CONFERENCE ABSTRACT

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ABSTRACT

SECTION 1: DIAGNOSTICS OF MICROBES FOR MEDICAL AND VETERINARY MICROBIOLOGY

M. Střítecká., J. Svoboda, L. Mejzlíková (Institute for Microbiology of Medical Faculty of Masaryk University and St. Anna's Faculty Hospital Brno, Czech Republic): **Difficulty of identification of -haemolytic *Streptococcus.***

Haemolytic streptococci are the most common human pathogens out of genus *Streptococcus.* They can initiate acute disease of respiratory tract, skin or urogenital tract.

When determining group of streptococcus, serological methods should be primarily used. Not only serological methods are used in routine practice for rapid identification of A-group, even though theirs reliabilities are lower. Bacitracin test make use of high sensibility of A-group Streptococcus to bacitracin. We have proved that some species of C and G-group are bacitracin sensitive and on the other hand found A-group Streptococcus bacitracin resistant.

Using latex agglutination (Pastorex strep, Biorad) we identified in total 262 strains of -haemolytic streptococci. Out of this amount 26 % of A-group, 18 % of B-group, 39 % of C-group, only 1 % of F-group (surprisingly) and 16 % of G-group.

A-group streptococci showed 9–23 mm zone around bacitracin (0,004 j, Itest plus s. r. o, Hradec Králové), about 4 % were bacitracin resistant. Only 4 % of B-group streptococci showed zone around bacitracin at all. However 31 % of C-group streptococci shoved 7–20 mm zone around the disc. No zones were observed with F and G group streptococci.

It is clear, that particularly C-group streptococci might be confused with A-group streptococci.

M. Sládeková (HPL Microbiological Laboratory, Bratislava, Slovakia): **The proof of toxin A producing by the strains Clostridium difficile.**

Clostridium difficile (next only C. d.) is a recognised major cause of nosocomial antibiotic-associated diarrhoea and pseudomembrannous colitis. Importance of disease and next therapy charging the patient led to focus this problem. Monitoring of the complications and proof of the production of toxin A by strain colonizating gastrointestinal tract of the patients has to find out the

extent and prevalence of certain strains *C. d.* circulating in European hospitals. The analysis of molecular and phenotypic methods, and the analysis of the antimicrobial susceptibility of the strains isolated from patients provide surveillance and recommendation of the antibiotic therapy of disease caused by toxigenic strains of *C. d.* across Europe.

The authors focus on the qualitative detection of the strains *C. d.* producing toxin A in faecal samples by dot-blot method. The aim of the work is find out the extent of *C. d.* toxin A strains in our region of Bratislava before joining to ESGCD European Study Group on *C. d.* We present the analysis of the occurrence of the strains *C. d.* producing toxin A according to the age category and sex, based on the type of diagnosis and hospital department with respect to the antibiotic therapy.

To assemble new knowledge of the occurrence toxigenic strains of *C. d.* and detailed analysis of these data could help to create guidelines on prevention, diagnosis, treatment and surveillance of *C. d.* infections.

Ľ. Gašparovičová[1], *Ľ. Jurgoš*[2], *D.Daniš*[3], *D. Hučková*[1], *Ľ. Vozárová*[1] ([1]HPL Ltd. Microbiological Laboratory, Bratislava, Slovakia; [2]Private Gastroenterological Laboratory, Bratislava, Slovakia; [3]Institute of Pathology of Teaching Derer's Hospital, Bratislava, Slovakia): **Asset of westernblot method in diagnostics and strategy of antimicrobial therapy of Helicobacter pylori.**

The discussion about etiopathogenetical importance *of Helicobacter pylori* (HP) virulence factors and their influence on determination of eradicational therapy start is still actual. The main dispute is over the toxic proteins CagA (cytotoxin associated with gene A) and VacA (vacuolating cytotoxin), pathogenetical or protective respectively significance. These are isolated from patients with active chronic gastritis and duodenal ulcers. HP virulence factors prevalence in reflux gastroesophagitis patients is identical to duodenal ulcers patients, but different in functional dyspepsic patients. Epidemiological studies show variable incidence frequency of CagA considering the gender, age and geographical distribution.

The incidence of discussed proteins was observed by indirect determination of antibodies of IgG class by western blot method (Euroimmun GmbH, Germany). The samples of 30 patients were examined by western blot method. 10 patients were HP type 1 positive and 20 patients were HP type 2 positive. The presence of CagA was evident in 17 samples. CagA together with VacA were present in 10 samples. No differences in subjective conditions and clinical status of HP type 1 and HP type 2 patients were observed. Western blot method evidence was compared to direct evidence of HP (urease test and microscopically confirmation by staining with Giemsa) in samples extracted from stomach mucous. There was 97 % (29 of 30 samples examined) identity of the results acquired by mentioned methods. HP was not proved in one biopsied sample, but nonrepresentative biopsy can not be excluded in this case.

Based on previous experiences, western blot method usage difficulty level is comparable to the other method ELISA, considering the sample preparation. But it has higher sensitivity and specificity. Due to use of western blot method the presence overview of toxic strains of HP in examined samples could be determined. It offers to gastroenterologist a possibility of more exact decision about the antimicrobial therapy in dyspepsia problems patients.

M. Kobidová[2], *K. Schwarzová*[1], *I. Čižnár*[1] ([1]Institute of Preventive and Clinical Medicine, Bratislava, Slovakia; [2]Faculty of Science, Bratislava, Slovakia): ***Borrelia burgdorferi*** **s. l. isolates from the vector and host comparison by immunochemical methods.**

Borrelia garinii K48 tick isolate and the CSF isolate from a patient with neuritis *Borrelia afzelii* LeMo were used for comparison of the phenotypical properties. Double diffusion methods in agaroses by Ouchterlony and immunoelectrophoresis with modifications were applied in the study.

Both isolates showed a different growth rate. The patient's isolate grew slowly and the growth curve phases have had a 2 days delay shift. With the increasing number of in vitro passages this differences were reduced.

By the precipitation of antigens with hyper immune rabbit antisera a partial homology of the isolates was confirmed, however simultaneously there was a relative high degree of heterogeneity

of Osp proteins and endotoxine like component. Further, the immunochemical analysis showed the presence of common antigens of LeMo isolate with *B. garinii* K48, but also with other *B. burgdorferi* s. l. genotypes.

K. Tomanová, J. Smola (Institute of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Brno, Czech Republic): ***Lawsonia intracellularis*: Its Determination in Horses In Czech Republic.**

Lawsonia intracellularis, previously known as ileal intracellular symbiont, is an obligatory intracellular micro organism, which was observed and described mainly in pigs as a contributor of swine proliferative enteritis complex (ileitis). The causative agent is detected by PCR method from intestinal epithelium or in the faeces of infected pigs. A specific and sensitive serological test was developed for fast diagnosis of swine ileitis, which enables to detect antibodies in the chronic forms of this disease and simultaneously in unapparent carriers.

For a long time, the occurrence of *L. intracellularis* in samples of section materials taken from horses was uncertain. While four cases were recorded in the USA and one positive PCR result was registered in our laboratory from 7 analyzed intestinal samples of horses. Last year for the first time, the occurrence of this disease with its clinical manifestations in horses was described only in Canada. Clinical materials, which were collected from horses (English pureblood breeds) with anamnesis of chronic diarrhoea in relatedness of *L. intracellularis*, were investigated in our laboratory without depending up on the previous results. We used PCR for direct identification of bacteria from the faeces and rectal swabs. And we used commercial kit of immunofluorescence for indirect detection of antibodies.

15.7 % of positive PCR results of *L. intracellularis* were registered from 51 analyzed samples taken from horses. 76.6 % of specific antibodies were detected from 64 analyzed blood sera. Identification of *L. intracellularis* from intestine and faeces of horses (English pureblood and half-pureblood breeds) has priority not only in Czech Republic but also in Europe.

Achieved results by detecting the causative agent suggest that beside domestic pigs, horses may be one of those very significant hosts of these bacteria. New opportunities of microbiological diagnostics of *L. intracellularis* create the possibilities of further investigations of etiologically significant micro organisms in the field of gastrointestinal diseases of horses.

P. Carasová, V. Celer (Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Brno, Czech Republic): **Porcine circovirus type 2 – possibilities of laboratory diagnostics.**

Porcine circovirus type 2 (PCV-2) is the cause of “postweaning multisystemic wasting syndrome” and “porcine dermatitis and nephropathy syndrome” in pigs. Laboratory diagnostics of this virus is performed almost exclusivelly by PCR and the virus itself has not been diagnosed in the Czech Republic so far. We have designed primers allowing specific detection of PCV-2 genome in clinical samples and we have succeeded in detecting the viral genome in lymphoid tissue of pigs with suspect of disease. The identity of resulting PCR fragments was verified by sequencing.

G. Vozárová, M. Lisalová, P. Milošovič, J. Hanzen (HPL, Ltd. Bacteriological Laboratory, Bratislava, Slovakia): **Using of selective media for isolation of *Yersinia enterocolitica.***

Yersinia enterocolitica is an important human pathogen who causes gastroenteritis, lymphadenitis, arthritis and other diseases.

Yersinia enterocolitica produces pinpoint colonies after 24 hours of incubation on the solid agar media. These colonies may be overlooked on commonly used nonselective cultivation media for examination of clinical specimens containing a multiplicity of bacterial species. The aim of our research was to use selective media that may enhance detection of pathogenic strains of *Yersinia enterocolitica* from clinical specimens in our region.

From the September 2000 we use a selective diagnostic agar medium Yersinia Selective Medium Oxoid. On this medium *Yersinia enterocolitica* produces the typical red colonies with a

transparent border. For differentiation from other mannitol-positive microorganisms that may give a colonial morphology resembling *Yersinia enterocolitica* we identify all suspect isolates by biochemical tests. In all strains that were determined as *Yersinia enterocolitica* we performed serotyping by slide agglutination with rabbit antisomatic O antisera.

The results of isolations on selective medium Yersinia Selective Medium Oxoid prove that its using allow the detection of *Yersinia enterocolitica* who is the important enteric pathogen especially in children and young children.

SECTION 2: MORPHOLOGICAL AND PHYSIOLOGICAL PROPERTIES OF MICROORGANISMS

I. Sládková, O. Zahradníček (Microbiological Institute of Medical Faculty of Masaryk University and St. Anna's Faculty Hospital in Brno, Czech Republic): **Comparison of delta-haemolytical activity of coagulase-negative staphylococci of blood cultures and from the respiratory system.**

Coagulase-negative staphylococci are an important group of microbes, that is able to act sometimes as normal flora (in skin, including outer part of nasal cavity, external ear etc.), on the other hand, they are known as heavy pathogens in blood system, escpecially as hospital infections – often in form of a "catheter sepsis". It is questionable, whether the strains found in outer respiratory ways (i. e. common flora) differ from the strains from blood cultures (i. e. probably valuable pathogens) in production of any virulence factors. One of such factors is delta-haemolysin (or, more precisely, delta-like haemolysin). We compared the delta-haemolytical activity of 60 strains of coagulase-negative staphylococci from bloodstream and 60 strains of coagulase-negative staphylococci from nasal cavity. We found that majority of respiratory strains showed zero or weak haemolytical activity; among the strains form blood cultures, on the other hand, the ratio of strains with a strong delta-haemolytical activity was much higher. Nevertheless, the results are not very clear. There are many confusing factors, at least following ones should be taken into account: 1) the quality of the used agar medium is not constant, despite all the standardisation; 2) the group of "coagulase-negative staphylococci" is not homogenous – in the blood culture strains many species were found, the ratio of S. haemolyticus and S. hominis was considerably high; in respiratory strains, S. epidermidis was the only really frequent species; 3) only part of the blood culture strains are real parhogens - it is very difficult to differentiate between real causative agents of septicaemia and contaminants from skin.

O. Zahradníček (Microbiological Institute of Medical Faculty of Masaryk University and St. Anna's Faculty Hospital in Brno, Czech Republic): **Interaction of deltahaemolytical activity of staphylococci with haemolytical activity of *Staphylococcus hyicus* and *Staphylococcus chromogenes.***

Unlike *Staphylococcus aureus* with its several types of haemolysins, majority of non-aureus (mostly coagulase-negative) staphylococci show at most one type of haemolytical activity. This activity is partially analogical to the activity of delta-haemolysin of *Staphylococcus aureus*. Nevertheless, there are some differences between delta-haemolytical activity of Staphylococcus aureus and haemolytical activity of at least some of coagulase-negative staphylococci. Some phenotypical ones are shown in this work. The intaraction between several staphylococcal strains, producing "delta" activity (both *S. aureus* and non-aureus staphylococci) and strains of *Staphylococcus hyicus* and *Staphylococcus chromogenes* was tested. **S. hyicus** and *S. chromogenes* are well known for their production of another type haemolysin. The presence of this haemolysin inhibits all types of delta activity, but the extent of this inhibition is different in *S. aureus* (inhibition is weak) and non-aureus staphylococci (a strong, complete inhibition).

M. Heroldová., J. Vytlačil, P. Ondrovčík. (Microbiological Institute of Medical Faculty of Masaryk University and St. Anna's Faculty Hospital, Brno, Czech Republic): **Relation between production of slime and elastase coagulase-negative staphylococci and their ability to adhere on endoprothese surfaces.**

Coagulase-negative staphylococci (CoNS) start to play more important role as causative agent of serious complications in orthopaedic operations. Danger of infection is getting bigger with using of plastic and metal catheters, prosthetic cardiac valves and endoprothesis. CoNS are characteristic by their ability of adherence and making of biofilms on surface of the medical appliances. Production of polysaccharide slime and elastase seems to be backup factor of adherence and following invasion agents to organism.

The aim of our study was to find out how the production of these exosubstances is connected with measure of adherence CoNS to single types of materials used for production of endoprothesis. If there is an important difference in production of slime and elastase between strains of CoNS isolated directly from orthopaedic infection and strains from other clinics.

We studied 30 strains CoNS isolated from orthopaedic materials and 30 strains isolated from other departments. We tried to find out production of elastase by sprinkle method with elastin and Congo red. Slime production was tested on CRA agar. We did not find out any differences in adherence, and production of slime and elastase between both CoNS groups. Our results suggest that there are differences in adherence to individual type of endoprothesis materials.

