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ABSTRACTS

Prof. Tomášek's Life

O. Zahradníček, V. Woznicová (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic*):* **Some moments of Prof. Tomášek's life – Part 1**.

Prof. Václav Tomášek, the long-time head of the Institute of Microbiology of the Faculty of Medicine, Masaryk University and the Faculty Hospital, Brno, was an admirable personality. It is not accidental that the conference of young microbiologists was given his name. To commemorate Prof. Tomášek's 40th anniversary of death (he died on 20 June 1962), the organizers have decided to contribute several papers to him. As it is not possible to describe his whole life, the contributions mention only a few interesting points of Prof. Tomášek's life and work. The main source for the papers was the archive of Prof. Tomášek at the Institute of Microbiology.

Moment No. 1: Prof. Tomášek – an international researcher. In this part, we present Prof. Tomášek as an intensively communicating scientist, receiving and sending letters in several languages and exchanging information throughout the world. He was a member of international societies and took part in major microbiological congresses.

Moment No. 2: Prof. Tomášek – an official and an academic dignitary. In spite of his inborn modesty, during his life Prof. Tomášek served as an official in many organizations. He was, for example, president of the Medical Chamber for Moravia and Silesia. Nevertheless, his most important function was that of the Dean of the Faculty of Medicine, Masaryk University in the years 1946–1947.

Moment No. 3: Prof. Tomášek – a human being**.** Prof. Tomášek was an example of personal bravery and loyalty to his motherland. During World War II, he took part in the anti-Nazi resistance movement. In the years 1941–1945, he was interned in the concentration camps of Mauthausen and Oświęcim (Auschwitz). Even in these conditions, he could make use of his bacteriological knowledge. – Also after World War II, Prof. Tomášek did not lose his sense for humanity and mercy, which he showed many a time.

O. Zahradníček, V. Woznicová (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic): **Some moments of Prof. Tomášek's life – Part 2.**

This paper adds some more moments of the life and work of Professor Václav Tomášek to those shown in Part 1 (see Wednesday, after the Ceremonial Opening of the Conference). Unlike the first part, this one is less serious.

Moment No. 4: Prof. Tomášek – a good pupil and student. This moment does not lead us to the 20th, but directly to the 19th century. The first school report of Professor Tomášek from the elementary school at Koryčany was issued in 1899. The future Professor Tomášek was excellent in all subjects, except writing and singing. His school results were very good even later, including his university studies in Prague.

Moment No. 5: Prof. Tomášek – a fighter with the state bureaucracy between the Wars. Although the bureaucratic methods are often a target of criticism even these days, in Czechoslovakia between the Wars the bureaucracy was very strong. It was very difficult for Professor Tomášek (as head of the institute) to explain to the Ministry of Education that some elementary office and laboratory equipment was really needed.

Moment No. 6: Prof. Tomášek – a fighter with the state bureaucracy after World War II. The end of the war brought partial liberty to Czechoslovakia, but also the bureaucracy came back. The communist rule after 1948 made this bureaucracy even worse and politically engaged. A characteristic bureaucratic document is the "Request for an extraordinary premium for ordinary professors".

All these moments show that Professor Tomášek's name is the best for the conference. Therefore, it will bear Tomášek's name for the 12th time (probably 4th to 6th June 2003) again.

1. Direct and Indirect Diagnostics of Microbes, except Borrelia

M. Heroldová, R. Tejkalová, J. Svoboda (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic):**Detection of β-haemolytic streptococci on blood agar supplemented with amikacin.**

Beta-haemolytic streptococci cause secondary infections of various skin lesions. Other bacteria, not so fastidious (*Staphylococcus aureus, Pseudomonas aeruginosa*, and Gram-negative enteric bacilli), are also observed. These microorganisms on blood agar overgrow β-haemolytic streptococci; that is why the isolation of β-haemolytic streptococci is so difficult.

In our laboratory we initiated inoculation of skin swabs not only on blood agar, Endo agar and blood agar with 10 % NaCl, but also on blood agar supplemented with an antibiotic – amikacin.

Between November 2001 and May 2002, we examined 836 samples. We isolated 86 strains of β-haemolytic streptococci. We found out that other bacteria participated in the infection in 87 % of samples. We demonstrated that 37 % of isolates of β-haemolytic streptococci were seen only on blood agar with amikacin.

E. Vlková, J. Medková, V. Rada (Department of Microbiology and Biotechnology, Czech University of Agriculture, Prague, Czech Republic): **Identification of bifidobacteria isolated from fermented milk products and food supplements.**

Bifidobacteria are Gram-positive non-spore-forming irregular anaerobic rods. The typical habitat of this genus is human and animal intestinal tract. It has been suggested that *Bifidobacterium* species are important in maintaining general health. Potential health benefits of bifidobacteria to the host have led to their wide application in dairy products. Bifidobacteria tend to be host specific. Therefore, it is necessary to select the strain that is compatible with the host (e. g. human) for which the probiotic product is intended. The aim of the presented work was to identify the bifidobacterial strains isolated from fermented milk products and food supplements. An additional aim was to find out whether the strains used are of human or animal origin.

Eighteen products were tested. Bifidobacteria were isolated using TPY agar modified by the addition of mupirocin (100 mg . l^{-1}). Isolates were identified to the genus level by the detection of fructose-6-phosphate phosphoketolase. Bifidobacteria were characterised using API 50 CHL and API ID 32 A Rapid tests (BioMérieux, France). In addition, the growth at 46°C was tested. Subsequently, all strains were identified to the species level using computer program Bacter (INRA, Lille, France).

Sixteen strains were identified as *Bifidobacterium animalis* and two strains as *Bifidobacterium longum*. Our results showed that most of the bifidobacterial strains currently used in food products are of animal origin.

The work was supported by a grant of the Czech Ministry of Education, Youth and Sports, MSM 412100003.

J. Prodělalová, A. Španová, B. Rittich (Department of Microbiology, Faculty of Science, Masaryk University, Brno, Czech Republic): **Testing of different methods for preparing the DNA template for the identification of wild Salmonella strains using the PCR**.

The polymerase chain reaction (PCR) is a specific, sensitive and time-saving method, which has been used successfully in many branches including microbiology. However, the usefulness of PCR to detect bacterial cells is limited by intracellular inhibitors that can be the cause of misleading negative results.

In this work we studied 8 wild strains of *Salmonella* which were classified as belonging to different serovars. PCR was performed using primers ST 11 and ST 15 which enable to amplify a 429 bp long DNA fragment. This PCR product was detected by agarose gel electrophoresis. As a DNA template we used crude cell lysates extracted by boiling the cell suspension in distilled water for various periods of time (10–30 minutes) and the DNA purified by phenol-chloroform extraction.

The results of the experiments showed that wild strains of *Salmonella* differed in the content of intracellular PCR inhibitors. The method of preparation and storage of the DNA template is of great importance for the result of PCR. With the strain *Salmonella arizonae* 18/98 a visible PCR product was obtained only after 30 minutes of boiling the cell lysate or by using DNA purified by phenol-chloroform extraction. With the other species studied the sensitivity of PCR increased already after 10 minutes of boiling the cell suspension. The sensitivity of PCR was increased also by the use of proteinase K. It has been showed that for PCR the appropriate DNA template was the DNA of *Salmonella* cells purified by phenol-chloroform extraction or crude cell lysates stored at -20 °C. In the case of storage at + 4 °C it is possible to use non-lysed cells only (6 days of storage were tested).

The results presented document the importance of intracellular inhibitors for the detection of Salmonella cells by PCR. Preparing the DNA template according to the amount of inhibitors may eliminate the influence of inhibitors and storage of the prepared samples.

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H. Hrbáčková, M. Zajac, M. Šrumová (Section of Medical Virology, Department of Medical Microbiology, University Hospital Motol, and Second Faculty of Medicine, Charles University, Prague, Czech Republic)**: The contribution and limitation of PCR method in the diagnostics and control of the therapy of active CMV infection.**

An analysis of the advantages and limitations of PCR diagnostics in monitoring the activity and therapy of CMV infection in immunosuppressed and immunodeficient patients. Demonstration of the results on typical cases obtained from monitoring the development of the activity of CMV infection.

