out of 80 accounting (82.5%) of total cases examined. Nine fungal species belonging to 5 genera were identified and collected from 80 cases of ringworm examined. Dermatophytes identified from examined cases of human tinea diseases were *Microsporum canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum* and *T. violaceum*. The isolated closely related fungi to dermatophytes were *Chrysosporium keratinophilum*, *C. tropicum*, *Malassezia furfur* and *Candida albicans*. *Malassezia furfur* and *Trichophyton mentagrophytes* were the main causative fungi of tinea versicolor. However, only the first fungus was sufficient to be detected by direct microscopic examination of the specimens. *Microsporum canis* and *Trichophyton violaceum* were the most prevalent dermatophytes caused tinea capitis and tinea corporis, while *T. mentagrophytes* and *T. rubrum* were the most common dermatophytes caused tinea cruris, tinea pedis and tinea manuum. *Candida albicans* a closely related fungus to dermatophytes was considered the main causative pathogen of tinea cruris.

YOUSSEF, M. S.

Antidermatophytic activity of some medicinal plant essential oils and aqueous extracts against isolated human skin pathogenic fungi

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Antidermatophytic activity of both essential oils and aqueous extracts of 16 different medicinal plant kinds was estimated against seven species of dermatophytes isolated from human skin mycotic diseases in Sohag Governorate, Egypt. These pathogenic fungi were *Microsporum canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. violaceum*, *Chrysosporium keratinophilum* and *C. tropicum*. The data clearly elucidated that both of essential oils and aqueous extracts of Ceylon cinnamon and greater galangal had a wide-spectrum highly antidermatophytic activity, whereas the two tested extracts of sweet flag had wide- and mediate-spectrum highly antidermatophytic activity, respectively. The essential oils of wild tea, garlic and thyme had mediate-spectrum highly active, whereas their aqueous extracts possessed wide-spectrum moderate antidermatophytic activity. On the other hand, only the aqueous extract of cabbage seeds proved to be have a mediate-spectrum highly inhibitory effect, whilst the two tested extracts of cardmom and the essential oils of eucalyptus and glinus in addition to the aqueous extract of radish were of limited-spectrum highly antidermatophytic activity.

ZALA, J.

Antifungal drug susceptibility/resistance in the medical mycology

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In the 1990's the antifungal drug resistance became a very important problem in the treatment of the systemic mycotic infections. As, because of the increasing population of immunocompromised patients, the number and the severity of these infections show a rising trend the successful therapy should be essential.

Earlier the Amphotericin B was the unique tool against the life threatening systemic mycoses, but due to its strong undesirable side effects the research turned for finding new antifungal agents. The introduction of the new generation of azoles (triazoles) led to better results, but a quite new problem - the resistance – developed. Triazoles (fluconazole, itraconazole) inhibiting the biosynthesis of ergosterol the major and essential membrane compound of the fungal cells influence the function of many enzymes. Because of the direct membrane damaging effect of amphotericin B the decreased susceptibility is very rare. The fungal cell could protect itself against the indirect enzyme based effect of azoles by a lot of ways, resulting to many type of azole resistant strains. Several factors can led to the presence of a resistant strain in a patient: intrinsic resistance of the colonising strain, replacement of the sensitive endogenous strain with a more resistant *Candida albicans* or other *Candida* species, genetic alteration and transient gene expression, changes in the structure and

composition of the cell membrane, alteration in the cell type and in the enzyme production, etc.. Since these processes are very complicated and the data between the in vitro and clinical findings are controversial, there must be a very effective co-operation among laboratories and hospital wards.

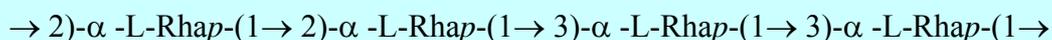
ZDOROVENKO, E. L.^{1,2}, Yu. A. KNIREL¹, V. V. OVOD³

Structures of O-polysaccharide chains of *Pseudomonas syringae* pv. *garcae* LPS

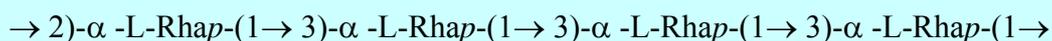
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Pseudomonas syringae pv. *garcae* is cause of coffee diseases. Lipopolysaccharides (LPSs) from microbial cells of *P. syringae* pv. *garcae* (three strains) were extracted by saline solution. O-specific polysaccharide fractions (OPSs) were obtained by gel-filtration the products of LPSs mild acid hydrolysis. Using ¹H- and ¹³C-NMR spectroscopy, including two-dimensional spectroscopy, sugar and methylation analyses and Smith degradation the structures of OPSs were elucidated. It was found that in all strains studied they belong to a structural group that is characterized by L-rhamnan backbone with tetrasaccharide O repeats. OPS of one strain had linear structure. Others were branched in different positions with a monosaccharide side chain of 3-acetamido-3-deoxy-D-fucose (D-Fuc3NAc).