D. Kotrba, M. Siglová, J. Masák, A. Čejková, V. Jirků, L Rybariková, P. Hron (Department of Fermentation Chemistry and Bioingeneering, ICT, Prague, Czech Republic): **Determination of biofilm growth and factors influencing adhesion of selected microbes (*Candida maltosa*).**

Biofilm formation is common phenomenon in many environmental conditions. Set of metodic approaches, which allow us to determine amount of biofilm biomass is required for characterization of conditions both enhancing and decreasing biofilm formation. Therefore we developed procedure allowing observation of adhesion on particle carriers. We employed chemical treated glass spherical particles. Procedure included incubation of culture in presence of carrier, during procedure quantitative and qualitative development of adhesion and stage of formed biofilm, were observed, optionally morphological alterations were watched too during adhesion process. Fluorescence microscopy gives appropriate informative view for qualitative determination of biofilm formation. If images are stored, it can be achieved easy comparison and qualitative evaluation of adhesion on various types of carriers or in various intervals. Fluorescence microscopy brings several advantages in observing adhesion process. It enables locate even individual microbes, while preparation of samples is relatively simple. Fluorescence microscopy is applicable even for opaque samples. Thanks to fluorescent dye SYTO 13 specifity it is possible to make visible only viable microbes.

Determination of microbial proteins amount by modified Bradford's method was used for accurate quantification of microbial biomass bounded on carrier's surfaces. Sonication, which is very efficient method was applied to detach microbes attached on carrier surfaces. Concurrently is sonication simple and fast method. Interval 10 minutes appeared long enough to detach bounded cells from carrier surface by ultrasound with frequention 35 kHz.

Determination of microbial proteins is very sensitive method, which make possible to realize even small amount of microbes. Its micro-volume design in connection with Bioscreen C device allowed us to process relatively vast variety of samples in quite short time. For quantitavely determination of larger part of biomass, what is the case of *Fusarium proliferatum*, it is possible to use simple determination of dry biomass.

Important characteristic influencing adhesive behaviour of microbes is hydrofobicity both of cell outer layers and carrier surface. Hydrofobicity measurement of microbes was carried out by BATH technique, which is unsophisticated but accurate. For carrier surface hydrophobicity it is feasible to select contact angle measurement by the sessile drop technique.

Results obtained by approach described above provided us with overview about ability of microbes to colonize various types of surfaces under various conditions. Based on the performed

experiment we could estimate fundamental carrier characteristics, which are required for successful biofilm formation.

F. Růžička (Microbiological Institute of Medical Faculty of Masaryk University and St. Anna's Faculty Hospital, Brno, Czech Republic): **Ability of adherence of Candida on epithelia of healthy persons and persons with diabetes.**

The ability of genus *Candida* to adhere into epithelial cells is considered an important factor of virulence. It makes possible the colonization of mucosal surfaces by this microorganism. Its adherence into the epithelial cells of the healthy people and into the epithelial cells of the people with diabetes mellitus was observed at 96 strains of *Candida albicans* and 135 strains of other species within this genus.

The good ability to adhere into the epithelial cells of the healthy people was proven in 70.8 % strains *C. albicans*. In *C. tropicalis, C. parapsilosis* and *C. glabrata* strains. the proven ratio of this ability was smaller. The adherence of other tested species was only rare.

In the comparison of the average number of the yeast cells which adhered into the epithelial cells of the healthy people and the epithelial cells of the people with diabetes we did not find a statistically significant difference in most of the observed species. Only in *C. parapsilosis* and *C. lusitaniae* the number of the yeast cells which adhered into the epithelial cells of the people with diabetes was higher.

P. Matějková, J. Šmarda (Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Effects of colicins E1 and K on spheroplasts.**

We investigated inhibition effects of colicins E1 and K on glycine and lysozyme spheroplasts from a sensitive strain *E. coli* K12 Row, from mutant strains in proteins BtuB, TolC, OmpA, OmpF and tolerant strains tolA, tolB, tolQ. The colicins E1 and K have the same mechanism of the lethal effect, they are ionofores. However, they differ by outer membrane receptors and by one of the translocation system proteins.

The ability to form spheroplasts was reduced in the following order: Row, btuB, ompA, ompF, tolC, tolB, tolA, tolQ. Glycine spheroplasts preparation in the latter two mutants was not successful at all.

The effect of colicins on glycine spheroplasts was evaluated according to the degrees of their ability to regenerate rods on a thin agar layer in the presence or absence of colicin. We followed the degree of lysozyme spheroplasts lysis expressed as a decrease of light absorbancy of their suspension in time.

Mutants resistant to colicin E1 – strains btuB and tolC (total insensitivity of rods) –converted into glycine spheroplasts showed total insensitivity to it. However, lysozyme spheroplasts showed rapid lysis in its presence.

Mutants in porins OmpA and OmpF (decreased sensitivity of rods to colicin K) if converted into glycine spheroplasts appeared to be almost insensitive to this colicin. Lysozyme spheroplasts showed rapid lysis just as the resistant mutants.

Tolerant mutant tolB was fully sensitive to colicin E1 and showed a decreased sensitivity to colicin K. Glycine spheroplasts had the same parameters. Lysozyme spheroplasts were also fully sensitive to colicin E1. Colicin K enhanced the lysis, but just in a insignificant degree. Tolerant mutants tolA and tolQ were fully insensitive to both colicins and retained their insensitivity even if converted into lysozyme spheroplasts (glycine spheroplasts could not be considered).

Glycine spheroplasts of all of the investigated strains retained properties similar to those of intact cells in the presence of both colicins. Lysozyme spheroplasts of the mutants in proteins of the outer membrane gained full sensitivity, which could be explained by damage of the outer membrane carrying receptors by the preparation technique in the presence of EDTA. Hypertonic sucrose medium could also cause plasmolysis and insufficient contact of the outer and cytoplasmic membrane. Lysozyme spheroplasts of the tolerant strains retained their sensitivity or insensitivity typical for the intact cells. So no desicive damage of colicin translocation system takes place during

spheroplast preparation with lysozyme and EDTA (!), even though protein TolB is directly associated with the peptidoglycane layer.

K. Kollárová, H. Kožuchová, I.Čižnár (Institute of Preventive and Clinical Medicine, Bratislava, Slovakia): **Common proteins of *Plesiomonas shigelloides* and members of families *Enterobacteriaceae* and *Vibrionaceae*.**

We applied SDS-PAGE and Western-blot (WB) analysis to study protein profile of *Plesiomonas shigelloides* and members of families *Enterobacteriaceae* and *Vibrionaceae*. The aim of this study was to compare protein profile and its similarity to antigenic profile of the studied specieses.

For this purpose we have applied SDS-PAGE and WB analysis with hyperimmune rabbit polyclonal antibodies specific for *Plesiomonas shigelloides*.

SDS-PAGE showed that members of family *Vibrionaceae* revealed more protein fractions of high molecular range (45 - 205 kDa). In members of family *Enterobacteriaceae* besides this high molecular range, two low molecular fractions (up to 29 kDa) were also identified. WB analysis showed a number of common antigens reacting with anti-*Plesiomonas shigelloides* polyclonal rabbit antisera were present in all tested strains from family *Enterobacteriaceae* and/or family *Vibrionaceae*. The high molecular antigens were dominating in both families. Members of family *Enterobacteriaceae* contained three fractions of a lower molecular weight. Protein and antigenic profile of *Plesiomonas shigelloides* was closer to profile of members of family *Enterobacteriaceae*.

R. Ďurana, S. Bystrický (Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia): **Isolation of the cell-surface antigens from three pathogenic yeasts of the *Candida* genus and investigation of their chemical properties for potential neoglycoconjugate synthesis.**

Three representatives of the pathogenic yeasts of the *Candida* genus (*C. albicans* CCY 29-3-32, *C. tropicalis* CCY 29-7-6, and *C. glabrata* CCY 26-20-1 from the Culture Collection of Yeasts and Yeast-like Microorganisms (CCY), Institute of Chemistry, Slovak Academy of Sciences) were grown on a semi-synthetic yeast liquid medium containing 2 % D-glucose as described previously. Cell wall a-D-mannans and peptidomannans, which are believed to be cell-surface antigens determining immunological properties of the yeasts were isolated by two different methods, a-D-mannans by precipitating with Fehling's reagent and peptidomannans without the use of Fehling's reagent.

In the synthesis of neoglycoconjugates, the most common way how to attach the protein to polysaccharide unit is the use of an appropriate linker. The most widely used one is adipic acid dihydrazide (ADH). In our investigation, we focused on the determination of the possible cross-linking effect of homobifunctional linker ADH in polysaccharide derivation, which is usually not considered and which may participate in changes of the steric structure of the epitope of the biomolecule with subsequent consequences in alternations of immunobiological properties.

For this purpose, cell wall a-D-mannans were oxidized and the carbonyl groups were derivatized with ADH. The degree of the cross-linking was determined using colorimetric methods (Park-Johnson assay, trinitrobenzesulfonic acid assay), elemental analysis and size exclusion chromatography.

We have confirmed relatively high degree of the cross-linking effect of ADH in the polysaccharide derivation. Our investigation indicates that the cross-linking is probably dependent on the degree of branching of the polysaccharides and that the cross-linking of the polysaccharides is rather intramolecular than intermolecular. We also confirmed that milder oxidized polysaccharides do cross-link less than more oxidized polysaccharides.

These findings should be taken in account in the preparation of immunoconjugates.

I. Rudolf, B. Rittich (Department of Microbiology of Faculty of Science of Masaryk University in Brno, Czech Republic): **Properties of DNase I immobilized on magnetic carriers.**

Immobilization of enzymes on magnetic carriers brings a lot of advantages. They could be easily separated from reaction mixture and used repeatedly. Suitable particles of small diameter are used in molecular diagnostics which enables manipulation with small volumes.

Aim of presented work was to determine activity of DNase I immobilized on two types of carriers in presence of different ions (Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}). DNase I was immobilized on magnetic perl cellulose with diameter 63 to 125 m and on microparticles poly(HEMA-co-EDMA) with diameter 1.2 m. Activity of DNase I was determined by spectrophotometer and by agarose gel electrophoresis. High polymer and plasmid DNA was used as substrate.

It was found that DNase I immobilized on both carriers is biologically active and degradate DNA. Activity of enzyme changes with type of used ion. The most optimum results were obtained for acetate buffer in presence Ca^{2+} ions and Mg^{2+} ions (pH = 5.0) and in presence of Mn^{2+} and Co^{2+} (pH = 5.5). Possibility of repeatable usage of immobilized enzyme in presence of above mentioned ions was also analyzed.

It was prooved, that immobilized DNase I can be repeatedly used in presence of Co^{2+} ions at least 20 times any statistically decrease of activity.

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SECTION 3: EXPERIMENTAL MICROBIOLOGY

D. Molinková, V. Celer (Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Brno, Czech Republic): **Expression of glycoprotein gD of EHV-1 virus in bacterial cells.**

Glycoprotein D (gD) is an important component of herpesvirus particle. It is involved in the binding of virus on the surface of host cells and contains antigenic structures responsible for induction of neutralising antibodies. Gene coding for glycoprotein D of equine herpesvirus type 1 (EHV-1) was cloned into pTrcHis plasmid vector and bacterial cells were transformed with this DNA construct. Resulting protein was expressed as a recombinant molecule with polyhistidine domain. Purification of insoluble protein fraction was performed using appropriate methods based on affinity chromatography.

L. Strašák, V. Vetterl, J. Šmarda (Faculty of Science and Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Effect of low-frequency magnetic fields on bacteria *Escherichia coli;* dependence on the frequency. Attempt to synchronize bacterial culture, the combination of effects of the magnetic field and UV irradiation.**

Magnetic fields can influence the living systems. Our contemporary work is based on results presented here in last years. Bacteria *Escherichia coli* react adversely on short-time exposure by the magnetic fields. We found that 50 Hz magnetic fields (B = 2–10 mT, $t_{exposure}$ = 2–12 min) kill the bacteria. We concentrated on other frequencies in the low-medium-frequency range (2 Hz–20 kHz). We have found that these frequencies act similarly as the 50 Hz one, but we have found no dependence of CFU number on the frequency of the magnetic field applied.

At measuring the growth curves, we had found that the number of CFU of exposed cells is lowered to the number of CFU of unexposed ones, but both divide with a similar rate. We have made an attempt to synchronize the cultures by a thermal shock and to compare the time constants of dividing. However, we were not able to prepare well synchronized cultures. On the other hand, we could see clearly the difference between exposed and non-exposed cells.

We have tried to examine the effect of a combination of two harmful factors: magnetic field and UV irradiation. We have found that UV irradiation is much more destructive to bacteria than magnetic fields. Magnetic fields exposures before or after UV irradiation showed only a small difference in the number of CFU.

E. Janouškovcová[1], *A. Žákovská*[1], *M. Dendis*[2] ([1]Faculty of Science, Masaryk University, Brno, [2]Genetic laboratory, CKTCH, Brno, Czech Republic): **Identification of Ag structures of *Borrelia burgdorferi* and protein changes of individual passages using SDS-gradient PAGGE.**

The aim of the study was to identify 27 spirochete isolated strains and determinate their protein composition. The second task was to find the protein changes in Ag structure of *Borrelia* in individual passages.

In 1997–1999 a total of 1990 *Ixodes ricinus* ticks, 72 small mites and fleas from wild-living rodents, parts from 219 rodent tissues and 200 mosquitoe larvae were collected at four localities: Pisárky (land-register Brno), Bílý potok (l.-r. Velká Bíteš), Bažantula (l.-r. Studénka), Vysoké Mýto (l.-r.)

A total of 27 isolates were yielded (8 from ticks, 1 from small bites, 15 from tissues of wild - living rodents, 3 from mosquitoes larvae). All isolated strains were identified by comparison to standard stems *Borrelia burgdorferi* sensu stricto, *B. afzelii, B. garinii* and were further tested for the protein composition changes with the SDS-PAGGE method. 7 isolated strains were compared in the interval of 4–7 passages. SDS-PAGGE electrophoresis proceeded in gradient polyacrylamid gel in the presence of sodium dodecylsulfate.

Isolates were further identified for the presence of DNA of pathogenic species of *Borrelia burgdorferi* using nested PCR method.

Results: With the aid of SDS-PAGGE electrophoresis all of isolated strains compared to standards were detected. Changes in protein composition of isolated strains, during the passages in the frame of one species was confirmed. There was also a change in an individual protein fraction concentration. 2 isolated strains from Ixodes ticks were determined as *B. garinii*, others as *B. afzelii*. All izolated strains from rodents were *B. afzelii*, those from mosquitoe larvae were non *Borrelia burgdorferi*. Results of nested PCR confirmed PAGGE results.