PCR diagnostics of CMV genome based on the detection of a part of the gene coding matrix protein pp65. The source of material for PCR is the quantitative isolation of peripheral leukocytes. The extraction of DNA is realized by a QIAamp DNA Blood Mini Kit. PCR reaction is realized in a nested arrangement and the results are detected by agarose gel electrophoresis. The results of the qualitative PCR method are compared with the parallel detection of pp65 antigenemia in peripheral leukocytes and in some cases with the results of a quantitative PCR method realized by a cooperating laboratory.

Two newborns, one child after the heart transplantation, a group of risk adults after the lung transplantation, and a group of children after the transplantation of hematopoietic cells were examined. Both children who were tested for clinical suspicion of congenital CMV infection were repeatedly detected as positive. CMV DNA outlasted in a period of 4 or 5 months after the diagnosis and/or after the beginning of therapy. PCR positivity of a patient after the heart transplantation continued for 5 months from the time of CMV diagnosis, too.

Four episodes of CMV infection were detected in a group of 5 examined patients after the lung transplantation.

PCR diagnostics revealed 7 CMV episodes in 6 patients in a group of 35 patients after the transplantation of hematopoietic cells. In addition, a fluctuation of CMV activity around the cut-off value of this method was detected in a half of monitored patients. This fluctuation is manifested by isolated positivity of one or two samples.

Nested PCR diagnostics is so sensitive that it is even able to detect clinically indifferent benign reactivations or fluctuations of a latent virus infection. As a result, one or two PCR positive results are not a motive for the start of therapy if patients are regularly monitored. After successful therapy, a positive result also outlasts for a long time without implying another episode of virus activation.

On the other hand, the high sensitivity of the method provides the physicians a long time period for an optimal decision about the beginning, type and intensity of therapy. It corresponds with the highest aims of clinical diagnostics. In cases where the patient is not monitored continuously and the examination is realized basing on clinical suspicion, we obtain the best results when combining it with the detection of antigenemia. In case of positive results in both methods this combination is a successful solution for controlling therapy. In other cases, when the results of antigenemia are negative, we can control the therapy by quantitative PCR reaction only.

Y. Boriskin, P. Rice, R. Stabler, J. Hinds, H. Al-Ghusein, P. Butcher (London, UK): **DNA microarray as a diagnostic tool for viral CNS infections.**

To develop a DNA microarray for rapid diagnosis and specific pathogen identification in patients with meningitis and encephalitis. Initially developed for viral pathogens, once the utility has been assessed, the array can be extended to non-viral pathogens causing CNS infections.

A DNA microarray was constructed comprising 39 unique gene sequences for 14 viral causes of meningitis and encephalitis, namely, cytomegalovirus (CMV), herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), human herpesviruses 6A, 6B and 7 (HHV-6 and HHV-7), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), papovaviruses BK and JC, measles, mumps, Coxsackie B and ECHO viruses. PCR-amplified and purified viral probes representing a mean of three (range 1–5) different regions of the viral genomes were printed on glass slides and arranged to create an easily recognizable virus-specific pattern. CSF samples from patients with viral CNS syndromes, diagnosed previously by nested PCR and gel electrophoresis detection, were subjected to DNA and RNA extraction followed by a single round of multiplex DNA PCR or RT-PCR respectively. The amplified DNA was purified and enzymatically labelled using random primers and the Cy3 fluorescent dye. As an internal control, in every reaction the mixture of original DNA probes was with the Cy5 fluorescent dye. A co-purified two-dye target mixture was hybridized to the array and the fluorescence emitted by bound-labelled DNA was measured in the control and sample channels. By superimposing the two alternatively labelled images the pattern of spots appeared characteristic for a specific virus found in the specimen. All positive targets were validated by direct sequencing.

So far, all CSF samples diagnosed previously as CMV, HSV-2, HHV-6A and 6B, HHV-7, VZV, EBV, JC, Coxsackie and ECHO viruses have been confirmed as such by the microarray. Although not every target within the viral genomes was amplified, at least one target per virus yielded a specific pattern.

This pilot DNA microarray is capable of detecting at least 14 different viruses in a single test and is as sensitive as nested PCR.

2. Direct and Indirect Diagnostics of Borrelia

L. Čechová[1], M. Němec[1], J. Halouzka[2] ([1]Department of Microbiology, Faculty of Science, Masaryk University, Brno, Czech Republic, [2]Institute of Vertebrate Biology, Czech Academy of Science, Department of Medical Zoology, Valtice, Czech Republic): **Application of polyacrylamide gel electrophoresis to the analysis of *Borrelia burgdorferi* sensu lato cell proteins.**

The frequency of the important illness Lyme borreliosis increases nowadays. For that reason, early identification of the causative agent of this disease, the bacterium *Borrelia burgdorferi* sensu lato, is necessary. From the point of view of immunology, the differentiation of individual genome species

within the *B. burgdorferi* s. l. complex is also inevitable. The method of cell protein analysis was chosen for the differentiation of the individual genome species representatives.

Borrelia strains were cultivated in BSK-H medium supplemented with rabbit serum and antibiotics – rifampicin and phosphomycin. Different growth conditions of spirochetes were the cause of growth in the medium without antibiotics. These conditions were studied, too. After cell lysis the spectrum of proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) was analysed. The samples were separated on discontinuous 12 % polyacrylamide gels. Proteins were fixed with silver nitrate. Protein spectra were analysed with software using cluster analysis (GelCompar II).

The proteins of 18 borrelia strains were analysed. Some of those belonged to the taxons undoubtedly associated with the Lyme disease (*B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*; on the whole, 7 strains). Others were 1 strain of *B. valaisiana* and 10 strains still not identifiable (those strains were isolated from arthropods (ticks and insects) and from rodents). There were determined proteins of molecular weight of about 7 – 200 kDa using the SDS PAGE method. The strains were grouped into three separated protein profile clusters at $r \geq 0.7$. The results show the differences between protein representations in the individual genome species of *B. burgdorferi* sensu lato. *B. afzelii* shows a higher variability in protein spectrum than other strains. Different growth conditions (growth in BSK-H medium without antibiotics) affected the profile of proteins in all the observed strains.

The results obtained indicated that analysis of cell protein profile could contribute to the study of *Borrelia burgdorferi*. A more detailed analysis of the bacteria in question will require the use of other methods.

E. Janouškovcová[1], A. Žákovská[1], J. Halouzka[2], M. Dendis[3] ([1]Department of Comparative Animal Physiology and General Zoology, Faculty of Science of Masaryk University, Brno, [2]Institute of Vertebrate Biology, Czech Academy of Sciences – Department of Medical Zoology, Valtice, [3]Genetic Laboratory, Centre for Cardiosurgery and Transplants, Brno, Czech Republic): ***Borrelia burgdorferi* – identification of isolated strains using gradient PAGE, PCR, and RFLP methods.**

Borrelia burgdorferi sensu lato is a pathogenic spirochete bacterium. There is the typical occurrence of two genome species in the Czech Republic – *Borrelia garinii* and *Borrelia afzelii,* transmitted most widely by *Ixodes ricinus* ticks. After the transmission to human hosts, dogs and many other animals this bacterium causes Lyme disease. Both genome species differ in their protein composition as a result of the genetic structure difference. The aim of our study was to identify spirochete isolates which were acquired successfully from different sources: vectors (ticks), potential vectors (mosquitoes), and hosts (wild rodents) using RFLP, single-tube nested PCR, and SDS gradient PAGE methods.

In the years 1996–2000 a total of 2398 ticks, 72 mites (from wild rodents), 1336 mosquitoes, and parts of 216 rodent organs were collected from six localities in the Czech Republic. A total of 33 spirochete isolates were obtained finally. All these isolates were identified by comparing the protein composition to standard strains of *Borrelia burgdorferi* sensu stricto, *B. garinii,* and *B. afzelii* using SDS-gradient PAGE. For the genome species pertinence confirmation, single-tube nested PCR and restriction fragment length polymorphism (RFLP) methods were used.