All of them included O repeats with structures 1 and 2, which differ in the position of substitution of one of the rhamnose (Rha) residues.



1



2

This unique type of structural heterogeneity in the main OPS chain has been previously demonstrated for OPSs of some other *P. syringae* pathovars studied which have L-rhamnan main chain with Fuc3NAc as the lateral substituent. Of them, the OPS that of *P. syringae* pv. *coriandricola* GSPB 2028 (W-43) most closely resembles the OPSs studied.

This work was partially supported by grant from Tampere University Hospital Medical Research Fund and grant INTAS-UKRAINE 95-0142.

ZDOROVENKO, G. M.¹, Yu. A. KNIREL², L. M. YAKOVLEVA¹

Composition and structure of lipopolysaccharide macromolecule as taxonomic criteria in classification of *Pseudomonas syringae*

Pseudomonas syringae is a group of widespread phytopathogenic bacteria, which include of about 40 pathovars with not fixed taxonomic rank.

We have isolated and studied the LPSs from *Pseudomonas syringae* strains (total 35), representing different pathovars (total 17). Due to peculiar of LPS macromolecule architecture, their parts (lipid A, core, O-chain), having the different conservative levels, gives information on evolutionary aspect. We studied separately the different parts of LPS-macromolecule obtained after it was cleaved by mild acetic acid hydrolysis.

In lipid A fractions of all strains glucosamine, ethanolamine-phosphate and similar fatty acids were detected. The set of oxyacids identified (3-OHC10:0, 2-OHC12:0, 3-OHC12:0) is common for the typical representatives of *Pseudomonas* genus.

Studies on the core oligosaccharide fractions also showed their composition usual for pseudomonads. Rhamnose, glucose, glucosamine, galactosamine, KDO, alanine, phosphate as a typical components were detected. Composition of core oligosaccharide was more variable in comparison with that of lipid A. 5 chemotypes of core were detected.

On analysis of monosaccharide composition 7 chemotypes of O-chains were distinguished. And thus, the comparative study showed the divergence in the course of evolution of the LPS-macromolecule composition, in the strains studied, from 1 to 7 chemotypes on the levels: lipid A – core – O-chain.

The O-chains structures were determined on the basis routine and modern methods, first of all, different variants of N.M.R.-spectroscopy. From these data follows that in all strains studied, independent on their taxonomic rank, the O-chains are built up by a general principle, that their backbones are represented by rhamnans: *L*-, *D*- or mixed (*DL*-) with tri- or tetrasaccharide O-repeats, having identical structures in the majority of the strains. This is in a good agreement with inclusion of the host-plant differentiated species into *Pseudomonas syringae* as conditionally presented in Bergey's Manual. Presence and nature of the lateral branch substituents (*D*-fucose, *D*-rhamnose, *N*-acetyl-*D*-glucosamine, *N*-acetyl-3-amino-*D*-fucose) and the mode of binding of those with the main O-chain determined the serogrouping of the strains.

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Investigation of the *sep15* gene in *Schizosaccharomyces pombe*

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An attempt to isolate field strains of a very virulent Infectious Bursal Disease Virus on chicken embryonal fibroblasts

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Infectious bursal disease (IBD) is an acute virus induced infection characterised by the destruction of lymphocytes in the bursa of Fabricius and other lymphoid organs. The pathogenesis of viral infection may be induced by several mechanisms, including immune response such as antibody production and cell-mediated reactions.

Seven field isolates of IBD virus (IBDV) obtained from field outbreaks of IBD in Slovenia from 1993 to 1995 were examined. The disease was confirmed by pathological examinations and in some cases also by challenge test on SPF chickens.