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D. Blažek, V. Celer (Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Brno, Czech Republic): **Selection of recombinant scFv antibodies against p25 protein of Maedi – Visna virus.**

We have isolated anti-p25 antibodies from a human synthetic phage antibody scFv library (Human Synthetic VH + VL scFv Library). Recombinant p25 protein of ovine lentivirus was used as antigen. After four cycles of panning, clones that recognized p25 protein were isolated. The isolated phage antibodies were characterized by immunoblotting, and the nucleotide sequences of selected clones were determined.

M. Monincová[1,2], *Z. Prokop*[1], *S. Marvanová*[1], *J. Damborský*[1] ([1]National Center for Biomolecule Research, [2]Institute for Microbiology, [3]Institute for Environmental Chemistry and Ecotoxicology, Faculty of Science, Masaryk University, Brno, Czech Republic): **Kinetic characterisation of mutant dehalogenases using the gas chromatography.**

Haloalkane dehalogenases are hydrolytic enzymes catalysing cleavage of the carbon-halogen bond. Halogenated aliphatic hydrocarbons serve as the substrates for these enzymes. Haloalkane dehalogenases can be used in practise for bioremediations and chemical syntheses. Several different haloalkane dehalogenases have been isolated, but their substrate specificity and catalytic properties are sometimes not optimal for the practical use. Because of this we have to look for the new enzymes or improve currently available dehalogenases.

LinB is the haloalkane dehalogenase from *Sphingomonas paucimobilis*. It takes part in biochemical pathway for degradation g-hexachlorocyclohexane. g-hexachlorocyclohexane is a insecticide and due to its resistance to biological degradation it cumulates in the environment. Amino acid sequence, structure and catalytic properties of LinB are known. Another potential haloalkane dehalogenase, Rv2579 from *Mycobacterium tuberculosis*, was found in a genetic database. Amino acid sequence and structure of Rv2579 are similar to LinB. However, *Mycobacterium tuberculosis* is a human pathogen and its growth rate is very low, so it is impossible to isolate and characterise the enzyme from *Mycobacterium tuberculosis*. Computational analysis

detected six amino acids that can play an important role in the catalytic properties of these enzymes. Mutations Q146A+D147V, +L177A, + I211L, + L248I and +F143W were introduced into the *linB* gene cumulatively by site-directed mutagenesis. The plasmids with containing mutated genes were transformed to *Escherichia coli* JM109, the proteins were expressed and purified. Pure enzymes were used for kinetics measurement with 1-chlorobutane and 1,3-dibromopropane as the substrates. Concentrations of products were determined by gas chromatography. Kinetic constants – dissociation constant (K_M) and catalytic constant (k_{cat}) were calculated.

1,3-dibromopropane inhibits enzymatic reaction in its higher concentrations. Activity of every mutant constructed was lower compared with the wild type enzyme. The lowest activity was observed with +L177A and +I211L mutants. Interestingly, affinity of the substrates in +L177A mutant increased 400-times compared to the wild type enzyme. The mutant +F146W, which contains six cumulative mutations, was still active. Six cumulative mutations reconstructed the dehalogenase Rv2579 from the dehalogenase LinB. Mutants with high affinity could be used for the degradations of pollutants present in the environment in low concentrations.

A. Fialová, A. Čejková, J. Masák, R. Řásná (University of Chemical Technologies, Institute of Fermentative Chemistry and Bioingeneering, Prague, Czech Republic): **Study on properties of phenol by the yeast *Candida maltosa.***

Phenolhydroxylase is an inductive enzyme responsible for the first step of biodegradation pathway of phenol and phenolic compounds. These are widely spread contaminants of the environment, where they get into chiefly from industrial plants and accidents. Toxicity and bactericide effects of these aromatics often cause e. g. problems with the process of wastewater treatment. Biodegradation, as one of the most effective methods of decontamination of hereby fouled environment, means a process of pollutant utilisation by the micro-organisms and with this attached removing of such a harmful compound from the environment.

By the yeast *Candida maltosa*, which is able to use phenol as a sole source of carbon and energy even in higher phenol concentration in the YNB cultivation medium (up to ca 1.4 g/l), was followed the characteristics of biodegradation of phenol and its derivatives with the relation to the observed enzyme (participates on the reaction phenol to catechol). The enzyme phenol hydroxylase, monooxygenase with the cofactor NADPH, was after the microorganisms disruption determined in the cell free extract. The enzymatic activity was observed in the dependence on various outer conditions (the way of biomass disruption, type and concentration of the substrate, the microorganisms growth phase) and changes of phenol hydroxylase activity were recorded during the process of batch and fed-batch cultivation respectively.

The reached results are on the one hand a groundwork for further researches on the field of enzymatic assessment of the yeast *Candida maltosa* and on the other hand for potential practical application of this microorganism for decontamination of localities contaminated by phenolic compounds

A. Ryčovská (Department of Biochemistry, Faculty of Natural Sciences Comenius University, Bratislava, Slovakia): **Mitochondrial diseases: Yeast *Yarrowia lipolytica* as a model system for the study of respiratory complex I deficiencies.**

Mitochondriocytopathies represent a heterogeneous group of serious human diseases, which are manifested by myophaties and neurophaties associated with lactic acidosis. They are caused by mutations in the mitochondrial DNA and/or nuclear genes participating in the biogenesis of mitochondria that result in defects of respiratory chain. In the last decade yeasts became suitable model for study of many biological phenomena including various genetic diseases. These single-celled microorganisms are a unique model of higher eukaryotes and their biochemical mutants became instrumental in the analysis of many complicated processes such as oxidative phosphorylation. The obligately aerobic yeast species *Yarrowia lipolytica,* due to well elaborated molecular genetic tools (i.e. systems for genetic transformation, wide range of mutant strains, cloning vectors, gene libraries) represents an excellent model system for the study of the biogenesis

of respiratory complex I. We have isolated the gene *NUO51* coding for a 51 kDa subunit of the first respiratory complex that binds NADH and FMN. The subsequent analyses indicated that the *NUO51* is constitutively expressed on media with different carbon sources but the expression is dependent on the growth phase and the presence of iron in the cultivation medium. These analyses represent the first step in the study of respiratory complex I biogenesis in the yeast *Y. lipolytica*.

Š. Vejsadová, I. Holubová, M. Weiserová (Microbiolgical Institute, Czech Academy of Sciences, Prague, Czech Republic): **Localization of the Type I Restriction-Modification Enzymes *Eco*KI and *Eco*R124I in Bacterial Cell.**

Restriction – modification (R-M) systems protect the host bacteria by restriction of invading foreign DNA. The cellular DNA is protected from restriction by modification – methylation at the specific sequence recognised by the restriction enzymes. The type I R-M systems are the most complex so far discovered and are classified into four distinct families: IA, IB, IC and ID. Type I enzymes are encoded by three genes, *hsdR,hsdM* and *hsdS.* The products of all three genes are required for restriction, while for modification (methylation) only the products of the *hsdS* and *hsdM* genes are absolutely required.

To localise the type I R-M enzymes *Eco*KI and *Eco*R124I within the bacterial cell, the Hsd subunits present in subcellular fractions were analysed using immunoblotting techniques. The endonuclease (ENase) as well as the methylase (MTase) was found to be associated with the cytoplasmic membrane. HsdR and HsdM subunits produced individually were soluble, cytoplasmic polypeptides and only became membrane – associated when co-produced with the insoluble HsdS subunit. The release of enzyme from the membrane fraction following benzonase treatment indicated a role for DNA in this interaction. Trypsinization of spheroplasts revealed that the HsdR subunit in the assembled ENase was accessible to protease, while HsdM and HsdS, in both ENase and MTase complexes, were fully protected against digestion. We postulate that the R-M enzyme *Eco*KI and *Eco*R124I are associated with the cytoplasmic membrane in a manner that allows access of HsdR to the periplasmic space, while the MTase components are localised on the inner side of the plasma membrane.

The significant difference in the accessibility to trypsin of the HsdR subunit of both *Eco*KI and *Eco*R124I system reflects various regulation of their function in the bacterial cell.

SECTION 4: GENETICAL APPROACHES IN MICROBIOLOGY

P. Kašpárek, J.Doškař, R. Pantůček (Department of Genetics and Molecular Biology, Faculty of Science Masaryk univesrity Brno, Czech Republic): **Genom analysis of staphylococcal polyvalent bacteriophage 812.**

The phage 812 is a polyvalent virulent bacteriophage belonging to the family *Myoviridae*. It shows strong lytic activity on a broad host range of *Staphylococcus aureus* subsp. *aureus* strains as well as on some other staphylococcal species. The phage 812 is closely related to staphylococcal phages U16, SK311 and SK131 classified with the phage species Twort. The object of our study is the analysis of genome regions exhibiting chromosome rearrangements (insertions, deletions and inversions) in the host-range mutants of the phage 812. This analysis should contribute to the explanation of molecular mechanisms leading to the host range changes. Part of this study is focused on identification and characterization of genes and sequences of the phage 812 presumably relating to the host range changes. We determined the nucleotide sequence of a 913 bp insertion identified in the restriction fragment PstI-O of the host-range mutant 812b. The putative gene coding for the major capsid protein of the phage 812 has been found. This protein shows 65 % identity with the major capsid protein of the phage A511 growing on *Listeria monocytogenes* strains. Hybridization probe prepared from the intron sequences of phage Twort does not hybridize with the phage 812 DNA, nor with that of its host-range mutants, which indicates absence of the introns in these phages.

J. Prodělalová, A. Španová (Masaryk University in Brno, Faculty of Science, Department of Microbiology, Brno, Czech Republic): **Intracellullar PCR inhibitors in bacteria *Salmonella* sp.**

The polymerase chain reaction (PCR) is a method which is enables identification of microorganisms in various samples. The advantage of this method is a consequence of sensitivity, specifity of determination and time saving. from user vview is important, that PCR is not demanding on purity of isolated DNA. Only cruded cell lysates suffice for the identificatio of the target microorganisms. hiwever, tne presence of inhibitors can negativelly influence the censitivity of detection.

The aim of our work was determine the sensitivity of detection of bacteria cells. There were used cells *Salmonella* Typhimurium LT 2-18, *S.* Typhimurium LB 5000 and selected *Salmonella* strains of human origin (with 1 exception of rabbit meat). The primers ST 11 and ST 15 (Aabo et al., 1993) were used for amplification of 429 bp long PCR products. The amplification products were detected using agarose gel electrophoresis Two different DNA polymerases (RecTaq and LA DNA polymerases) were used for amplification.

Various procedures of the preparation of DNA matrix for PCR were use:

pure DNA prepared using the phenol extraction method

crude cell lysates prepared by boiling of cells for different time (10 - 30 minutes)

cells washed by sterile water.

The sensitivity was dependent on type of the DNA polymerase. The higher sensitivity was reached by means of LA DNA polymerase. Marked differences in sensitivity of PCR were detected by using various procedures of the preparation of DNA matrix.

Intracellurar PCR inhibitors are heat - resistant and removable by boiling.

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S. Šikutová, K. Pejchalová., J. Halouzka.[1]*, A. Žákovská* (Faculty of Sciences, Masaryk University, Brno, [1]Institute of Vertebrate Biology, Department of Medicine Zoology, Valtice, Czech Republic): **Monitoring of presence of spirochetes in haematophagous arthropods focused on *Borrelia burgdoferi* using DFM and PCR methods.**

The aim of our study was to find and identify spirochetes in selected groups of arthropods by DFM methods. Positive samples were examinated for the presence of *Borrelia burgdorferi* sensu lato by PCR method. Isolates were further analysed by gradient SDS-PAGGE method and determined their protein composition.

A total of 424 ticks (*Ixodes ricinus*) - 2 larvae, 279 nymphs, 73 females, 70 males) were collected in Brno-Pisárky locality in the year of 1998. During the summer seasons of the years 1999 and 2000 were collected 1035 mosquitoes from family *Culicidae* (larval stage, pupa and imago) and 61 females from family *Tabanidae* in three localities (Břeclav, Valtice and Lanžhot). During the winter season of the years 1999/2000 and 2000/2001 were collected 1391 hibernating females of mosquitoes in Břeclav locality.

The midgut of each individual was examinated by DFM (Dark-field microscopy) method and there was found amounts of spirochetes. Then positivite samples were analysed by one-tube nested PCR method for the presence of *B. burgdorferi* s. l. and for further details to the species of *B. burgdorferi* and determining protein composition was used by SDS-PAGGE method.

Results: From the total amounts of 424 ticks was with DFM method 51 positive (12.03 %), 36 (8.50 %) positive with PCR method. From summer mosquitoes 216 positive individuals from 1035 were positive (20.87 %), from winter mosquitoes from 1393 were 55 positive (3.95 %). There were found no spirochetes in insect from family *Tabanidae*. 2 isolates from ticks (*B. garinii, B. afzelii*) and 2 isolates from mosquitoes larvae were prepared.

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I. Holko, J. Urbanová, M. Kantíková, V. Kmeť (State Veterinary Institute, Dolný Kubín; Institute of Animal Physiology of Slovak Academy of Science, Košice, Slovakia): **The Detection of *Listeria monocytogenes* in Foods by Nested PCR.**

The possibility of PCR Detection of *Listeria monocytogenes* in various foodstuffs (milk and cheese) shows a way of specific, effective and rapid diagnostics of *L. monocytogenes* contamination in food – control laboratories. This method is useful as a possibility to differentiate nonhemolised strains of *Listeria monocytogenes* from non-pathogenic *L. innocua* with same results of biochemical and serological typisation (serotype 4ab).

For PCR detection we used two pairs of primers (PRFA and LIP) with affinity to *prf A* gene. As a result we gained 1060 bp product in the first step of PCR and 273 bp in nested PCR. The modified method of *Simon et al.* (1996) was used for investigation.

This work was carried out within the 5. FW project "Validation and standardisation of diagnostic PCR for detection of foodborne pathogens" in the Institute of Animal Physiology, Slovak Academy of Sciences.