A total of 13 isolates from ticks, 1 isolate from mites, 15 isolates from rodents, 3 isolates from mosquito adults, and 1 isolate from mosquito larva were obtained. The protein composition was determined and tick isolates were identified as *B. garinii* and *B. afzelii*; the isolate from the mite was identified as *B. afzelii.* All the rodent isolates were identified as *B. afzelii.* One mosquito adult isolate was identified as *B. afzelii*; the larval isolate from the mosquito and 2 mosquito adult isolates do not pertain to the *Borrelia burgdorferi* group. SDS-gradient PAGE results correlate with PCR and RFLP results.

This work was partially supported by CEZ: J 07/98: 143100010, 143100008.

P. Nejedlá [1], A. Holíková [1], M. Dendis [2], A. Žákovská [1] ([1]Faculty of Science, Masaryk University, Brno, [2]Genetic Laboratory, Centre for Cardiosurgery and Transplants, Brno, Czech Republic): **Determination of tick positivity for pathogenic Borrelia burgdorferi by using PCR-RFLP methods.**

The aim of the study was the monitoring of the presence of *Borrelia burgdorfer*i in ticks in the Pisárky park (Brno, Czech Republic) in 1996–2001. Ticks were being collected for one hour by flagging the vegetation in regular two-week intervals. In the year 2000 a total 979 Ixodes ricinus ticks were collected (127 females, 111 males, 486 nymphs and 73 larvae). The mid-gut of each tick was removed and homogenized in a 0.9 % NaCl solution. The homogenate was analysed for the presence of borreliae by dark-field microscopy (DMF). Dark-field microscopy positive homogenates were subsequently analysed by the PCR method. The samples with a greater mass of borrelia DNA were analysed by the RFLP method and transferred into BSK-H medium available for spirochete growth and cultivated.

Laboratory findings: The mean annual proportion of infected ticks in the year 2000 was 4.1 %. (in larvae 1.37 %, in nymphs 2.67 %, in females 7.87 %, in males 7.21 %.) Using the PCR method, microscope positive homogenates were 68 % PCR-positive.

Unlike in 1996–1999, in 2000 there was a possibility to determine the individual species of *Borrelia burgdorferi* sensu lato. Of 19 positive samples confirmed by the PCR method there were 7 *Borrelia garinii*, 6 *Borrelia afzelii*, and 6 samples could not be amplified.

A total of five spirochete isolates was gained from positive ticks, which were further analysed by gradient SDS-PAGE and the PCR-RFLP method. All isolates were tested for their protein composition and identified by comparison to standard strains (*B. burgdorferi sensu stricto, B. afzelii, B. garinii*, and *B. lusitaniae*). Four of them were determined as *B. afzelii*; one of them was *B. garinii.*

This work was partially supported by CEZ: J 07/98: 100010

M. Mejzlíková, H. Martiníková, A. Žákovská, O. Šerý (Department of Comparative Animal Physiology and General Zoology, Faculty of Science of Masaryk University, Brno, Czech Republic): **Detection of the positivity of *Borrelia burgdorferi* in *Ixodes ricinus* by PCR.**

Our scientific work is focused on the activity of *Ixodes ricinus* ticks and their positivity for the pathogenic spirochete *Borrelia burgdorferi*, causing the Lyme borreliosis disease. We observed whether there is a correlation between the temperature and atmospheric humidity and the positivity of ticks.

We collected ticks at regular two-week intervals in the Brno-Pisárky park area by the flagging method. In this case the activity means the number of ticks, which are collected for a constant period of one hour.

For the genome species confirmation a single-tube nested PCR has been developed in our laboratory. All ticks were homogenised in the buffer of the isolation kit and identified by comparison with the protein composition of the standard strains of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii,* and *Borrelia afzelii.*

Activity (8/15–11/10/2001):	Larvae	78
	Nymphs	82
	Females	9
	Males	32
	Total	201
Positivity (8/15–11/10/2001):	Larvae	1.3 %
	Nymphs	7.3 %
	Females	22.2 %
	Males	6.3 %
	Total	5.5 %

Conclusion: The mean activity of ticks for one-hour flagging was 29. The highest activity was 54 (8/29/2001) and the lowest activity 3 (11/10/2001). The annual average tick positivity in 2001 was 5.5 %. The highest positivity was in the year 1998 (12 %), the lowest positivity was in the year 2000 (2 %).

The most important phenomenon is the increased number of larval activity compared to the years 1996–2000. Our work will be continued till the end of July 2002.

This work was partially supported by CEZ: J 07/98: 143100008

K. Vostal, A. Žákovská (Department of Comparative Animal Physiology and General Zoology, Faculty of Science of Masaryk University, Brno, Czech Republic): **Detection of the presence of antibodies against *Borrelia burgdorferi* in the blood of wild-living rodents.**

A total of 352 rodents were caught in wild nature at three localities in the years 1997–1999. A total of 328 heart rinses and 28 sera were examined for the presence of antibodies against *Borrelia afzelii* by the indirect ELISA method.

A total of 127 rodents were caught at four localities in the year 2001. A total of 125 heart rinses and 2 sera were examined by the indirect ELISA method too, but with a differently prepared antigen. All the localities are in Moravia, the Czech Republic.

The aim of the study was: 1) Detection of antibodies against *B. afzelii* in the blood of wild rodents. 2) Comparison with results obtained in previous years.

Conclusion:

1) A total of 75 heart rinses and sera (59.1 %) were positive for the presence of antibodies.

2) A total of 120 heart rinses (36.6 %) and 12 sera (50 %) were positive for the presence of antibodies. As a result, a total of 132 out of 352 (37.5 %) was positive. It means nearly 1.5 × higher number than in the years 1997–1999.

As to the grouping by sex, we found that a total of 40 (42.9 %) of 67 males and a total of 35 (58.3 %) of 60 females were positive in the year 2001. Compared to the positivity of the years 1997–1999, there was a positivity of 42.9 % (54/155) of males and 42.9 % (67/156) of females. We can see a similar positivity inside each sex. However, the positivity of each sex increased heavily during the time period studied.

These differences could be caused by using a differently prepared antigen, or there is the reality of a higher number of more positive rodents in nature for this year.

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G. Rašková, A. Žákovská (Department of Comparative Animal Physiology and General Zoology, Faculty of Science of Masaryk University, Brno, Czech Republic): **Western blot analysis of rodent sera positive to pathogenic *Borrelia burgdorferi* sensu lato.**

In the year 1999, 12 wild-living rodents from two areas in the Czech Republic were entrapped. Vaccination was executed three times, each after a three week interval. After the application of the antigen tail blood was taken. The obtained sera were examined by indirect ELISA and Western blot methods. We searched for the presence of antibodies against *Borrelia afzelii*, *Borrelia garinii,* and a mixture of both antigens.

Results:

1. During the nine-week period of immunisation, the production of antibodies increased continually.

2. If we use *B. afzelii* antigen for Western blot: rodents immunised to *B. afzelii* showed the immune response mainly against the following antigens: Osp (all types), flagellin and Hsp. Individuals immunised to *B. garinii* responded especially to Osp proteins and Hsp. The same reaction is also perceptible in the immunisation process with the blended antigen (afz/gar).

In Western blot reaction with antigen *B. garinii*: rodents immunised to *B. afzelii* developed antibodies against OspA, OspB, OspD, p83, and Hsp. The individuals immunised to *B. garinii* reacted similarly but, in addition to this, they expressed a band for flagellin. The reaction is not different from that in which we use the blended antigen; in this case the rodents developed antibodies also against OspC and periflagellin.

From these experiments it can be seen that the stimuli of *B. garinii* antigen are weaker than those of *B. afzelii* and that is why the proteins are not expressed so strongly, and also that *B. garinii* responds to proteins with higher molecular weight.

Although both the antigens (*B. afzelii* and *B. garinii*) are very similar in their protein spectrum, there were some differences in the production of antibodies in the individual experiments.

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3. Virulence Factors of Microbes

I. Sládková, O. Zahradníček (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic): **Comparison of haemolytic properties of coagulase negative staphylococci in various localizations**

In the *Staphylococcus* genus*, Staphylococcus aureus* is considered to be the most pathogenic taxon. Among the clinically important taxons it is the only plasma coagulase producer, and thus the other taxons may be described as "coagulase negative staphylococci". These taxons – unlike *S. aureus* – have only one type of their haemolytic activity, described as the "delta-like" activity, as it is similar to the *Staphylococcus aureus* delta-haemolysin activity.