For the isolation on tissue culture bursal homogenates were used. Isolates were serially passaged on the chicken embryonal fibroblasts. The first appearance of cytopathic effect (CPE) was found after the sixth passage. The time required to see the CPE was approximately six days. With the passages this was shortened to 3 to 4 days. The strength of the isolates was determined by TCID₅₀. Also the plaques under agar were made. To confirm the virus, the neutralisation test on chicken embryonal fibroblasts was done. As a control the Winterfield strain of IBDV and antiserum against it was used. The cross-neutralisation data showed that field isolates belong to the IBDV type 1.

ZUTIC, M.¹, Z. LEPSANOVIC², D. KRNJAIC³, R. ASANIN³

Antimicrobial susceptibility and plasmid profiles of *Actinobacillus pleuropneumoniae* strains isolated from swine

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Eight strains of *Actinobacillus pleuropneumoniae* were isolated from the lungs of pigs (weighing 25-35 kg) in the outbreak of pleuropneumonia in Yugoslavia in the spring of 1998. The isolated strains were subjected to antibiotic sensitivity testing and plasmid profiling. The susceptibility of the isolates to antimicrobial agents was determined by the agar disc diffusion method. Seven of the eight isolated strains were sensitive to all antimicrobial agents. Only one strain was resistant to ampicillin, amoxicillin and tetracycline. In order to determine the genetic basis of this drug-resistant strain we undertook to isolate the plasmid DNA and subsequently subjected it to electrophoresis in agarose gel. That strain possessed plasmids of 3,5; 6,2 ; 10 and 50 megadaltons. As we had isolated only plasmids

from the resistant strain *Actinobacillus pleuropneumoniae* we came to the conclusion that the present resistance to beta-lactams and tetracycline was the consequence of the existence of the specific plasmid profile.

ZSOMBIK, L., G. J. KÖVICS

Preliminary data for overwintering of *Diaporthe helianthi* (anam.: *Phomopsis helianthi*) causing brown spot (stem cancer) of sunflowers in Eastern Hungary

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Almost two decade passed since a destructive sunflower pathogen *Diaporthe helianthi* Muntanola-Cvetkovic, Mihaljcevic & Petrov (anamorph: *Phomopsis helianthi* Muntanola-Cvetkovic, Mihaljcevic & Petrov) has observed (Mihaljcevic et al., 1980) and mycological described in Yugoslavia (Muntanola-Cvetkovic et al., 1981). First record of brown spot (stem cancer) was published by Németh et al. (1981) in Hungary. Since than this fungus has become the most serious problem of the sunflower growers to manage. Early experiences showed that the fungus can remain viable and infective only on infected stem pieces that overwintered above the soil surface, no pathogen was reisolated from stem pieces buried in the ground 5, 15, or 30 cm deep (Vörös et al., 1983). Both *Phomopsis conidiomata* (pycnidia) and *Diaporthe ascomata* (perithecia) can overwinter on stem fragments of sunflowers depending on ecological influences. *In vitro* only beta-conidia were found (Franc-Mihajlovic et al., 1994) however sterile alpha-conidia can also occur *in vivo*. The role of ascospores as the main source of primary inoculum is argued. Ascospores first appear mainly in June (Jinga et al., 1987). Ascospore traps was described for monitoring *Diaporthe helianthi* epidemics (Delos et al., 1995).

In a fungicide application experiment we observed an early epidemic of *Diaporthe-Phomopsis* disease with leaf necrotic symptoms on 12 June 1998. Later on a heavy epidemic developed on stems causing serious losses in yield in spite of fungicide applications. In the case of early infection seems more reasonable to apply fungicides preventively at an early growth stage (BBCH 16-18) than stage of inflorescence emergence (BBCH 51-55). Two spraying is more advantageous because effectiveness of the early treatment is over by the harvesting stage (Kövics, unpublished).

Aims of our present experiments to identify the overwintered forms of fungus and primary sources of inocula which can contribute to an early epidemic situation. Stem debris were collected from five sunflower plots of trans-Tisza region (Eastern Hungary) in early March 1999. Conidiomata and spores of 100 samples were examined by light microscopy. 30 conidiomata and conidia were measured by each samples for identification.

All pycnidia produced beta-conidia except Debrecen/01 sample which yielded alpha-conidia beside beta-ones. We also observed pycnidia and conidia of *Phoma macdonaldi* the causing agent of black stem at 1/3 of samples.

Infested stem residues were put in wet chambers to stimulate an early ascomata and ascospores production. After 10 days incubation period formation of perithecia and ascospores have started. Another part of samples serve for weekly monitoring of ascomata/ascospores production *in vivo* which is in progress by ascospore traps.