M. Pejchalová, K. Harsová, M. Indrová, J. Vytřasová (Department of Biological and Biochemical Sciences, University of Pardubice, Czech Republic): **Determination of *Arcobacter* spp. using duplex PCR technique.**

Arcobacter spp., previously identified as the aerotolerant *Campylobacter cryaerophila* are differentiated from *Campylobacter* spp. in 1991 by their ability to grow at 15 °C and by aerotolerance. Two *Arcobacter* species are found in association with human disease, they are *A. butzleri* and *A. cryaerophilus.*

Classical cultivation methods based on phenotypic trails are often time consuming in view of the fastidious growth characteristic of these organisms. They are also limited in specificity. Cultivation methods utilise primary enrichment in liquid nonselective media (5–7 days) followed by cultivation on selective solid media and consequent confirmation using biochemical and morphology tests. Subjective interpretative criteria, lack of standardisation and the prevalence of biochemical atypical strains have fuelled interest in molecular approaches of identification.

Prevalence of *Arcobacter* was verificated in raw meat, meat products, and raw poultry and as well as using of smears from manufacturing process. For the species-specific detection of *Arcobacter* the duplex PCR technique was optimised. The test uses two primer sets. Set I target a section of the 16 S rRNA genes of *Arcobacter* spp. Set II amplifies a position of the 23 S rRNA genes unique to *A. butzleri*. Upon PCR amplification, all of the *Arcobacter* isolates yielded a 1223 bp product, whereas *A. butzleri* exhibited both 1223 and 686 bp, respectively. Determination of Arcobacter can be reduced by 3 day using this method, in contrast with cultivation method. Possibility of direct determination of *Arcobacter* in real samples after enrichment in liquid medium was also tested.

K. Hrochová, L. Plíšková, I. Ryšková., E. Pozlerová, R. Kračmarová, S. Plíšek, V. Palicka (University Hospital, Hradec Králové, Czech Republic): **Rapid diagnostic of bacterial meningitides by using molecular biology methods.**

Bacterial meningitis is a life–threatening infection. Rapid and accurate diagnosis is therefore of fundamental importance for effective treatment. Polymerase chain reaction (PCR) based diagnostic assays have been used to improve the detection of bacterial agents in cerebrospinal fluid (CSF).

Biological samples (n = 32) were obtained from different patients who were admitted to Teaching hospital in Hradec Králove from Jun 2000 to November 2001 due to suspected bacterial meningitis.

The multiplex PCR method was used for *Haemophilus influenzae and Streptococcus pneumoniae* (16S rRNA) detection. Standard PCR was used for *Neisseria meningitidis* (conservatory regulatory gene) detection, nested PCR was used to detect Listeria monocytogenes (hemolysin gene) and *Escherichia coli* (conserved regulatory gene).

From all samples were 15 PCR positive (*Neisseria meningitidis* 6 samples, *Streptococcus pneumoniae* 5 samples, Listeria monocytogenes 2 samples and *Streptococcus* spp. and *S. agalactiae*

1 sample). Culture positive were 11 specimens. We could not identified 4 culture positive samples due to other species. Culture was negative but direct smear and/or latex agglutination were positive in 4 cases.

PCR identified *N. meningitidis* in 2 and *S. pneumoniae* in 1 culture negative samples.

These techniques are able to confirm the presence of bacterial DNA in a CSF samples of patients (clinical symptoms correspond to bacterial meningitis) with culture – and/or microscopy (latex agglutination) negative cerebrospinal fluid. These diagnostic methods are considered to be very sensitive, specific and rapid.

SECTION 5: ENVIRONMENTAL MICROBIOLOGY

P. Švec[1], *I. Sedláček*[1], *L. A. Devriese*[2], *M. Baele*[2], *M. Vancanneyt*[3] *and J. Smola*[4] ([1]Czech Collection of Microorganisms (CCM), Masaryk university Brno, Czech Republic; [2]Laboratory of Bacteriology, Faculty of Veterinary Medicine, Ghent University, Belgium, [3]BCCM/LMG Bacteria Collection, Ghent University, Belgium, [4]Institute for Microbiology and Immunology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic): **Analysis of atypical *Enterococcus casseliflavus* strains isolated from intestines of the garden snail *Helix aspersa*.**

Enterococci are gram-positive and catalase-negative cocci inhabiting commonly human as well as various animals. They can cause various human infections as well as nosocomial infections. Moreover, enterococci are often isolated from waters, plants, soil and foods.

Twelve yellow-pigmented and motile enterococci isolated from intestines of the garden snail *Helix aspersa* were studied in this work. They were phenotypically close to *E. casseliflavus*, but they showed certain unusual biochemical characteristics (positive sorbitol, melezitose, D-tagatose, amidon; negative Methyl-D-Mannosidase). tDNA-PCR (tRNA intergenic length polymorphism analysis) divided all strains studied into three groups in full agreement with biochemical test results and differed them from the other *E. casseliflavus* strains included in the analysis as well. Because the results obtained implied that the strains analysed could represent a new species, 16S rRNA gene sequences of two representative strains were determined (they were deposited in GenBank database as AF367977 and AF367978). Both sequences were very similar each other as well as to the other *E. casseliflavus* and *E. gallinarum* sequences deposited in the gene databases (> 99 % gene sequence similarity). DNA-DNA hybridizations were done to clarify taxonomic position of these strains. Representative strains were hybridised with *E. casseliflavus* LMG 10745^T and *E. gallinarum* LMG 13129^T. DNA-DNA homology values obtained were 73–93 % for all strains hybridised with *E. casseliflavus* LMG 10745^T and 17–20 % with *E. gallinarum* LMG 13129^T.

Our results unambiguously showed that the enterococcal strains studied belonged to the species *E. casseliflavus* although they showed some unusual biochemical characteristics. We suppose that they represent source-specific *E. casseliflavus* ecovars inhabiting intestines of snails. Moreover, this work confirmed that enterococcal strains isolated from „unusual" sources could differ in their phenotypical traits. The representative strains were deposited in the Czech Collection of Microorganisms (CCM) as *E. casseliflavus* CCM 4868, 4869, 4870 and 4871.

S. Doškařová[1], *I. Sedláček*[1] *a R. Pantůček*[2] ([1]Czech Collection of Microorganisms (CCM), Masaryk University Brno, [2]Department of Genetics and Molecular Biology, Faculty of Science, Masaryk University Brno, Czech Republic): **Biochemical and molecular taxonomy of gram-positive cocci isolated from skin of llamas.**

Macrococci are gram positive, catalase and oxidase positive cocci inhabiting skin of horses and ponnies. Their pathogenicity is unknown. The genus *Macrococcus* was earmarked from the genus *Staphylococcus* and described in 1998.

Eight strains isolated from the skin of *Lama glama* breeded in the ZOO in Brno were described and characterized in this work. All strains studied were gram positive and catalase and oxidase positive, mostly orange-pigmented cocci. Their biochemical traits were in full agreement with the description of the genus *Macrococcus*. They differ from all hitherto described macrococci by their positive nitrate

reduction, negative aesculin and DNA hydrolyses and negative glycerol acidification. The GC content of genomic DNAs were 45.1–52.2 %. Following molecular-genetic methods were done to clarify their taxonomic position – ribotyping, macrorestriction analysis and 16S rRNA gene sequencing.

All phenotype and genotype analysis results obtained divided studied strains into two homegeneous groups and confirmed that they represent strains of the genus *Macrococcus*. Moreover, all strains were clearly dictiguished from all hitherto described macrococcal species. Our results imply that they represent two new species of the genus *Macrococcus*. This suggestion will be confirmed by DNA-DNA hybridisations. All strains studied were deposited in the Czech Collection of Microorganisms as *Macrococcus* sp. CCM 4808 – CCM 4815.

J. Janda (Czech Collection of Microbes, Brno and Department of Microbiology, Faculty of Science, Masaryk University Brno, Czech Republic): **Gram negative bacteria in the cast waters.**

The object of the research was one-year monitoring of bacterial habitat of the Jedovnice stream by the three groups of gram negative rods - *Enterobacteriaceae, Vibrionaceae* and gram negative nonfermenting rods. Only members of the family Enterobacteriaceae were identified to the species level. There were chosen 30 water sources of isolation (7 from the surface waters, 13 from the underground, 10 from the stalactite waters). From these localities there were taken the samples of waters four-times in one year. The three groups of gram negative bacteria were compared separately in each from the three types of localities (stalactite waters, surface waters and underground parts of stream). This procedure was done in every season of the year.

The stalactite waters have showed as relative very conservative. Gram negative nonfermenting rods formed over 50% of the all isolates, in the rest of the isolates dominated *Enterobacteriaceae* over the members of the family *Vibrionaceae*. Water temperature showed stable year value about 10 C. In the underground waters there was occurrence of the three groups of gram negative bacteria almost the same, except of the spring, where gram negative nonfermenting rods dominated. Water temperature was changed dependently on the year season in the relation between 5–15 °C. The surface waters were the most variable, except winter testing (gram negative nonfermenting rods dominated), the occurrence of the species of the family *Enterobacteriaceae* was the highest. At the same time the species of the family *Vibrionaceae* were in balance between spring and summer testing. Temperature in these waters was changed between 4–22 °C (one year period).

15 genera were identified as the members of the family *Enterobacteriaceae*, from which the three - *Serratia, Klebsiella* and *Enterobacter* - were isolated from the all types of waters and from the all isolation sites and they represented more than 50 % isolated strains of the family *Enterobacteriaceae* together. The other genera were found only in the some types of waters, or some season of the year. They were following genera: *Hafnia, Kluyvera, Escherichia, Yersinia, Citrobacter, Providencia, Morganella, Ewingella, Cedecea, Buttiauxella, Rahnella* and *Pantoea*.

The strains of the most frequent genera *Serratia* were studied in detail. They were studied on the base of biochemical and physiological tests with the assistance of cluster analysis. There were identified four species: *S. fonticola, S. rubidaea, S. proteamaculans* subsp. *proteamaculans, S. proteamaculans* subsp. quinovora, *S. liquefaciens* sensu stricto. One group of the isolates forming homogenic cluster and occurring in the all types of waters and taking seasons, was not identified into any known species. It was determined into the complex named *S. liquefaciens* sensu lato and its detail taxonomy position remains unclear and it will be the object of the further study.

L. Čapková, [1]*R. Horváth, A. Žákovská* (Faculty of Sciences, Masaryk University, Brno, Czech Republic [1]Genetic laboratory, CKTCH, Brno, Czech Republic): **Identification of *Borrelia burgdorferi* spirochetes in midgut of haematophagous and non haematophagous arthropods.**

During the period from March 1999 to April 2000 the presence of spirochaetes was observed in the midgut of haematophagous and non haematophagous arthropods using the method of dark field microscopy (DFM).

In positive samples spirochaetes were further detected by method PCR into species *Borrelia burgdorferi.* If the number of observed spirochaetes in visual field overreached one hundred, the

cultivation in BSK-H medium has been made. All together 725 haematophagous and nonhaematophagous arthropods were examined, 34 of them were positive (4.69 %).

DFM positivity of haematophagous: family *Ixodidae:* 7 Ixodes ricinus ticks (from total number of 182, which is 3.84 %); family *Culicidae:* 13 winter mosquitoes (from total number 218–5.9 %) and 7 summer mosquitoes (total number 159–4.4 %); family *Simuliidae:* 1 Simulium equinum (total number 9–11.11 %). DFM positivity of non haematophagous: family *Trichoceridae:* 5 Trichocera regelationis (total number 54–9,26%); family *Chironimidae:* 1 sample (of total number 27 - 3.7 %). In the the families *Lymoniidae* (8), *Anisopodidae* (5), *Bombilidae* (5) and *Muscidae* (12) no spirochaetes were observed.

In 10 arthropods the occurence of *Borrelia burgdorferi sensu lato* were found by methods PCR: 1 *Culex pipiens molestus* - winter mosquito, 4 *Ixodes ricinus* ticks (3 nymphs and 1 female), and 5 *Trichocera regelationis* nonhaematophagous. 2 *Ixodes ricinus* ticks isolated strains were taken to detect using method SDS-PAGGE and determined as *Borrelia afzelii*.

Acknowledgement: J07/98:143100008

M. Gódyová (Department of Soil Sciences, Faculty of Science, Comenius University, Bratislava, Slovakia): **Microscopic fungi - agent of biodeterioration in environment.**

Microscopic fungi are very adaptable organisms, more often spreading to new and atypical biotops. They are the significant agent of material biodeterioration and their decompose activities cause a lot of problems. Four historical objects presented in this study belong to atypical biotops. Two late-baroque chapels in Trnava, depositories at the Slovak National Museum in Martin, a crypt of Chatam Sófer in Bratislava and the late-gothic church in Okoličné were analysed. They all were very intensively contaminated by microscopic fungi.

Samples were collected by wiping with sterile cotton-wool from wall-paintings, wooden sculptures, altars, pictures and tombstones and carried onto media in Petri dishes in the laboratory. The sedimentation method of removal was used for air contamination. The testing of three petrification solutions (Solakryl BT-55, oil of cloves, beeswax in toluene) using in restoration was also the part of the presented study.

Altogether 26 genera and 52 species of microscopic fungi were isolated. The highest contamination of micromycetes was recorded in the church of Okoličné (19 genera and 33 species) and in the crypt of Chatam Sófer (19 genera and 24 species). In these objects two rare and for Slovak mycology also new species, *Penicillium arenicola* and *Merimbla ingelheimense*, have been found. *Penicillium* sp. and *Aspergillus versicolor* were the common contaminants of all analysed objects. Genera and species *Cladosporium* sp., *Ulocladium* sp., *Alternaria* sp., *Aspergillus* sp., A. flavus, A. ustus, A. versicolor, A. wentii, Penicillium sp., *P. chrysogenum* and *P. expansum* had the most often occurrence. The results of petrification solutions testing showed their low resistance against micromycetes colonisation.