This work is a continuation of our previous work, where the delta-haemolytic activity of two groups of strains of coagulase-negative staphylococci was compared: staphylococci from blood cultures and from upper respiratory ways. The aim of the new work was to compare a specific sample of isolates from urine with both these groups. Only isolates with a quantity of at least 10 000 CFU per millilitre were studied, e. g. probable urinary infection causative agents. The hypothesis was that these strains would differ from both previously studied groups.

That is why we tested 44 strains of coagulase negative staphylococci from urine and have compared them with 60 strains from the blood cultures and 60 strains originating in the nasal cavity. All of these strains had their origin in specimens sent to the Institute of Microbiology, Faculty of Medicine, Masaryk University and St. Anne's Faculty Hospital in Brno. In urine strains haemolytic activity was proved in 39 strains (89 %). Among them, 31 strains (70 % of the total), showed a strong haemolytic activity. These results may be compared with blood culture results (haemolytic activity in 39 strains – 65 %, out of which strong activity in 38 strains – 63 %) and nasal swab strains (haemolytic activity in 50 strains – 83 %, out of which strong activity in 23 strains – 38 %).

The results show that the strains from urine specimens produce haemolysin more often than the strains isolated from blood cultures and nasal swabs.

O. Zahradníček (Institute of Microbiology, Faculty of Medicine, Masaryk University and St. Anne's Faculty Hospital, Brno, Czech Republic): **Haemolytic interactions of *Staphylococcus aureus* and coagulase negative staphylococci with *Staphylococcus hyicus* and *Staphylococcus chromogenes*.**

As has been proved before, the haemolytic synergism between a beta-haemolysin-producing strain of *Staphylococcus aureus* and delta(-like)-haemolysin producing strains of various staphylococci is sometimes inhibited by *S. hyicus* or *S. chromogenes*. The aim of my work was to examine whether any differences between *S. aureus* and any species of coagulase negative staphylococci might be found at this point. Twenty-two strains of *S. aureus*, 41 strains of *S. epidermidis*, 16 strains of *S. haemolyticus,* and 19 strains of other staphylococci (all from clinical samples) were tested for their haemolytic properties. Among the strains of all taxons, some delta(-like)-haemolysin-negative strains were found (except *S. haemolyticus*). Among delta(-like)-haemolysin producing strains, the haemolytic synergism with a beta-haemolytic strain of *S. aureus* was sometimes inhibited by *S. hyicus* and/or *S. chromogenes*, sometimes it was not. This was found both for *S. aureus* and for coagulase negative staphylococci. These results show that the inhibitability of haemolytic synergism of delta(-like)-haemolysin and beta-haemolysin is not taxon-specific and is rather accidental. Thus, if there are any phenotype differences between delta-haemolysin of *S. aureus* and the delta-like activity of coagulase negative staphylococci, the interaction with *S. hyicus* and *S. chromogenes* is no example for this.

L. Černohorská (Institute of Microbiology, Faculty of Medicine, Masaryk University and St. Anne's Faculty Hospital, Brno, Czech Republic): **Detection of slime production by microbes isolated from various clinic materials.**

In the recent few years the virulence of coagulase negative staphylococci isolated from blood cultures as well as from various clinical materials was connected with their slime production. Slime producers are more resistant to antibiotics, host immune reaction, and can adhere to important medical devices such as catheters, artificial heart valves and orthopaedic implants.

The aim of this study was to prove slime production by other bacteria. By means of agar with Congo red and the Christensen method used for slime detection of *Staphylococcus epidermidis,* about 80 samples from blood cultures and also from other clinical materials were examined. The most investigated bacterium was *Pseudomonas aeruginosa* but other bacterial members were also examined. The results of both methods were compared.

F. Růžička[1], M. Votava[1], M. Dendis[2], M. Heroldová[1], R. Horváth[2], V. Woznicová[1] ([1]Institute of Microbiology, Faculty of Medicine, Masaryk University and St. Anne's Faculty Hospital, Brno, [2]Genetic Laboratory of the Centre for Cardiosurgery and Transplant Surgery, Brno, Czech Republic): **Production of biofilm and presence of *ica*-operon in *Staphylococcus epidermidis* strains.**

The production of the polysaccharide intercellular substance (so-called slime) genetically encoded by *ica*-operon enables the strains of *S. epidermidis* to form a multilayer biofilm. The biofilm increases the adherence of bacterial cells to artificial materials or to damaged surfaces of bloodstream. It also protects the bacteria against the effects of antibiotics or against the immunity system. The biofilm is therefore considered as one of the most important factors of virulence of these microbes. The slime production was demonstrated by the characteristic growth on the Congo red agar and by the Christensen test-tube method. The presence of *ica*-operon was proved on the polymerase chain reaction followed by electrophoresis. Sixty-two *S. epidermidis* strains isolated from blood cultures and 61 *S. epidermidis* strains isolated from the skin of healthy individuals were examined by the above-mentioned methods. Out of 62 blood isolates, 30 (48.4 %) were positive for slime production. Out of 61 skin strains, 18 (29.5 %) formed the slime. Ica-operon was detected in 33 (53.2 %) blood strains and in 18 (29.5 %) strains from the skin. The presence of ica-operon and the production of slime were proved more often in *S. epidermidis* strains isolated from blood cultures than in strains isolated from the skin. The ability to form the biofilm indicates a higher clinical danger caused by the corresponding strain of *S. epidermidis*. The proof of forming the biofilm may help to distinguish the strains of *S. epidermidis* that cause the infection of bloodstream from saprophytic strains contaminating blood cultures.

J. Chumchalová, J. Šmarda (Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Occurrence of colicinogeny and lysogeny in two new genera of the family *Enterobacteriaceae*.**

Several genera of the *Enterobacteriaceae* family including *Escherichia*, *Shigella*, *Salmonella,* and *Serratia* are known to produce bacteriocins, i.e. proteins able to kill sensitive bacterial strains of the same or closely related species. Moreover, some strains are lysogenic and thus able to produce temperate phages. Bacteriocinogeny and lysogeny are not mutually exclusive bacterial genetic markers. We examined bacteriocin and phage production in two relatively new enterobacterial genera *Leclercia* and *Kluyvera*. We tested 50 strains of *L. adecarboxylata,* 34 strains of *K. ascorbata,* and 21 strains of *K. cryocrescens* using cross-inhibitory agar plate test. Each strain was tested as a potential producer and indicator against all other strains. In addition, 6 standard *E. coli* and *S. sonnei* colicin and phage indicator strains were used. All *Leclercia* and *Kluyvera* strains used were obtained from the Czech Collection of Microorganisms in Prague. Experiments were performed on nutrient agar plates under standard and iron-limited conditions. 0.2 mmol . l^{-1} 2,2´-dipyridyl was used as an iron-chelating compound. No bacteriocinogenic strain of *L. adecarboxylata* was found and 5 strains (10 %) produced temperate phages. Each phage infected 1 to 4 indicator strains of *L. adecarboxylata* and none of the 6 standard indicators was sensitive. One strain was found to produce wide zones (15 mm) on standard indicators under iron-restricted conditions. This is likely because of the production of iron-chelating

siderophore. One strain of *K. ascorbata* was found to produce phages active against one indicator of *K. cryocrescens*. No bacteriocinogenic strains were found among *Kluyvera* strains. All in all, these results demonstrate the differences in bacteriocinogeny and lysogeny within *Enterobacteriaceae*. In *E. coli* and *E. fergusonii*, approximately 40 % and 12 % of the strains are colicinogenic. However, no colicinogenic strains were found in *E. hermanii* and *E. vulneris* species. In addition, 57 % of *E. hermanii*, 22% of *E. fergusonii,* and 10 % of *E. vulneris* strains were found to be lysogenic.