J. Sedláková, M. Ďuranová (University of Constantine the Philosopher, Department of Botany and Genetics, Nitra, Slovakia): **Evaluation and quality classification of surface water of National nature reserve Parížske močiare – the swamps.**

In the article we focus on evaluation of surface water quality from National nature reserve Parížske močiare – the swamps, in relation to biotic components of landscape. Evaluated area is an important wetland biotope of international importance legislatively protected as a national nature reserve (NNR), and it also belongs among Ramsar localities. Concerned area represents a lower part of flood plain of Paríž stream between villages Nová Vieska and Gbelce with soils of high humus horizon and organogenic fen sediments. Dominant vegetation type is common reed (Phragmites), which is locally cut out. For evaluation and classification of surface water quality in the NNR Parížske močiare – the swamps, we used the norm STN 75 7221, valid from January 1999. The markers that we have chosen and analysed are: markers of oxygen regime (O_2, BOD_5, CHOD-Mn), basic physical-chemical markers (pH, water temperature, Cl^-), nutrients (NH_{4+}, NO_{2-}, NO_{3-}, PO_4^{3-}) in mg. l^{-1} and microbiological markers (coliform bacteria, thermotolerant coliform bacteria,

enterococci, psychrophile bacteria and myxobacteria) in colony-forming-units / ml (CFU / ml). In the year 2000 we took water samples in April, July and September and in the year 2001 in January and at the beginning and at the end of March. The analyses show that the surface water of NNR Parížske močiare – the swamps, belongs to the water quality class V. In the monitored sampling areas, together six, we noticed increased level contents of ammonia (measured maximum is 7.6 $mg.l^{-1}$), nitrate (in intervale 10,1–18,30 mg $.l^{-1}$) and phosphate (measured maximum – 1.6 $mg.l^{-1}$). Water pH is in the intervale 7.41–9.01. The number of coliform bacteria is in untervale 0–60 000 CFU ml^{-1}, thermotolerant coliform bacteria 0–18 $CFU.ml^{-1}$, enterococci 0–6 $CFU.ml^{-1}$, psychrophile bacteria 78–420 000 $CFU.ml^{-1}$ and myxobacteria with the maximum of 64 $CFU.ml^{-1}$. Measured results indicate a high level of general, fecal and agricultural pollution

SECTION 6: BIOTECHNOLOGICAL APPLICATIONS IN MICROBIOLOGY

M. Strouhal[1], *R. Kizek*[2,3], *M. Němec*[1] ([1]Department of Microbiology; [2]Department of Theoretical and Physical Chemistry, Faculty of Science, Masaryk University Brno; [3]Institute of Biophysics of Czech Academy of Science, Brno, Czech Republic): **Sorption of selected heavy metals by cells of *Yarrowia lipolytica* CCM 4510.**

The strain used in experiments was isolated from the soil contaminated by crude petroleum. This strain utilizes different sources of carbon and energy as petroleum distillates, sugars and organic acids etc. It is used in bioremediation and biodegradation processes. Recovering soil would have high concentration of different pollutants, e.g. heavy metals. Therefore we affected the cells by selected heavy metals. The cells were cultivated in Malt Extract Broth (MEB) with metals for 20 hours at 25^{O}C and intensively shaken. Chloride salts of cadmium, nickel, cobalt and zinc were added into the medium at start of cultivation. After cultivation the amount of heavy metal bound to different parts of the cells was measured. Amount of the metal linked onto the surface of cultivation bottle was established. The amount of metals was detected voltametricaly in acetate buffer and in MEB. Established concentration was correlated to control and calibration. Influence of heavy metals on growth parameters and minimal inhibition concentration (MIC) were established too.

Used metals had effect on measured parameters at different rate. MIC for this strain was 9.62 mmol/l of zinc, 2.45 mmol/l of nickel, 2.43 mmol/l of cobalt and 0.39 mmol/l of cadmium. Biomass production was directly proportional to used metal concentration. Cadmium decreased wet biomass from 12.6 to 6.3 mg/ml of medium (50 %) at concentration 37.5 mmol/l. 1000 mmol/l of nickel and 1500 mmol/l of cobalt produced the same effect. Zinc decreased biomass about 50 % at much higher concentration (4800 mmol/l, 12.6 to 5.9 mg/ml of medium). Specific growth rate was approximately half at the presence of all used metals at concentrations ahead MIC than control. Used metals were bounded onto various parts of cells at different rate. Percent ratio of bounded metals onto the cell parts was perhaps the same for every used concentration of metals. Concentration of cadmium measured in cytoplasm was 1.61 % only. Cell surface bounded cadmium at concentration under measurable limit. 1 g of wet biomass sorbed 76.4 % of total embedded cadmium. Under measurable limit were concentrations of nickel in cytoplasm and bounded onto the cell surface. 1 g of wet biomas linked similar concentration of nickel as cadmium - 74.4 %. Wet biomass (1 g) unbinded from medium 59.1 % of cobalt and 50.1 % of zinc. Zinc was sorbed onto the cell surface and in cytoplasm in proportion of about 5 %. Cobalt was bounded onto the cell surface in higher concentration than other used metals, 35.8 % was unbind from medium. Cytoplasm linked 4.8 % of total amount of cobalt in medium.

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Haloalkane dehalogenases are the enzymes naturally occuring in the microorganisms colonising contaminated environments. They are involved in biochemical pathways of many bacterial species

and play primary role in detoxication of halogenated aliphatic compound by hydrolitic cleveage of carbon-halogen bond. It is more suitable to use molecular methods based on the proof of gene encoding the enzyme for the screening of specific enzymes in bacteria rather than using the biochemical methods based on the proof of specific enzymatic activity. Thus optimalized molecular methods can serve as a quick instrument for screening of a large number of samples in a short time.

On the basis of the previous study (*Jesenska et al.*, 2000) providing the evidence of dehalogenases in *Mycobacterium tuberculosis* H37Rv and other clinically important mycobacteria and on the basis of the sequence comparison in genetic databases demonstrating the presence of genes encoding two putative haloalkane dehalogenases in *Mycobacterium tuberculosis* H37Rv named Rv2579 and Rv2296, the study was initiated for screening of putative haloalkane dehalogenases in selected strains of *Mycobacterium avium* subspecies *paratuberculosis*. Isolates of *Mycobacterium avium* subspecies *paratuberculosis* originated from variuos sources of animal tissues and were acquired from the collection of Veterinary Research Institute.

The aim of this work was screening of *rv2579*-like gene and *rv2296*-like gene in 48 selected strains of *Mycobacterium avium* subspecies *paratuberculosis* using polymerase chain reaction and DotBlot hybridisation. DotBlot hybridisation and polymerase chain reaction are the molecular methods which enable a quick identification of specific DNA sequences in the entire bacterial genom. DotBlot hybridisation is based on the complementary matching of specific DNA probe labeled with the easy detectable marker with the unknown source of DNA. PCR products from *Mycobacterium bovis* 5033 were used as the probes with myc1 and myc3 primer combination in the case of *rv2579*-like gene and with TBC1 and TBC2 primer combination in the case of *rv2296*-like gene. Polymerase chain reaction is based on the amplification of the short region of DNA. The specific primers flanking required region, the termostable DNA polymerase and the source of DNA as a template are used for PCR amplification. Primer combinations myc1 + myc3 and myc5 + myc6 were used for detection of *rv2579*-like gene and combinations TBC1 + TBC2 and TBC3 + TBC4 were used for screening of *rv2296*-like gene. The results acquired from these methods will be compared with biochemical methods to verify validity of both these approaches.

H. Hronská, M. Rosenberg, Ľ. Krištofíková (Department of Biochemical technology, Faculty of Chemical Technology, Slovak Technical University, Bratislava, Slovakia): **Microbial production of tartaric acid.**

L-tartaric acid is organic acid which is widely used in the food and pharmaceutical industry. Nowadays, it can be produced by two technological processes – chemical synthesis and fermentative method. It can be produced from the less and argols, by-products of the wine industry, and by catalytic epoxidation of maleic acid.

The increasing demand for tartaric acid has initiated the search of the new fermentative methods for its production. It could be produced by bacterial fermentation either from saccharidic substrates (glucose or gluconic acid) or from epoxysuccinic acid. Glucose or 5-oxogluconic acid can be utilized by bacterial strain of the genera *Acetobacter*, especially *Acetobacter suboxydans*. The low yields of L-tartaric acid and the formation of by-products, such as glycolic acid (the inhibitor of cell growth) are the disadvantages of those fermentations.

Another method of L-tartaric acid preparation is bioconversion of cis-epoxysuccinic acid or its salts by various species of *Nocardia, Acinetobacter, Agrobacterium, Rhizobium* and *Pseudomonas*. Bioconversions are conducted under non-growth conditions without formation of the by-products and the yields vary in the range from 92 to 100 %.

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L. Sláviková (Department of Biochemical Technology, Faculty of Chemical Technology, Slovak University of Technology, Bratislava, Slovakia): **Strategy of solid state fermentations and their industrial use.**

An increased interest in solid state fermentation (SSF) has been observed over the last decade. These processes are important from ecological and economic point of view. Moreover, attention is nowadays focused on finding of new alternative energy sources and materials, and thus, SSF as microbial technologies which utilize raw materials or co-products from other industries and transform them to useful products could satisfy market claims. The result of SSF could be either substrates enriched in nutrients (proteins, vitamins, polyunsaturated fatty acids) or production of large scale of compounds as enzymes, organic acids, lipids, biologically active metabolites and fermented foods (cheeses, tempeh, koji).

M. Ďuranová, J. Sedláková (University of Constantine the Philosopher, Department of Botany and Genetics, Nitra, Slovakia): **Changes in content of aminoacids during the solid-state cultivation of microorganisms (*Trichoderma viride*).**

The aim of the work was to find out the changes in concentration of aminoacids during the solid-state cultivation. We monitored the changes in content of single aminoacids in seven variants after 0 hour, 72 hours, 120 hours and 168 hours. Substrates used for solid-state cultivation were rich on glutamic acid (10.50–31.50 %) and proline (4.00–10.40 %). It is well known that prolamine fractions of protein complex in grains of cereals have a low content and unbalanced representation of essential aminoacids. During the solid-state cultivation, the content and proportion of single aminoacids was changing according to quality of different substrate variants, which influenced the growth and reproduction of filamentous fungi *Trichoderma viride* and also the further transformation of saccharide substrates to microbial protein. High changes in content of single aminoacids in combination with straw were monitored in variant 160. The content of threonine was highly increased after 120 hours, it was of 77 % and after 168 hours, it was increased of 80 %. The content of isoleucine was also highly increased after 120 hours. It was increased of 65.70 %. The increase of limiting aminoacid from the point of view of the monogastric animal – lysine – we noticed after 120 hours, of 32 %. On the other hand, from the non-essential aminoacids we noticed decrease of glutamic acid of 23.30 % and proline of 39.90 % after 168 hours but, the content of asparagine was increased of 91.70 %, alanine of 69.30 % and serine of 47.50 %.

In variant 260 was higher increase of essential aminoacids and decrease of non-essential aminoacids. The content of essential aminoacids increased of 30.40 % from initial 36.138 mg . g^{-1} to 47,104 mg . g^{-1}. High increase was noticed in content of threonine, it was of 61.10 % after 168 hours. The content of leucine increased after 168 hours of 56.70 % and the content of lysine of 35.50 %. The content of non-essential aminoacids declined of 5.50 % from initial 62.677 mg.g-1 to 59.230 mg . g^{-1}, while higher decrease was noticed in the content of glutamic acid of 21.20 % after 120 hours and after 168 hours of 26.20 %. The content of proline decreased after 120 hours of 23.10 % and after 168 hours of 26.70 %. The content of alanine highly increased after 168 hours of 42.70 %.

I. Voštiar, E. Šturdík (Department of Biochemical Technology, Faculty of Chemical Technology, Slovak University of Technology, Bratislava, Slovakia): **Applications of biosensors for monitoring of fermetation processes.**

Monitoring of fermentation processes is very significant factor necessary for active driving of the course of these processes. Knowledge of the actual state of the fermentation process allows more effectively influence the course of process. Demand for a fast and accurate monitoring open wide opportunities for applications of the biosensors.

Biosensors are analytical devices with incorporated biological component. Biospecific interaction of biological component and analyzed substrate is a source of analytical signal. Highly specific detection allows application of biosensors in analysis of the matrices with complex composition, such as fermentations broths.

Different aspects of the biosensors applications in monitoring of fermentation processes are discussed. In a first part is described interfacing of biosensors into bioprocesses with respect to a choice of appropriate sampling strategy which is simple and applicable in flow-through devices for on line monitoring (e. g. SIA, FIA, CFA). Discussed are also problems which are limiting for biosensors applications (e. g. operation stability, sensitivity to the effect of extreme physical and chemical factors etc.)

At the final part there is listed compendium of the published realizations of the fermentation monitoring using of amperometric, calorimetric or optical biosensors in off-line and on-line configurations.

SECTION 7: MICROBIOLOGY IN EPIDEMIOLOGICAL RELATIONS

R. Hubač, M. Hartmanová (Department of Epidemiology, PMMA Hradec Králové, Czech Republic): **Decontamination possibilities in conditions of the Czech Army.**

This short comunication informs about the current decontamination possibilities in conditions of the Czech Army, disinfection is especially stressed. In the personal outfit the soldiers have got DIKACIT that serves to individual drinking water treatment. A mobile 2-chamber disinfection apparatus PDP-2 together with a sterilization-distillation set ZSD are used for the linien disinfection. The medical material is sterilized in a field autoclave Aut-41 or in ZSD. Sterile apyrogenic distilled water is prepared using a small distillation set (MADES) or ZSD. The personnel operating with PDP-2, ZSD should be trained for work with moderate-pressure steam boilers. Dry and wet procedures are used for decontamination of vehicles, technology and the spaces. The dry method is an action of rapid hot aircraft engine combustion products into which a liquid hypochlorite suspension is sampled. The wet procedure is based on depositing decontamination mixture using a spraying line of flow or a spraying frame.

A hypohlorite mixture is used for disinfection. Application of foams is a developmental trend for disinfection in the Czech Army.

H. Jelínková, P. Pazdiora, M. Švecová, K. Fajfrlík, G. Čertíková, R. Bícová (Department of Epidemiology, Faculty of Medicine in Pilsen, Czech Republic): **The Occurence of Pneumoniae in the Teaching Hospital in Pilsen in The Years 1996–2000.**

Pneumonia is a weighty sanitary problem and a part of them have be treated during the hospitalization. Their etiology isn't as a rule determined, the diagnosis leans on a physical and rentgenology finding.

In the year 1996 discovered the possibility to use new serological diagnostic methods. And so with the aim to map the situation and to extend the serological diagnostic we began in autumn 1996 the observation of "community acquaired" and nosocomial pneumonias in the Department of Tuberculosis and Respiratory Diseases and in the Department of Medicine II of the teaching hospital in Pilsen.