4. Experimental Microbiology

L. Strašák[1], V. Vetterl[1, 2], J. Šmarda[3] ([1]Institute of Biophysics, Czech Academy of Sciences, Brno, [2]Faculty of Science, Masaryk University, Brno, [3]Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Low-frequency electromagnetic fields and bacteria *Escherichia coli* and *Leclercia adecarboxylata***

This work is a continuation of the works presented at this forum in last years. We investigate the effect of low-frequency magnetic fields (50 Hz, up to 20 mT, time of exposure up to 30 min) on bacteria. The magnetic field kills *E. coli*. We found a threshold value of magnetic induction of a statistically significant decrease of the number of bacteria in a culture. It is about 0.84 mT. We compared the behaviour of *E. coli* and *L. adecarboxylata* in the magnetic field. There is a decrease of the number of cells in the sample after the exposure in both cases. *L. adecarboxylata* is more resistant against magnetic fields. By means of induction of the bacteriophage from a lysogenic strain of *L. adecarboxylata* we verified the fact that magnetic fields have no effect on the DNA of exposed bacteria.

D. Šmajs[1], G. M. Weinstock[2] ([1]Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [2]Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, USA): **DNA library of *Treponema pallidum* in *Escherichia coli*: use for function genomics.**

Treponema pallidum subspecies *pallidum* (Nichols) is the causative agent of the sexually transmitted disease syphilis. The construction of genomic libraries represents a powerful resource for genetic studies of bacteria including non-culturable organisms. Chromosomal DNA of *T. pallidum* was used to construct a large insert bacterial artificial chromosome (BAC) library in *Escherichia coli* DH10B using the pBeloBAC11 cloning vector. The 678 individual insert termini of 339 BAC clones (13.9 x coverage) were sequenced and the cloned chromosomal region in each clone was determined by comparisons to the genomic sequence. In addition to that, the restriction mapping was performed to verify the chromosomal position and integrity of the cloned fragments. A single 15.6-kb region of the *T. pallidum* chromosome was missing in the BAC library, between bp 248727 and 264323. In addition to the 12 open reading frames (ORFs) coded by this region, one additional ORF (TP0596) was not cloned as an intact gene. Altogether, 13 predicted *T. pallidum* ORFs (1.25 % of the total) were incomplete or missing in the library. Three of 338 clones mapped by restriction enzyme digestion had detectable deletions and one clone had a detectable insertion within the insert. These data and the fact that some regions of the *T. pallidum* chromosome are over- and underrepresented suggest that there is a selection bias against some treponemal genes in *E. coli*. Out of the mapped clones, 19 were selected to represent the minimal set of *E. coli* BAC clones covering 1026 of the total 1040 (98.7 %) predicted *T. pallidum* ORFs. Using this minimal set of clones, at least 12 *T. pallidum* proteins were shown to react with pooled sera from rabbits immunised with *T. pallidum*. These data indicate that at least some *T. pallidum* genes are transcribed and expressed in *E. coli* and that this library can be used for functional genomic screenings.

A. Yansanjav[1], M. Němec[1], I. Hollerová[2] ([1]Department of Microbiology, Faculty of Science, Masaryk University in Brno, Czech Republic, [2]Institute of Brewing and Malting in Prague, Czech Republic): **The comparison of API 50 CHL test and whole-cell protein profiles in the diagnostics of lactic acid bacteria isolated from beer.**

Lactobacillus strains are the most common contaminants in breweries and include beer spoilage and non-spoilage strains. In general, the API 50 CHL test is a basic method for identification of lactic acid bacteria, but sometimes it is not reliable enough and other tests are often recommended.

The strains isolated from beer were submitted to the analysis of total soluble protein profiles using SDS-PAGE. The Gelcompar II computer program was used to analyse the protein banding patterns.

All 13 strains, identified as *Lactobacillus plantarum* by API 50 CHL, were put in the group of the protein profile cluster at r > 0.79, and the strains of *Lactobacillus brevis* were grouped into another separated protein profile at r > 0.85 including at least extra two strains of *Lactobacillus* identified as *Lactobacillus collinoides* using the API 50 CHL test. One of the *Lactobacillus collinoides* strains showed a 96.7% similarity with *Lactobacillus brevis*.

On the whole, the evaluated results of API 50 CHL and evaluation of protein profiles by using SDS-PAGE are very similar.

Further analyses, such as PCR and ribotyping of all the above-mentioned strains, are to be carried out.

T. Moško[2], V. Ďurmanová[1], J. Košovský[1], M. Kúdelová[1], J.Rajčáni[2] ([1]Institute of Virology, Slovak Academy of Sciences, Bratislava, [2]Department of Microbiology, Jessenius Medical Faculty, Martin, Slovakia): **Expression and isolation of selected non-structural herpes simplex virus 1 (HHV 1) proteins.**

We expressed the herpes simplex virus 1 (human herpesvirus 1, HHV-1) immediate early protein ICP 27 (IE63, Vmw63) encoded by gene UL 54, the early ori-binding protein (UL9), and early thymidine kinase protein (UL 23) in Escherichia coli JM 109 cells. The cells were transformed with the PinPoint Xa-1 (Promega) plasmid construct coding for a biotin labelled fusion protein. This expression system could be induced by IPTG to start the production of the given fusion protein. The resulting biotinylated products were purified in appropriate amounts by a monomeric avidin-conjugated resin (SoftLink™ Soft Release Avidin Resin, Promega). This proprietary resin allows the elution of biotinylated fusion protein under nondenaturing conditions. The isolated proteins, although partially digested, were suitable antigens for immunization of mice (BALB/c). Mice immunized with immediate early protein ICP 27 were partially protected (the LD_{50} value increased 100 times) against infection with HHV 1 (strain SC 16) as compared to non-immunized animals, but less protected than mice immunized with a mixture of envelope glycoproteins (HHV 1, strain HSZP; the LD_{50} was over 10,000 times higher than for non-immune mice).

5. Environmental Microbiology and Biotechnology

I. Melenová, V. Spěváková, P. Lovecká, J. Trogl, K. Demnerová (Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Czech Republic): **Screening of toxicity and mutagenicity of chosen substances present in groundwater.**

Our work was aimed at evaluating possible toxicity and genotoxicity of the substances PCE, TCE, benzene, toluene and m-xylene, zinc and arsenic and mixtures due to their presence in groundwater used as a source of drinking water.

We used BIOSCREEN, MICROTOX, and LUMAC apparatuses to estimate the toxicity of the above-mentioned substances. BIOSCREEN was used as kinetic measurement of the growth of bacteria *Pseudomonas* sp. P2 in the presence of toxicants added in various concentrations. As a blank, an inoculated sample without any toxicant was used. Specific growth speed (μ) was then compared.

MICROTOX and LUMAC were used to determine EC_{50} (15 min) by measuring the bioluminescence of *Vibrio fisheri* dependent on the toxicant concentration.

The Ames *Salmonella* Typhimurium his^- test was used to determine the mutagenicity of the substances mentioned above. The Ames test is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations. The test employs several histidine-dependent *Salmonella* strains (selected from *S.* Typhimurium LT2), each carrying different mutations in various genes in the histidine operon. These mutations act as hot spots for mutagens that cause DNA damage via different mechanisms. When *Salmonella* tester strains are cultivated on a minimal media agar plate containing traces of histidine, only those bacteria that reverted to histidine independence (his^+) are able to form colonies. The number of spontaneously induced revertant colonies per plate is relatively constant. However, when a mutagen is added to the plate, the number of revertant colonies per plate was proved to rise, usually in a dose-related manner.

The number of colonies that reverted to histidine independence was counted. The results were compared using the parameter Rt/Rc. Rt represents a total number of revertants of a particular concentration of a tested substance whereas Rc is a total number of spontaneous revertants on control plates. An important criterion was a value of Rt/Rc = 2. Only values of Rt/Rc > 2 were presented.

V. Spěváková, K. Demnerová (Department of Biochemistry and Microbiology, Institute of Chemical Technology in Prague, Czech Republic): **Isolation of soil strains from aquifer and their genetic characterisation .**

This work was carried out as a part of an international project focused on the development and study of feasibility of multibarrier concept. The concept deals with the worldwide problem of contamination of ground water, as this is the main source of drinking water in Europe. Moreover, it is exceedingly important to prevent pollutants from spreading widely in the environment.

Remediation technologies of contaminated aquifers are based on physical, biological and/or chemical treatment; however, in-situ treatment is becoming more interesting recently. A very attractive multibarrier system is based on "permeable barrier technology" where a trench made perpendicular to the groundwater flow and filled with a coarse material will treat pollutants in the passing groundwater. Apart from sorption of some compounds the coarse material can be also used to develop a well-performing biofilm able to degrade organic substances, e.g. BTEX (benzene, toluene, m-xylene), perchloroethylene (PCE), and trichloroethylene (TCE), which were found to be the most common groundwater toxicants.

My aim was to carry out phylogenetic studies of microbial community in the soil sample contaminated by BTEX as well as to isolate particular strains enabled to utilize the pollutants as a sole source of carbon and energy. During the enrichment on particular substrates (excluding PCE and TCE where no growth was detected) and their mixture, a rapid change in bacterial community was observed. One culture independent method for studying microbial communities is the analysis of very conservative PCR amplified 16S rDNA region of the rRNA operon by denaturing gradient gel electrophoresis (DGGE) in order to search for any difference in the DNA sequence. The method was first optimized using control strains. Purified genomic DNA obtained from each isolate was subjected to PCR reaction using universal primers for the V3 region of the 16S rDNA (carrying GC clamp). In a DGGE gel the number, precise position, and intensity of the bands in a gel track give an estimate of the number and relative abundance of numerically dominant ribotypes in the sample.

M. Vošahlíková, J. Pazlarová (Faculty of Food and Biochemical Technology, Institute of Chemical Technology, Prague, Czech Republic): **Effects of different organic acids on biodegradation of polychlorinated biphenyls.**

Polychlorinated biphenyls (PCBs) belong to recalcitrant xenobiotics, which were involuntarily released into the environment. Usage of soil microorganisms degrading PCBs represents one of the possible ways of their removal from the environment. The strain *Pseudomonas* sp. 2 was isolated from the polluted soil in the Czech Republic and proved a very good capacity for degradation of PCBs. The test of PCBs degradation involved two weeks of aerobic cultivation (rotary shaker) in standard mineral medium (MM) with Delor 103 (0.05 g . l^{-1}) and biphenyl (BP) (5 g . l^{-1}) at 28 °C. Quantitative estimation of PCB reduction was done after the extraction of the reaction mixture by hexane, followed by GC chromatography.

In our previous research we found the formation of chlorobenzoic acids as main products of biodegradation. This research followed the effect of some organic acids: 2,5-dichlorobenzoic acid (CBA), mixture of amino acids (AA), and humic acids (HA).

Our results show that addition of CBA and AA stimulates biodegradation of PCBs. The presence of 0.4 g/l of HA does not inhibit the growth of *Pseudomonas* sp. 2. and did not serve as a carbon source for the strain studied. Then an alternative biodegradative system was tested: MM, Delor 103, AA, and HA. Our strain, *Pseudomonas* sp. 2, was able to degrade PCB under the above-mentioned conditions with optimal concentration of HA.

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L. Chromá, K. Francová, T. Macek, M. Macková (Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic): **Rhizoremediation of polychlorinated biphenyls**.

The methods of biological removal of xenobiotics from the environment have recently replaced in some cases expensive physicochemical procedures. The cooperation of plant roots and microorganisms is called rhizoremediation.

In our study we monitored plants of different species in an area contaminated with PCBs and we tested the ability of biological systems to degrade PCBs over several months. In pot experiments, we followed differences in microbial growth between the non-vegetated and vegetated soil, and in the rhizoplane and rhizosphere. This study showed a beneficial effect of the growth and cultivation of both types of organisms. Cultivation experiments showed a higher number of microorganisms in the rhizoplane of all plants than in the bulk soil. Species isolated from rhizosphere and rhizoplane areas were identified as: *Pseudomonas sp*., *Pseudomonas stutzeri, Pseudomonas fluorescens, Alcaligenes* sp. Certain isolated bacterial consortia were able to degrade PCBs in the presence of biphenyl co-substrate used at laboratory conditions but also in the presence of phenolics isolated from roots. This phenomenon proved the important role of plant compounds and individual plant species in metabolic processes leading to decontamination of polluted soil.

Not only microorganisms but also plants are able to transform PCBs to various metabolites – e. g. hydroxychlorobiphenyls and hydroxybenzoic acid. These compounds are more soluble in water and for that reason they are more accessible for e. g. microorganisms. Now, the PCB degraders are tested for the ability to metabolise also the plant products of PCB pathway.

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E. Ryšlavá, M. Macková, T. Macek (Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Czech Republic): **Biodegradation of PCB's in real contaminated soil.**

This is part of the research work focused on the study of possible mutual relationships of microorganisms and plants in PCBs metabolism. Polluted soil was obtained from a site that has been contaminated by PCBs for a long time. This soil was used for isolation of bacteria that potentially participate in biodegradation of PCBs. Bacterial consortia and individual strains were isolated and their capability to degrade PCBs in the presence of biphenyl as co-substrate was tested under laboratory conditions. The 4B consortium and the JAB1 strain, which showed the best capability, were re-cleaned by isolation techniques and individual bacterial strains were taxonomically identified by the method 16S of ribosomal DNA. Presently, tests for biphenyl operon presence are being carried out, presuming degradative capabilities of isolated strains. The tests are done by PCR amplification of the *bphA1* and *bphC* genes.

The same contaminated soil was used for experiments under real conditions. Within six months, three plant species were cultivated – tobacco (*Nicotiana tabacum*), black nightshade (*Solanum nigrum*), and alfalfa (*Medicago sativa*) – and decrease of PCBs was observed. At the same time changes in the participation of rhizospheric microorganisms in vegetated and not vegetated soil were studied. Besides their own capability of PCB transformation, the plants release exudates stimulating growth and degradative capability of associated microorganisms via their root system. An HPLC analysis of tobacco and black nightshade tissue extracts proved that the exudates were phenolic acids (vanillic, ferulic, caffeic, cinnamic, and 4-hydroxybenzoic acids). The experiment in laboratory conditions has proved 20–30 % degradation of PCBs by isolated microbial consortium in 14 days, using the plant extract as the only source of carbon.

R. Teplá, L. Kotoučková, J. Navrátilová (Department of Microbiology, Faculty of Science, Masaryk University in Brno, Czech Republic): **Biodegradation of nitroaromatic compounds by Gram-positive irregular rods.**

Bacterial strain *Rhodococcus opacus* J3 was isolated from soil for its capability to degrade 4-nitrocatechol (4-NC). 4-NC is a nitroaromatic compound and an environmental pollutant produced by industry (human activities). Some microorganisms are able to degrade this compound completely.

Strain J3 was cultivated on mineral medium with yeast extract (YE) and with 4-NC. The growth of cells was accompanied by a decrease of the concentration of 4-NC and by an increase of nitrite ions in the medium. The cells of the strain undergo a typical life cycle in the course of cultivation.

The influence of 4-NC on the morphological shape of cells was examined by microscope and by a LUCIA G software. Circularity was chosen as the best parameter for comparing the morphological stages. If 4-NC was in the medium, fragmentation of filaments was more rapid and the medium value of circularity was higher in comparison with cells cultivated with YE.

J. Navrátilová[1], L. Kotoučková[1], J. Neča, [2] E. Durnová[3] ([1]Department of Microbiology, Faculty of Science, Masaryk University in Brno, [2]Veterinary Research Institute, Brno, [3]Department of Bacteriology, Regional Institute of Public Health, Ostrava, Czech Republic): **Characterization of bacterial strain J7 and study of its degradation abilities.**

Nitroaromatic compounds are widely distributed in the environment because of their extensive use as drugs, herbicides, pesticides, explosives, dyes, and solvents. Nitroaromatics and products of their complete degradation have relatively high acute toxicity and some of them may be potential carcinogens. Many microorganisms are capable of degrading these compounds. The aim of this study was to isolate bacterial strains capable of degrading these nitroaromatics.

The bacterial strain J7 was isolated from soil by selective enrichment in mineral medium with 4-nitroguaiacol. The strain J7 was identified as *Rhodococcus percolatus* on the basis of morphology, physiology, biochemical characterization, whole cell fatty acid composition, and determination of partial sequence of genes for 16S rRNA. At this strain there was an extended ability to degrade nitroaromatic compounds. The strain J7 degrades e. g. 4-nitrocatechol, 4-nitrophenol, 4-nitroguaiacol, and others.