In the period November 1996 – December 2000 were caught up in this two departments 253 pneumonias. In this set were found out 241 "community acquaired" and 12 nosocomial pneumonias. By the patients were performed the serological examination of 1–2 specimens of a blood. Laboratory was the diagnosis of the "community acquaired" pneumonia acknowledged by 123 (51.0 %) patients. The most frequent etiological agents were *Chlamydia pneumoniae* (57%), *Pneumococcus* (14%), virus of influenza A (7%), *C. psittaci* (6%), *Mycoplasma pneumoniae* (6%), coronaviruses (3%), RS viruses (1%), *Coxiella burneti* (1%). In 28 cases was catched up the mixed infection. In the etiology of nosocomial pneumoniae asserted most frequent *C. pneumoniae* and pneumococcus. We watched the seasonal occurence of this agents too. With some microorganisms we could meet during all the year, by some others was found out only the seasonal occurence.

The results of the serological study were compared with the routinely diagnostics. During the observation significant improved the routinely laboratory examination in the department of Tuberculosis and Respiratory Diseases above all.

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A total of 2030 *Ixodes ricinus* ticks (264 females, 238 males, 1115 nymphs and 112 larvae) were collected by flagging in park Pisárky (Brno) in 1997–2000. The midgut of each tick was removed under stereomicroscope, homogenized in a 0.9 % NaCl solution and observed by dark-field microscopy.

In 1997 the positivity of 448 *Ixodes ricinus* ticks was 9,6%

In 1998 the positivity of 424 *Ixodes ricinus* ticks was 12,3%

In 1999 the positivity of 360 *Ixodes ricinus* ticks was 3,6%

In 2000 the positivity of 1232 *Ixodes ricinus* ticks was 2,1%

From all samples 125 tick homogenates (6.85 %) were found to be infected by *Borrelia* species, from which positivity of 2.7 % was observed in larvae; 7.0 % in nymphs; 6.8 % in females; 10.9 % in males. Most of positive samples were further analysed by PCR method with *B. b.* sensu lato specific primers designed for the flagellin gene and afterward also nested PCR method was developed.

From positive ticks we prepared 6 spirochaete isolated strains, which were determined by comparison of protein composition to standard stems (*B. b.* sensu stricto, *B. afzelii, B. garinii, B. lusitaniae* and two non *Borrelia spirochaete* strains) using gradient SDS-PAGE method. These isolated strains were determined as *B. garinii* and *B. afzelii.*

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J. Kadeřábek[1], *A. Souček*[1], *M. Sedláčková*[2] ([1]Department of Medical Microbiology, 1st Medical Faculty, Charles University Prague, [2]Department of Paediatrics, 1st Medical Faculty, Charles University Prague, Czech Republic): **Seroprevalence of *Helicobacter pylori* infection in asymptomatic children and adolescents.**

128 children and adolescents with no gastrointestinal disorders, aged 0–19 years (mean age 10,73 years), have been examined for presence of specific serum antibodies IgG against *Helicobacter pylori.* The sera were acquired from the biochemical laboratory where they had been sent because of another examination.

The following methods were used for detection of serum antibodies against H. pylori:

1. The ELISA test with antigen from whole cell bacterial sonicates of *H. pylori* strains. This modified method had been correlated with direct microbiological examination (culture) and histological finding before. Values of S-IgG antibodies were significantly higher ($p < 0.0001$) for the microbiologically positive findings ($x = 3.94$, $s = 1.26$) than for the negative findings ($x = 0.72$, $s = 0.71$). The specificity of our test was 96.1% and the sensitivity 90.3% (discrimination analysis, correlation coefficient = 0.856, $p < 0.001$). The resultant value was expressed with the coefficient P/N. P was the absorbance of the tested serum, N was the mean absorbance of two negative sera. Positive sera had the coefficient $P/N > 2.5$, negative ones $P/N < 2.0$. The result between these values was considered as indeterminate – belonging to the 'grey zone'.

2. The Western blot (BLOT *Helicobacter pylori* IgG and IgA, Test-Line Ltd.) was used only for selected sera.

The level of antibodies IgG, reflecting presence of H. pylori infection, was ascertained in 3 children (2.34 %) with the ELISA test. 2 children belonged to the 'grey zone'. To get more precise results, the Western blot IgG was used for the sera with P/N greater than 1.5. After uniting the results of ELISA and Western blot, 8 children (6.25 %) were found in the whole group that we can consider to be infected by H. pylori because of presence of specific antibodies IgG. We also determined IgA antibodies against H. pylori with the Western blot to exclude persistence of maternal IgG antibodies in 2 children. Specific antibodies IgA were found in both children.

IgG positivity of serum antibodies was found in age groups of children:

- to 5 years in 8.7 % children (2/23),
- 6–10 years in 3.1 % (1/32),

• 11–15 years in 6.7 % (3/45),
• 16–19 years in 7.1 % (2/28).

The presented results show relatively low prevalence of specific IgG serum antibodies in our group. This corresponds well with results obtained in similar studies conducted on western Europe children. Unlike previous studies we have not observed increase of seropositivity in elder children.

M. Vrba (Department of Clinical microbiology, Faculty Hospital Brno, Czech Republic): ***Escherichia coli* O 157.**

E. coli O 157 is one of the most significant pathogenic *E. coli*. The ruminants, especially cattle, are a reservoir of this serotype. Man is infected by contaminated foods and water and also by direct contact. *E. coli* O 157 causes not only diarrhoea and haemorrhagic colitis but even haemolytic uremic syndrome (HUS). This disease is caused by the effect of verotoxins that are produced by *E. coli* O 157.

The microbiological diagnostic is founded on the cultivation of stool and on the detection of verotoxins in cultures and stool. We isolated one strain *E. coli* O 157 in our laboratory from stool of a child with HUS last year. This strain produced verotoxin VT1 and VT2.

SECTION 8: MICROBIOLOGY IN CLINICAL RELATIONS

J. Zahradníčková, L. Elstnerová, M. Janková, Z. Šedová (Department of Neonatology, 2nd Children Internal Clinic of Faculty Hospital in Brno, Czech Republic): **A peracute *Klebsiella* sepsis in a premature neonate.**

Neonatal sepsis is a clinical syndrome of systemic illness acompanied by bacteremia occurring in the first month of life. In considering the first symptoms of neonatal sepsis, two clinical situation may be defined: early-onset (the first 5–7 days) and the late-onset (as early as 5 days of age). The first of them is characterized by a sudden onset and fulminant course that can progress rapidly to septic shock with a high mortality rate. Praematurity is the single most significant factor correlated with septicaemia.

We report an immature girl with the peracute course of early-onset neonatal sepsis caused by *Klebsiella pneumoniae*. The first symptoms occured suddenly at 3 days of the girl's life, and they were: tachykardia, respiratiry failure, development of DIC with successive bleeding into the central nervous system and formation of a subdural haematoma that had to be treated using an operation.

The causative agent was found in the blood culture and in the respiratory system. In was probably a hospital infekcion. The therapeutic effect was likely due to cefotaxime, althougt other antibiotic (ampicilin, co-ampicilin, amikacin, netromycin) were also used.

I. Kocmanová (Department of Clinical Microbiology, Faculty Hospital Brno, Czech Republic): **Cryptococcal Meningitis – a case study**.

Nowadays the cryptococcal infection arouses an interest especially in connection with Acquired Immunodeficiency Syndrome (AIDS). Of course, it is not possible to omit other predisposing factors – especially lymphomas, collagenosis, diabetes mellitus, steroid therapy etc.

Rare symptoms often lead to a late diagnosis and a delayed therapy. The cytology and biochemistry findings are normal in 20 % of patients.

At the neurological clinic of FN Brno 47-year old patient was hospitalised for a several months lasting headache of an unknown origin. The patient status was continually worsening and the liquor sample was taken. After the cultivation *C. neoformans* was confirmed in the sample. In spite of the started relevant therapy the patient died.

P. Paterová (Department of Microbiology, Hospital Pardubice, Czech Republic): **Occurance of novobiocin-resistant staphylococci in clinical specimens.**

Recently coagulase-negative staphylococci have been considered potential pathogens and they can cause various kinds of infections (especially in immunocompromised patients). The simpliest test to diferentiate coagulase-negative staphylococci is the sensitivity to novobiocin.

During a six-month period we collected 50 isolates of novobiocin-resistant staphylococci. They were obtained from various specimens (23 specimens of urine, 11 swabs from wound infection, 6 blood isolates and 9 other specimens). Strains were speciated by the Lachema Staphytest identification system. The commonest isolate was *S. saprophyticus* ssp. *saprophyticus* (42.9 %), then *S. cohnii* ssp. *cohnii* (28.6 %), *S. sciuri* ssp. *sciuri* (10.2 %), *S. hominis* ssp. *novobiosepticus* (8.2 %), *S. cohnii ssp. urealyticum* (6.1 %), *S. xylosus* (4.1 %). *S. saprophyticus* ssp. *saprophyticus* was found in the adequate quantity in urine obtained from 9 females (aged 31 on average) and 5 males (2 aged under 11 years, 3 over 67 years). We found 15 novobiocin-resistant staphylococci in swabs from wound infections and aspirates from abscessus, however, only one strain was collected twice. Interesting strains were from haemocultures of patients with assumed bacteriaemia, where *S. hominis* ssp. *novobiosepticus* was identified in 3 of 6 cases. The sensitivity to all the investigated antibiotics displayed 30.6 % of collected strains, 51 % of izolates were resistant to 1 or 2 antibiotics (the most frequent erythomycin and tetracyclin). The rest was resistant to 3 and more antibiotics – this group includes all strains *S. hominis* ssp. *novobiosepticus.*

Novobiocin-resistant staphylococci are an interesting, but till this time, a little bit omitted group of clinical isolates.

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Pneumocystis carinii (PC) is a very particular fungal microorganism with a number of specific features; it cannot be equated with any fungal genus or species hitherto known. It is resistant to both fungistatic and fungicide agents. Pneumocystis pneumonia is found in patients receiving immunosuppressive therapy, in oncological patients, in patients with inborn immunodeficiency, and particularly in patients with AIDS.

In individuals with no immunity deficit, according to most literary data, PC seems to be an innocuous, ubiquitous microorganism. This opinion is also confirmed by the frequent occurrence of antibodies in the general population (75 %). Symptoms of PC pneumonia (PCP) most often cited in literature are non-productive cough, dyspnea, chest tightness, and fever. Bilateral infiltrates,interstitial in character, are the most frequent x-ray findings.

We decided to evaluate clinical findings of patients who had had PC proved in bronchoalveolar lavage.

In the year 2000, we indicated bronchoalveolar lavage (BAL), followed by the evaluation of bronchoalveolar fluid (BAF), and in 73 (100 %) patients with pathological X-ray and symptoms persisting despite repeated antibiotic therapy. Twelve (16.4 %) patients had hemato-oncological diseases, 3 patients (4.1 %) were treated for solid tumors, and 59 (79.5 %) had protracted pulmonary disease or respiratory symptoms without any precise pulmonary diagnosis yet made.

In all 73 patients, BAL was performed complete with a complex cytological and microbiological evaluation of BAF. To identify PC, BAF from each patient was evaluated by immunofluorescence using monoclonal antibodies (Institute of Microbiology of the Masaryk University Faculty of Medicine; St Ann’s University Hospital).

PC was proved in BAF in 27 (36.9 %) out of 73 patients. In 13 out of these 27, a disease was present which might have caused the deficit of immunity. In 12 further patients with a proof of PC in BAF, no reason for decreased immunity was found. PC was proved using PCR in 15 patients (20.5 %), using monoclonal antibodies in 11 (15 %), and using both in 1 female patient (1.37 %). Bilateral interstitial pulmonary infiltrates were seen in none of the patients. Antipneumocystis therapy was started in 15 patients (20.5 %) after the assessment, discussed in this paper, of the patients’ clinical findings. In 7 patients (9.6 %), control BAL was performed complete with control BAF evaluation after the termination of the treatment. After the antipneumocystis treatment’s end, PC was no more proved in any of the patients.

Before indicating BAL with the evaluation of BAF to prove the presence of PC, the indication of the examination has always to be reassessed meticulously. Once PC in BAF is proved, the decision is to be made concerning the treatment. This is made in the light of presence or absence of an immunocompromising disease, or, in patients with no unequivocally proved disorder of immunity, on the basis of clinical signs.

J. Bednářová, H. Štroblová (Dept. of Clinical Microbiology, Faculty Hospital Brno, Czech Republic): **Intrathecal Synthesis of Specific Antiviral IgG Antibodies in Patients with Multiple Sclerosis.**

The aim of our study was to detect the intrathecal synthesis of specific IgG antibodies against the viruses of measles, rubella and varicella zoster in patients with definite multiple sclerosis. Presence of these antibodies, called MRZ reaction (M – measles, R – rubella, Z – varicella zoster), is typical for chronic inflammatory autoimmune diseases of the nervous system.

We investigated a cohort of 20 patients: 17 patients were diagnosed as multiple sclerosis and 3 patients served as negative controls. Serum and CSF samples were analysed at each patient. The diagnostic kit of Human Company, Germany (M, R, and Z Virus Human ELISA IgG Antibody Test) was used for the detection of specific IgG antibodies. The intrathecal synthesis of specific IgG antibodies was evaluated as specific antibody index – AI according to Reiber's method. Values of AI > 1.4 indicated the intrathecal synthesis.

Intrathecal synthesis of IgG antibodies against measles, rubella and/or varicella zoster viruses (AI > 1.4) was detected in 95 % of patients with multiple sclerosis. Antibody index against measles was positive in 80% patients, against rubella in 50 % of patients and against varicella zoster virus in 35 % patients. Intrathecal synthesis of specific antiviral IgG antibodies was not detected in patients who served as negative controls (AI in the range 0.7–1.3).

MRZ reaction supplements diagnostic methods used in patients with multiple sclerosis. It is significantly valuable in diagnostically problematic cases where other methods (magnetic resonance, oligoclonal IgG bands) do not provide definite diagnosis.

SECTION 9: ANTIMICROBIAL THERAPY

Z. Šándorčínová, E. Bogyiová, V. Takáčová, L. Siegfried (Institute of Medical Microbiology, Medical Faculty of Šafárik University, Košice, Slovakia): **Evidence of extended-spectrum beta-lactamases (ESBL) in *Klebsiella pneumonie* on the basis phenotype tests and PCR.**

Klebsiella pneumoniae is prominent among the gram-negative bacteria causing nosocomial infections and an important source of transferable antibiotic resistance.

Bacterial strains (150) were isolated from immunocompromised patients hospitalised in the ICUs (University Hospital, Košice). In the ESBL-suspected strains ($MIC_{\text{third generation cephalosporins}} > 2$ μg/ml) double disc synergy test (DDST) was carried out and production of ESBL was verified by specialised Etest for ESBL. On the basis of both tests 17 strains of *Klebsiella pneumoniae* were included to the ESBL producers (11,3 %).

As the SHV family is prominent among beta-lactamases produced by *Klebsiella pneumoniae* and other *Enterobacteriaceae*, the PCR for evidence of genes encoded SHV beta-lactamases was carried out. We detected *blas*HV genes in all 17 ESBL-positive strains.

H. Žemličková, P. Petráš, P. Urbášková (National Institute for Public Health, Prague, Czech Republic): **Detection of oxacillin resistance in coagulase-negative staphylococci.**

A rapid slide latex agglutination test (LA test), MRSA-Screen (Denka Seiken), which detect PBP 2a, was tested for ist ability to differentiate between mecA-positive and mecA-negative coagulase-negative staphylococci. A total 74 clinical isolates from 4 species were examined for susceptibility of oxacillin by disc diffusion method, broth microdilution method and screening test. According to the NCCLS breakpoint for oxacillin resistance (ł 0,5 mg/l; Ł 17mm) were identified

60 oxacillin resistant isolates. The LA test was performed with oxacillin induced isolates and also without induction on parallel. The LA test without induction of PBP 2a required 1 to 9 min for positive reaction. With induction of PBP 2a, all 60 oxacillin resistant isolates were positive within 1 min. For reliable detection of oxacillin resistance by the MRSA-Screen in coagulase-negative staphylococci, induction PBP 2a is very useful.

K. Vaňová, M. Bednář (Department for Medical Microbiology, 3rd Medical Faculty, Charles University, Prague, Czech Republic): **The verification of conformity between the critical and minimal inhibitory concentration of antibiotics.**

The new method for an assesment of the antibiotic concentration under the forming inhibitory zone edge (critical concentration) by the disc method is based on the formula described by Humphrey and Lightbown in 1952. This formula has been changed to eliminate the antibiotic diffusion constant if two or more discs with various concentrations of antibiotic are used. So there is no need of preliminary calibration and that raises the flexibility of this new multidisc method. The method can be compared to E-test. Its result is the critical, not minimal inhibitory concentration of the drug. The prevailing proximity of critical and minimal inhibitory concentration allows thinking of practical exploitation of this new variant of multidisk method.

Critical and minimal inhibitory concentration regularly show their correspondence among broad spectrum of different grampositive and gramnegative bacteria and/or antibiotics. Only in methicillin- heteroresistant strains of Staph. aureus the results are doubtful: the critical concentration in these cases is usually much lower compared to MIC. It can be explained by growth of resistant colonies inside the inhibitory zone while the measured inhibitory zone edge is formed by sensitive population.

L. Ryšková, P. Čermák, R. Křížová (Department of Microbiology, University Hospital, Hradec Králové, Czech Republic):**A rapid metod for antimycobacterial susceptibility testing.**

The performance of the BACTEC MGIT AST SIRE antimycobacterial susceptibility testing of Mycobacterium strains to streptomycin, isoniazid, rifampin, and ethambutol were evaluated in our laboratory. The MGIT AST system is a rapid procedure based on analysis of fluorescence in a drug containing tube and in a growth control tube.

Twenty three clinical isolates were tested by this method and the results were compared with those of the method of proportion on Löwenstein-Jensen media, which was considered the reference method. Agreement rates between MGIT AST results and the method of proportion were 100 % for streptomycin, 78 % for isoniazid, 100 % for rifampicin and 96 % for ethambutol. The mean time for MGIT AST was 6,5 days (for the method of proportion it was 21 days).

MGIT AST is a time-saving method, useful to reduce the duration of antimycobacterial susceptibility testing. This method is a reliable for susceptibility testing to streptomycin, rifampicin, ethambutol, additional studies are needed for isoniazid.

Z. Krakovská (Institute of Preventive and Clinical Medicine, Bratislava, Slovakia): **Tolerance of environmental micromycetes to amphotericin B.**

Microscopic filamentous fungi – micromycets represent an important part of environment. Their negative effect (mycotic infections, mycotoxycoses, allergies) to human's health is significant.

The aim of this study was to test activity of amphotericin B – "the gold standard" used in therapy of deep mycoses - against soil fungi dependent on the cultivation medium (Sabouraud agar, malt extract agar, Czapek-Dox agar, dichloran-glycerol agar, potato-dextrose agar, Yeast Nitrogene Base agar).

Amphotericin B (0.1, 0.3, 0.5 μg/ml) were incorporated into a liquid medium with Bengal Rose (150 mg/l). Diluted soil samples (0.2 ml, 10^{-3}) were inoculated onto the surface of the plates and incubated at 25 and 37 °C for 7 days.

The highest number of amphotericin B–tolerant species were isolated onto Sabouraud agar, potato-dextrose and malt extract agar at 25 °C. We isolated *Absidia* sp., *Acremonium* sp., *Arthrinium* sp., *Alternaria* sp., *Aspergillus clavatus, A. fumigatus, A. nidulans,* aspergillus from

A. niger gr., aspergillus from *A ochraceus* gr., *A. ustus, A. versicolor, Cladosporium* sp., *Echinobotryum* sp., *Fusarium* sp., G*eotrichum* sp., *Gliomastix* sp., *Chaetomium* sp., *Chrysosporium* sp., *Mariannaea* sp., *Microsporum gypseum,* Mortierella sp., *Myrothecium* sp., *Myxotrichum* sp., *Paecilomyces* sp., *Paecilomyces lilacinus, Phialophora* sp., *Phoma* sp., *Penicillium sp., Scopulariopsis brevicaulis, Stachybotrys chartarum, Stemphylium* sp., *Talaromyces wortmanii, Trichoderma* sp. a *Veronaea* sp. At 37 °C, the situation was comparable. The strains of *Acremonium* sp., *Alternaria* sp., *A. fumigatus,* aspergillus from *A. glaucus* gr., *A. nidulans,* aspergillus from *A. niger* gr., *A. terreus, A. ustus, A. versicolor, Byssochlamys fulva, Eupenicillium brefeldianum, Fusarium* sp., *Chrysosporium* sp., *Mariannaea* sp., *Microsporum gypseum, Phialophora* sp., *Phoma* sp., *Penicillium sp., Scedosporium apiospermum, Scopulariopsis brevicaulis, Stachybotrys chartarum, Talaromyces wortmanii, Thielavia* sp., *Trichoderma* sp. and *Trichophyton terrestre* were isolated onto Sabouraud, potato-dextrose and malt extract agar with incorporated amphotericin B. It can be concluded that amphotericin B–tolerant strains should be considered as a rise-factor of the lifethreatening infections of immunosupressed patients.

SECTION OF POSTERS:

M. Stará, S. A. Bobegamage (Institute of Preventive and Clinical Medicine, Bratislava, Slovakia): **Experimental intraperitoneal and oral infection by coxsackie virus B4 (CVB4) using the murine model. Comparison of Serological Parameters.**

Experimental and epidemiological studies support the involvement of CVB4 as one of the etiological agents involved in the development of insulin dependent diabetes mellitus (IDDM). The intraperitoneal route of infection in the murine model is commonly used in the study of the pathogenesis of coxsackie B viruses. Little is known about the oral route which is the natural mode of infection of coxsackieviruses.

The aim of our present work was to compare some markers (glucose levels, antibody titers, levels of alpha, beta , gamma interferons and TNF alpha) in the mice infected by the intraperitoneal and the oral route. 3 - 4 weeks old Swiss albino mice were infected with 0.2 ml of $1x10^{2.5}$ $TCID_{50}$ (intraperitoneal) and 0.5 ml of $2 . 10^{9.7}$ $TCID_{50}$ (oral) of CVB4 and the control mice with PBS. The blood was collected by direct heart puncture at different intervals. Our results show differences in antibody titers and the cytokine patterns.

Š. Vejsadová, J. Felsberg , M. Weiserová (Microbiolgical Institute, Czech Academy of Sciences, Prague, Czech Republic): **The Role of the HsdS subunit for regulation restriction endonuklease *Eco*R124I.**

Restriction – modification (R-M) systems are interesting models for studying protein-DNA and protein-protein interactions. R – M enzymes are able to cleave invading foreign DNA and methylate cellular DNA. Type I R – M systems are encoded by three genes, which are required for production of restriction endonuklease. While *hsdM* and *hsdS* genes are capable of producing methyltransferase (MTase) required for modification.The HsdS subunit is responsible for DNA binding and contains conserved regions which are responsible for protein-protein interactions with the HsdM and HsdR subunits.

We used PCR-mutagenesis, in the presence of different concentration of Mn^{2+} to produce mutants of the central conserved region of the binding subunits of the type I restriction endonuklease *Eco*R124I. Complementation analysis between the plazmids carrying the PCR fragments and plasmid pKF650 (carries the *hsd* genes of the related *Eco*R124/3I system) was used to analyze the R – M fenotype, for discriminate DNA-binding mutations from subunit assembly mutations. From a total of two hundred transformants 71 mutants were obtained and of these mutants 61 were found to be able no restriction (no competition with the alternate HsdS subunit produced from pKF650 in the complementation assay). All mutants in the *hsdS* gene were used for DNA sequence analysis. The mutants were tested for their ability to bound with the HsdM subunit into the MTase. The test is based on the evidence that HsdM is

found to be soluble (in cytoplazmatic fraction) and HsdS insoluble (in membrane fraction) in bacterial lysates. In case of assembly HsdS with HsdM, the HsdS subunit is found in the soluble fraction as part of active MTase or as part ENase (komplex MTase with HsdR subunit).

Comparing of the DNA – sequence analysis, and tested R – M phenotype of the mutants, with results of biochemical analysis *in vitro*, the region of HsdS subunit altering function and assembly of the enzyme.

I. Voštiar[1]*, J. Tkáč*[2]*, E. Šturdík*[1]*, P. Gemeiner*[2] ([1] Department of Biochemical Technology, Faculty of Chemical Technology, Slovak University of Technology, Slovakia, [2]Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia): **Application *Gluconobacter oxydans* cell based biosensor with enhanced selectivity for monitoring of ethanolic fermentation.**

Gluconobacter oxydans possesses unique features including surface localization of oxidative enzymes, a broad range of oxidised substrates, partial oxidation and therefore high rate of substrate turnover. High efficiency of substrate oxidation is very promising for biosensor construction. Low selectivity of *G. oxydans* biosensor hampers its wider use. Recently a very sophisticated method for analysis of samples containing both ethanol and glucose was developed. This system consists of non-specific *G. oxydans* sensor, which detects both glucose and ethanol and of selective glucose sensor detecting only glucose. The concentration of ethanol must be subtracted from these two signals.

G. oxydans is able to oxidise glucose more efficiently than ethanol. Thus, a microbial sensor using *G. oxydans* cells is not suitable for ethanol monitoring during *Saccharomyces cerevisiae* fermentation without modification. For this purpose a very simple method was employed using size exclusion effect of a cellulose acetate membrane. Properly prepared cellulose acetate membrane is permeable only for molecules with molecular weight lower that 100 Da. In our biosensor construction a graphite electrode was modified by *G. oxydans* cells and covered by the cellulose acetate membrane. In a mediated system either ferricyanide or ferrocene was present in the bacterial layer. The sensitivity of the bacterial sensor increased in order unmediated, ferrocene mediated and ferricyanide mediated biosensor. Ferricyanide mediated microbial sensor is able to detect ethanol very efficiently with a sensitivity of 1.46 $mA.m.M^{-1}$ and response time of 60 s and did not react with glucose addition.

The effect of glucose interference on ethanol determination was strongly suppressed using the size exclusion effect of the cellulose acetate membrane. This system was successfully used in ethanol analysis of a fermentation broth containing high concentration of glucose.

P. Hejnar (Institute of Microbiology, Faculty of Medicine, Palacký University, Olomouc, Czech Republic): **Influence of cultivation temperature and incubation interval on values of minimum inhibition concentrations of -lactam antibiotic agents in *Stenotrophomonas maltophilia* strains of clinical origin.**

A standard dilution micromethod was used to determine the effect of cultivation temperatures (30 °C and 37 °C) and that of incubation intervals (24 and 48 hours) on in vitro effectiveness of ampicillin, ampicillin/sulbactam (AMS), cefoperazone (CPR), cefoperazone/sulbactam (CPS), ceftazidime (CTZ), piperacillin (PIP) and piperacillin/tazobactam (PPT) against 66 strains of *S. maltophilia* of clinical origin, After 24-hour incubation at 30 °C, the lowest occurrence of resistance was registered in CPS (4.5 % of resistant strains), and in CPR (45.5 %). After incubation prolonged to 48 hours, CPS (21.2 %) and CPR (81.8%) manifested again as the most effective agents. At cultivation temperature of 37 °C and incubation interval of 24 hours, resistant strains with the lowest frequency occurred at the application of CPS (21.2 %) and CPR (51.5 %). With the incubation interval increased to 48 hours, CPS (36.4 % of resistant strains) and CTZ (77.3 %) were found as the most effective agents. After prolonged incubation at 30 °C, the lowest increase in the number of resistant strains was evidenced in CTZ (by 4.5 %), the highest one in CPR (by 36.3 %); 48-hour incubation at 37 °C showed the slightest increase in AMS (by 10.6 %) and the greatest one in CPR again (by 34.9 %). The lowest average increase of the minimum inhibition concentration (MIC) was registered in CTZ at both temperatures (1.4 times at 30 °C, 2.1 times at 37 °C), the highest average increase was found in PIP

(11.3 times, and 5.0 times, respectively), The highest occurrence of *S. maltophilia* strains with maximally doubled MIC value after prolonged incubation interval and at both cultivation temperatures was proved in CTZ (100.0 %, resp. 82.6 %), the lowest one in PPT (27.8 %) at 30 °C, and in PIP (34.6 %) at 37 °C. CPS (with increasing effect in dependence on temperature decrease) was evaluated as the most effective one among the tested antimicrobial agents. The greatest "MIC stability" after prolonged incubation was found in CTZ, while CPR, PIP, and PPT appeared as markedly instable.