The degradation of nitroaromatics and the release of nitrites were measured spectrophotometrically on a Genesys spectrophotometer. The concentration of substrates was confirmed by HPLC analysis. The degradation of some nitroaromatics in concentration ranges of 0.025–0,1 mmol . l^{-1} was studied on an automatic microbiological system Bioscreen C. The resting cell suspension was used for determination of the degradation of 4-nitroguaiacol.

Currently, studies of the enzymatic activity of the bacterial strain J7 are in the centre of scientists' attention.

L. Kotoučková[1], J. Nečas[2] ([1]Department of Microbiology, Faculty of Science, Masaryk University, Brno, [2]Veterinary Research Institute, Brno, Czech Republic): **Degradation of 4-nitrocatechol, 5-nitroguaiacol and 3-nitrophenol by *Rhodococcus opacus* strain C6-1.**

Gram-positive irregular rods are bacteria with a wide spectrum of biodegrading activity. The strain *Rhodococcus opacus* C6-1 degrades 4-nitrocatechol, 5-nitroguaiacol, and 3-nitrophenol. Degradation of other 4-substituted nitrophenols was not proved – on the contrary, 4-nitrophenol and 4-nitroguaiacol are not degraded. Degradation of 4-nitrocatechol is not common, if the degrading strain is not capable of degrading 4-nitrophenol, either.

4-Nitrocatechol, 5-nitroguaiacol, and 3-nitrophenol were used as sole sources of carbon and nitrogen; substrates are degradable both separately and as a mixture, too. The strain is able to degrade 4-nitrocatechol up to 0.5 mmol . l^{-1}, degradation of 5-nitroguaiacol was determined to 0.1 mmol . l^{-1} and degradation of 3-nitrophenol to 0.05 mmol . l^{-1}. 3-Nitrophenol was the most difficult substrate to degrade for the strain studied: 0.05 mmol . l^{-1} 3-nitrophenol was degraded completely after 5 days of cultivation. 4-nitrocatechol (0.2 mmol . l^{-1}) and 5-nitroguaiacol (0.1 mmol . l^{-1}) were degraded in 24 hours.

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A. Fialová[1], T. Bley[2], E. Boschke[2] ([1]Institute of Chemical Technology, Dept. of Fermentation Chemistry and Bioengineering, Prague, Czech Republic; [2]Technische Universität Dresden, Institut für Lebensmittel- und Bioverfahrenstechnik, Dresden, Germany): **Monitoring the phenol-like compound biodegradation by the yeast *Candida maltosa* using BOD measurement provided by AQUALYTIC® System.**

Phenol and phenol-like compounds pose at present a considerable danger for the environment. These toxic aromatic contaminants, originating above all from petrochemical, pharmaceutical and chemical industry, often cause problems in the waste water treatment plants with their bactericidal effects. Understanding the biochemical background of the phenol-like compound biodegradation is of great interest because of future implication of the microorganisms into the contaminated site.

This work is focused on monitoring the biodegradation potential of the yeast *Candida maltosa*, which was isolated using modified methods of aerobic enrichment [1] from soil contaminated for a long period with phenol-like aromatics; therefore the yeast is adapted also to higher phenol concentration. A new method of BOD (mg . l^{-1} O_2) measurement was used, provided by AQUALYTIC® Sensomat System, which is based on a manometric measurement of the pressure decrease during the cultivation of aerobic microorganisms in the closed system (microbial oxygen consumption, the produced carbon dioxide is absorbed into potassium hydroxide).

It was found out that this user-friendly system is suitable for sensitive measurements of BOD. It is necessary to maintain constant temperature and eliminate possible oxygen limitation. *Candida maltosa* is able to utilize phenol (up to ca. 1.7 g/l), resorcinol (at more than 2 g/l) and catechol, *p*-cresol only in combination with phenol, and it cannot utilize benzoate and salicylate as the only source of carbon and energy. The higher BOD during the cultivation on phenol, compared with cultivation on glucose, corresponds with the theory (structure and ability to utilize phenol in the metabolism). *Candida maltosa* reaches the highest specific growth rate on catechol, phenol and resorcinol; the rate decreases in *p*-cresol, benzoate and salicylate series.

The results obtained confirm applicability of the AQUALYTIC® for biodegradation processe monitoring and may be utilized in the decontamination procedure.

M. Gódyová (Department of Soil Science, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia): **Soil micromycetes and their biodegradation activities in atypical biotopes.**

Soil microscopic fungi, in association with other groups of microorganisms, colonize various materials and objects in the environment. More often they are present in atypical biotopes like flats, industrial objects or cultural and historical monuments. Their presence in these atypical biotopes is undesirable not only because of aesthetic and hygienic reasons, but mainly for their biodegradation activities that cause damage or total destruction of the attacked material. Microscopic fungi colonize not only organic substrates like wood, paper, textile but they also grow on stone monuments that are characteristic by more difficult conditions for their growth and reproduction in terms of nutrition. Due to metabolites that they produce, soil microscopic fungi are able to penetrate this relatively strong material by their hyphae and so cause its chemical and mechanical degradation.

Detailed mycological analyses of two valuable monuments of history and art, the Crypt of Chatam Sofer in Bratislava and the Museum of African Art in Bratislava, and a comparison of wood and stone mycocenosis, were the main goals of the present work. The interior environment of these analysed objects was very intensively contaminated by micromycetes. Moulds on the individual objects were visible with the naked eye.

The samples of microscopic fungi were collected by wiping with sterile cotton-wool from objects of African art made of non-specific wood, ebony wood and from the so-called iron wood, and from the surface of the stone monuments made of limestone in the Crypt of Chatam Sofer. The samples were carried to the tubes with 10 ml of sterile distilled water and shaken out. The suspension was used

for inoculation of Petri dishes with medium. The comparison of wood and stone mycocenosis was computed according to Sörensen (Odum, 1977) and Jaccard (Lamée, 1976).

Altogether, 46 species of microscopic fungi were isolated from the objects of the Museum of African Art (26 species from non-specific wood, 32 species from ebony wood, and 18 species from ironwood). The highest species variety was characteristic of the *Aspergillus genera* (9 species), *Cladosporium* (3 species), and *Penicillium* (7 species). The common contaminants of all the analysed objects were the species of *Alternaria alternata, A. tenuissima, Aspergillus flavus, A. niger, A. ochraceus, A. versicolor, Cladosporium cladosporioides, C. herbarum, and Rhizopus stolonifer*.

From the stone monuments of the Crypt of Chatam Sofer 36 species of microscopic fungi were isolated. The species of the genera *Acremonium, Fusarium, Penicillium*, and *Trichoderma* were the most common.

The wood and stone similarity index value calculated by Sörensen was 0.5 and by Jaccard 0.3. Since two mycocenoses are considered similar when the index value is higher than 0.6, we can say the mycocenosis of the wood articles of the Museum of African Art and the stone monuments from the Crypt of Chatam Sofer are not particularly similar.

6. Didactics of Microbiology

R. Horký[1]*, L. Kotoučková*[1]*, D. Novotný*[2] ([1]Department of Microbiology and Czech Collection of Microorganisms of the Faculty of Science, Masaryk University, Brno): **Microorganisms and microbiology – an instructional CD-ROM for students.**

The CD-ROM is aimed especially at students of grammar and secondary schools. The CD-ROM consists of two basic parts – Microorganisms and Man, Microorganisms and Environment. The part Microorganisms and Environment will be presented. The topic is divided into the chapters: Soil, Water, Air, Fungi, School Experiments, and Test. Each of these individual chapters explains its topic basing on rich photographic material. Protocols for easy experiments are in the chapter School Experiments. The chapter Test serves for checking the knowledge.

The individual microorganisms are presented in macroscopic pictures of colonies and microphotographs of living or stained preparations taken with a light microscope. The pictures could extend the knowledge of microbiology at lower school levels.