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A. Liptáková, L. Pastvová., H. Sehnálková, H. Kerestešová, M. Molokáčová, L. Siegfried (Institute of Medical Microbiology of Medical Faculty of Pavel Jozef Šafařík University, Košice and Department of Clinical Microbiology, Faculty Hospital in Košice, Slovakia): **Diagnostica of *Helicobacter pylori* in clinical materiál.**

Helicobacter pylori is important gastrointestinal pathogen. It is involved in gastric carcinogenesis. That is why the early microbiological diagnosis is necessary. The prompt diagnosis helps to provide exact antiinfectious therapy. In our department we detect *H. pylori* using direct and indirect methods: microscopical biopsies examination using Gram method, detection of urea activity in biopsies using UREA-HP test, anaerobic cultivation on VL agara and serological IgG antibody determination using ELISA test. 1740 gastric and duodenal biopsies were examined in 2000, 732 of them (42.06 %) were positive using at least one direct method. IgG antibody titers were determined in 2129 patients, in 1283 of them (60.26 %) were detected positive titers (more than 1: 300).

Nowadays non-invasive methods are preferred throughout the world, e. g.: noninvasive antigen-based assay in stool samples. This method is ease to use, speed and noninvasive. Also there are recommendations for serial evaluation of IgG titres before therapy and 3–6 months after therapy. Our aim in future is to involve these methods among our diagnostic tools in our lab.

P. Pavlik, J. Hanzen, M. Lisalova, P. Milošovič, M. Sládeková, G. Vozárová (HPL microbiological laboratory, Bratislava, Slovakia): **Comparison between disk diffusion method and MIC for penicillin of penicillin-resistant *Streptococcus pneumoniae* isolated from respiratory tract.**

Streptococcus pneumoniae is an infectious agens especially of respiratory tract diseases, conjunctivitis, otitis media, but serious life endangers illnesses, like meningitis, perikarditis and sepsis too. Many authors put unvirulent Streptococcus pneumoniae in smooth phase in to physiological bacterial flora of upper respiratory tract, mainly by children.

Sensitivity of *Streptococcus pneumoniae* to penicillin in near past was considered like unproblematic. At the turn of 80–90 years of last century started occur the first notes about PNC resistance in Spain, Hungary and then in Slovakia too. Dr. Moravcik from OKM Martin described like first strains *S. pneumoniae* with MIC for penicillin more than 16 mg/l. Slovakia from a point of view of *Streptococcus pneumoniae* resistance to PNC became highly problematic area. Our laboratory for isolation of PNC resistant strains uses screening diffuse test with 1 mg oxacilin. But this method it is not possible to determine intermediate sensitive strain and to distinguish it from resistant one. Therefore is necessary to examine MIC for penicillin. Intermediate sensitivity allows successful treatment appropriate higher dose of penicillin. In this project I present study about actually the latest situation of resistance in our region.

H. Hrbáčková (Department of medical microbiology, Faculty Hospital Motol, Prague, Czech Republic): **The role of antigenemia detection in diagnostics of active CMV infection in transplanted patients.**

The aim of study: to compare of efficiency of quantitative antigen detection of cytomegalovirus (CMV) from blood (antigenemia) for timely and succesful therapy of active CMV infection after bone marow transplantation (BMT) and organ transplantation.

Description of method: quantitative determination of cellular nuclei in which CMV matrix protein pp65 is presented was detected in 200 000 leukocytes isolated from peripheral blood. Presentation of protein manifests activity of CMV infection. The method is more sensitive than shell vial cultivation and much quicker than classical cultivation. Detection of antigenemia provides higher predictive value for development of active CMV infection than qualitative PCR detection.

Determination of efficiency - it means success of therapy: 1.) after application of virostatic therapy the value of antigenemia falls down becomes negative and clinical symptoms disappear. 2.) A patient has gone through no more than two episodes of CMV active infections. Repeated activations correspond to insufficient suppression of infection. It means higher risk of negative influence on organism (development of GVHD, PTLD or rejection).

Results: blood of the children after kidney transplantation, adult lung-transplant recipients and children after bone marrow transplantation (BMT) was repeatedly tested. 10 active infections were detected from examples of 36 examined patients after renal transplantation. Correlation among value of antigenemia, development of clinical symptoms, necessity of virostatic therapy and its success was discovered. 7 active infections were found among 24 lung-transplant recipients. 2 infections were symtomatic and succesfully treated primoinfections and 5 once were asymptomatic untreated reactivations. Number of primoinfections is very low and therefore will be essential to examine them further. 26 CMV episodes were detected when 76 patients after BMT were monitored. 6 patients suffered from symptomatic infection and they were treated by ganciclovir. Other infections were asymptomatic with low values of antigenemia and mostly without therapy. These patients were periodically monitored and immediatelly treated after detection of antigenemia higher than 10 positive nuclei per 200.000 leukocytes but in spite of that therapy was at 33.3% unsuccessful. These results indicate insufficiency of antigenemia detection as method for timely diagnostics of active CMV infection after BMT. But the opposite conclusion ensues from results of antigenemia detection after renal and lung transplantation. Application of the method is legitimate and appropriate in this specialization of present diagnostics.

D. Lednická, K. Malachová (Department of Biology and Ecology, Faculty of Science, University of Ostrava, Czech Republic): **Isolation and Characterisation of Bacteria able to Degrade Natural Cellulose Fibres.**

In search for bacterial cultures that are able to rapidly degrade cellulose plant fibres in vitro, 77 celluloselytic strains were isolated from soil after enrichment on flax or sisal fibres as sole source of carbon.

The isolated strains were characterized by their fatty acid methyl esters fingerprints, separated by gas-liquid chromatography, and identified by MIS database. The data of the fatty acid compositions were analyzed and used to construct a dendrogram. Three major clusters A, B and C could be discerned and three strains were ungrouped, they were assigned to *Curtobacterium flaccumfaciens*, *Achromobacter piechaudii* and *Pseudomonas mendocina*. Cluster A could be further subdivided into three subclusters by their fatty acid profiles. The MIS database recognized the 9 strains from the first subcluster as *Cellulomonas gelida*, the 8 strains from the second subcluster as *Cellulomonas biazotea* and the 13 strains from the third subclusters *Cellulomonas cellulans.* Cluster B consisted of 9 strains, which were assigned to *Flavobacterium johnsoniae* on the basis of their fatty acid compositions. Cluster C could be further split into two subclusters C1, C2 and two separate strains on the base of the fatty acid profiles, but the strains were not recognized by the MIS database. To investigate further the possible identities of the strains of cluster C the API 20NE system and some additional biochemical tests were applied. All these phenotypic features meet the description of the genus *Cellvibrio.* But the strains from subclusters differed with regard to reduction of nitrate to nitrite: C1 (negative) and C2 (positive). To investigate the phylogenetic affiliation of the strains from cluster C, the 16S rRNA genes of four representative strains were sequenced and compared to the sequences available from the EMBL database. As no 16S rDNA sequence of *Cellvibrio* has been deposited yet in this database, this gene was sequenced from authentic *Cellvibrio mixtus* subsp. *mixtus* ACM 2603. The results show that strains from cluster C and *Cellvibrio mixtus* subsp. *mixtus* belong to the same branch. By the additional analysis of fatty acid from four authentic *Cellvibrio mixtus* subsp. m*ixtus* strains LMG 2847, LMG

2848, LMG 2849 and ACM 2603 it was proved that they have similar profiles to those of the strains from subcluster C2. However, the differences in fatty acid compositions between the subclusters C1 and C2, as well as the 16S r DNA sequence analysis suggest that potentially new *Cellvibrio* taxa were isolated, we tentatively classify all strains of cluster C as *Cellvibrio* sp.

In order to test the capacities of the isolates to degrade natural cellulosic fibres, 13 representative strains were tested in a standardized in vitro test. The best results had *Cellvibrio* strains. Strains assigned to *Cellvibrio* were able to degrade flax, broom and cotton fibres very rapidly, causing mass losses of 40 to 86% within 13 days of incubation.

This study was supported by Grant IGS OU 311057/01.

S. Kaderová, J. Šmarda, J. Chumchalová (Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Sensitivity of *Escherichia coli* and *E. fregusonii* strains to colicins of various mechanisms of action.**

The Grant Agency of Czech Republic supports extensive research projects into the incidence of colicinogeny among strains of various species within the *Enterobacteriaceae* family; vast differences are revealed. As a supplement to this research, we tried to check whether there also exists a different sensitivity of related species to type colicins. We applied the standard stitching test method on agar plates.

We tested two sets of 50 strains each, of *E. coli* and *E. fergusonii* (all strains selected at random from human sources), for sensitivity to colicins E1, E2, E3, Js, K and U.

No strain was found sensitive to *Shigella* colicin Js. In E. coli, the most frequent was the sensitivity to colicins U (16 strains) and K (14 strains), while the least frequent was the sensitivity to colicins E1 (9 strains) and E2 (9 strains). On the other hand, in *E. fergusonii* most strains were sensitive to colicins E1 (28 strains) and E2 (28 strains), while sensitivity to colicins K (0 strains) and U (6 strains) appeared with the least frequency.

As all colicins E1, K and U are of the ionophore killing mechanism, the differences revealed are not due to type specific mechanisms of inhibition, but to differences in the incidence of specific receptors or molecular mechanisms of colicin translocation across the cell wall.

L. Sláviková, S. Masrnová., M. Čertík, D. Slugeč, J. Šajbidor (Department of Biochemical Technology, Faculty of Chemical Technology, Slovak University of Technology, Bratislava, Slovakia): **Changes in fatty acid composition of cereal substrates during solid state fermentation by *Thamnidium elegans.***

Changes in fatty acid (FA) composition of cereal materials after solid state fermentation (SSF) by filamentous fungus *Thamnidium elegans* were investigated. As substrates were used spent waste fractions from noodle manufacture (wheat and rye brans, wheat sprouts), spent malt grains (SMG), rice, barley and noodles. As C-source in cereals served starch and partially lipid, whose content varied from 0.6 % (noodles) to 10 % (SMG), depending on employed substrates. Decrease in the lipid value of fermented mass during SSF was observed, but the w-6/w-3 FA ratio increased regarding the newly biosynthesized g-linolenic acid (GLA) especially. The result of SSF was the prefermented material containing lipid with maximum 20 % of biologically active GLA.

M. Gódyová[1], J. Ševc[2] ([1]Department of Soil Sciences, Faculty of Natural Sciences, Comenious University, Bratislava [2]Geological Institute, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia): **The relationship between mineral composition & pH of wall substrate and the occurrence of microscopic fungi.**

Organisms exist in very close relationship with the abiotic and biotic components of their environment and the character and composition of the substrate play an important part in these relationships.

This work presents the results of studying the relationship between micromycete species spectrum and the composition of the substrate.

The wall substrate from a historical church in Okoličné (Liptovský Mikuláš) was chosen as the object for research.

Four samples of this wall substrate from different parts of the church were collected.

and then used for mycology analysis, the determination of pH/H_2O, the determination of the mineral phases by rtg-diphractometric analysis and the determination of chemical elements content by semiquantitative spectrochemical analysis.

Solid, quite moist wall substrate has in all cases carbonate character (a significant occurrence of calcite-$CaCO_3$).Each of the collected samples had a different composition of chemical elements (Ca, Si, Al, Mg, Fe, Ba, Na). The values of pH/H_2O all fell within the range of 10,3 to 11,8. Despite these high basic values, the following genera and species of micromycetes were isolated: *Acremonium* sp., *Aspergillus flavus, A. versicolor, Aureobasidium pullulans, Cladosporium* sp., *Penicillium expansum, Trichoderma viride, Verticillium tenerum.*

Occurrence of these microscopic fungi in such extreme pH conditions is very interesting and exactly this fact confirms their already known high adaptability to extreme environmental conditions.

R. Filip[1]*, L. Chihu-Amparan*[2]*, G. Coman*[1] *and J. Silva-Sanchez*[2] ([1]University of Medicine and Pharmacy „Gr. T. Popa“ Iasi, Romania, Microbiology Department, [2]Centro de Investigaciones Sobre Enfermedades Infecciosas, Cuernavaca, Morelos, Mexico): **Detection of beta-lactamase resistance genes by PCR in non-typhoidic *Salmonella* isolated in Iasi, Romania.**

To investigate the resistance mechanism of 16 non-typhoidic *Salmonella* strains isolated from patients with acute diarrhoea admitted in the Pediatric Hospital „Sf. Maria“ Iasi, Romania. To check the good correlation between the results obtained by classical methods – isoelectric focusing (IF) – and genetic methods – polymerase chain reaction (PCR).

16 non-typhoidic Salmonella were obtained from patients with acute diarrhoea, admitted in the Pediatric Hospital „Sf. Maria“ Iasi, Romania from September 1998 to July 1999. As control for sensitivity testing was used *Escherichia coli* ATCC 25922. Identification and sensitivity testing was done initially by classical methods - multitest tubes and disk diffusion method (Bauer-Kirby) against the following antimicrobials: Ampicillin (AMP), Ampicillin/Sulbactam (SAM), Cefotaxime (TX), Ceftriaxone (CRO), Ceftazidime (CAZ), Ciprofloxacine (CIP) and Ofloxacin (OFX). Results were confirmed by DADE MicroScan System, Panel Combo 20, in the Bacterial Genetics Department, from CISEI, Cuernavaca, Morelos, Mexico. Isoelectric focusing was performed by the method described by *Matthew et al* (1976), with pre-formed minigels, pH range 3-10, supplied by Pharmacia LKB. Band were visualised with nitrocefin. PCR was done using the primers and conditions indicated by *Arlet* and *Philippon* (1991) for TEM, and for SHV, sequences were designated in the Bacterial Genetics Department.

Constantly, the studied strains exhibited resistance to AMP and SAM and variable resistance to TX, CRO, CAZ. These combinations classified the strains into four resistance phenotypes. IF showed two major beta-lactamse patterns: pI of 5.4, 7.6 (3/16) and 5.4, 8.2 (6/16). The strains expressing the beta-lactamase with pI of 5.4 correlated with PCR amplification for TEM_{bla}. Only one strain was positive for the PCR amplification of the SHV_{bla}.

This study shows that the main resistance mechanism against beta-lactam agents is the beta-lactamase production, with TEM type predominant, single and in association with an extended-spectrum enzyme. This fact should draw the attention to infectious disease practitioners, that have to prescribe the antibiotics very carefully, and according to the results of the sensitivity testing. We found a good correlation between the classical methods and PCR used in investigation of resistance mechanisms, but still the genetic tests are limited to research laboratories.