F. Růžička, V. Woznicová (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic): **Employing the illustrative videoclips in the education of medical microbiology.**

The aim of this project is to provide our students with new devices and diagnostic methods via short videoclips, which cannot be demonstrated for reasons of time and organization. The video material was taken with a Sony DCR-TRV 120D video camera. Nine illustrative videoclips several minutes long have been prepared, complemented with annotations of a lecturer, and scheduled into the education of microbiology.

O. Zahradníček, V. Woznicová, M. Votava (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic): **Teaching English terminology at the Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital in Brno**

The project reflected the existing need to improve the students' ability to work with international literature. The medical studies (not only) in Brno include two compulsory language-teaching programs and, for an absolute majority of the students, one of these is English. Nevertheless, this subject is not able to provide detailed information concerning problematic words in individual branches of medicine. This is logical, as the teachers are linguists but not surgeons, ophthalmologists, microbiologists, etc.

That is why we have decided to introduce these problems to the practical courses in microbiology. The practical course consists of demonstration of some words that are difficult to translate, and a translation of a text from a scientific journal in English, with attention paid to concentration of problematic words.

During the translation, the students learned to translate difficult words. When they met such words again, they were usually able to translate them properly.

A questionnaire among the students showed that the majority of them supported such an educational program and found it useful for the future scientific work.

The work was supported by a grant of the Czech University Development Fund F-0465/2000

Paper of the Best Young Czechoslovak Microbiologist of the Year 2001 (Awarded by the Czechoslovak Society for Microbiology)

M. Koblížek, C. Vetriani, P. G. Falkowski, Z. Kolber (Environmental Biophysics and Molecular Ecology Program, Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, New Jersey, USA): **Marine aerobic photosynthetic bacteria. Life on the edge of photosynthesis**.

Recently the presence of bacterial photosynthesis has been reported in the open ocean (Kolber et al. Nature **407**, 177, 2000; Kolber et al. Science **292**, 2492, 2001). We have isolated several strains of the photosynthetic bacteria from the marine environments. All of them are aerobic α-Proteobacteria belonging to the genus *Erythrobacter*. They contain functional bacterial photosynthetic units composed of the light harvesting complex 1 (LH1) and the bacterial reaction centre. The functionality of the reaction centres was proved by measurements of bacteriochlorophyll fluorescence transients and light-induced absorption changes. On the other hand, the bacteria are not capable of truly autotrophic growth. The presence of the photosynthetic apparatus can represent an ecological advantage in nutrient poor marine environment serving as an auxiliary source of ATP for the basal metabolism.

7. Experimentally Genetic Approaches in Microbiology

D. Gregorová, J. Doskočilová, A. Šebková, M. Pravcová, M. Faldynová, I. Rychlík (Veterinary Research Institute, Brno, Czech Republic): **Biological properties of low molecular weight plasmids in Salmonella Enteritidis.**

Salmonella enterica serovar Enteritidis (*S.* Enteritidis) frequently possesses plasmids of different sizes and roles. Besides the serovar specific virulence plasmid present in 95 % field strains, *S.* Enteritidis can also harbour plasmids of low molecular weight. Therefore we identified and characterized the most frequent low molecular weight plasmids in *S.* Enteritidis. Using DNA hybridization we found that there are at least three distinct groups of plasmids.

After sequencing 5 representative plasmids from each group we concluded that they belonged to ColE1, ColE2, and rolling circle replicating plasmids. The most widespread group turned out to be ColE1-like with plasmids pI, pC, and pK. The first characterized plasmid was plasmid pI (4053 bp) which encoded retron reverse transcriptase and influenced phage resistance. Plasmid pC (5269 bp) coded for a functional restriction modification that explains its nearly ubiquitous presence in highly phage-resistant *S.* Enteritidis PT14b strains. Plasmid pK (4245 bp) encoded a gene important for maintenance of plasmid in the cell and two ORFs with unknown functions. ColE2 type plasmid pP (4301 bp) encoded two proteins essential for replication and one ORF for which no function could be predicted. In the cell, pP was present predominantly in a single-stranded DNA form. The smallest plasmid pJ (2096 bp) encoded only a single protein essential for its own replication by the rolling circle mechanism.

Therefore we concluded that one of the frequent functions of low molecular weight plasmids in *S.* Enteritidis is protection against phage infection as observed in plasmids pI and pC, although in some cases, such as in the plasmid pJ, they could not be assigned any obvious function.

Acknowledgement: This work was supported by a grant of the Czech Ministry of Agriculture No. QC0195 and M03-99-01.

J. Doskočilová[1], I. Rychlík[1], D. Gregorová, A. Šebková[1], M. Pravcová[1], R. Karpíšková[2] ([1]Veterinary Research Institute, Brno, [2]National Public Health Institute, Brno, Czech Republic): **Low molecular weight plasmid of *Salmonella enterica* serovar Enteritidis encodes the gene for retron reverse transcriptase and thus influences phage resistance.**

Retron reverse transcriptases are unusual procaryotic enzymes capable of synthesising low molecular weight DNA by reverse transcription. All of the so far described DNA species synthesized by retron reverse transcriptase have been identified as multicopy single-stranded DNA (msDNA). We have shown that *Salmonella enterica* serovar Enteritidis is also capable of the synthesis of low molecular weight DNA by retron reverse transcriptase. Surprisingly, *Salmonella* serovar Enteritidis produced low-molecular-weight DNA which turned out to be a double-stranded DNA with single-stranded overhangs (small double-stranded DNA = sdsDNA). The sdsDNA was 72 nucleotides (nt) long, of which a 38 bp sequence was formed by the double-stranded DNA with 19-nt and 15-nt single-stranded overhangs, respectively. Three open reading frames (ORFs), encoded by the 4053 bp plasmid, were essential for the production of sdsDNA. These included an ORF with an unknown function, the retron reverse transcriptase, and an ORF encoding the cold shock protein homologue. This plasmid was also able to confer phage resistance onto the host cell by an undiscovered mechanism.

This work has been supported by the grant of the Czech Ministry of Agriculture QC0195 and M03-99-01.

J. Volf, M. Ševčík, H. Havlíčková, F. Šišák, I. Rychlík (Veterinary Research Institute, Brno, Czech Republic): **A role of *sdiA* in *Salmonella enterica* serovar Typhimurium physiology and virulence.**

Gene *sdiA* in *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) encodes a protein belonging to the LuxR family of transcriptional regulators. The initial computer analysis revealed the presence of a *fur* (ferric uptake regulator) box 19 bp upstream the start codon of the *sdiA* gene and a helix-turn-helix (HTH) motif in the carboxy terminal part of the SdiA protein. *fur* box is a sequence recognized by Fur protein, which can regulate iron metabolism, and the HTH motif is responsible for interaction with nucleic acid and is typical of transcriptional regulators. The deletion of the *fur* box resulted in the doubled *sdiA* transcription. Furthermore, the addition of dipyridyl, an iron chelator, to the culture media increased *sdiA* transcription to that observed in the *fur* box mutant, confirming that *sdiA* is suppressed in the presence of iron. When *S*. Typhimurium was grown in conditioned medium (previously used for 24 hour cultivation of *S.* Typhimurium and filter sterilized), *sdiA* transcription was repressed (4×) when compared to its transcription in fresh LB broth, and this repression was independent of the fur box. Oral infection of mice with a strain lacking the helix-turn-helix domain of sdiA indicated increased virulence of such *S.* Typhimurium mutant. *sdiA*, dually controlled by iron concentration and culture density derived signals, may therefore play an important role in *S.* Typhimurium virulence regulation. This work has been supported by a grant of the Czech Ministry of Education, Youth and Sports, LN00A016.

M. Ševčík, J. Volf, I. Rychlík (Veterinary Research Institute, Brno, Czech Republic): **Characterisation of yhjH in *Salmonella* Typhimurium**

The *Salmonella enterica* ser. Typhimurium (STM) mutant with a transposon inserted in the *yhj*H sequence was found to be unable to suppress the growth of a spectinomycin-resistant but otherwise isogenic STM strain. This gene shows homology to diguanylate cyclase and novel two-component signal transduction proteins though its function in STM is unknown. A transcriptional fusion was constructed which indicated that the *yhjH* was moderately expressed in the exponential phase of growth and up-regulated upon entry into the stationary phase. Expression of *yhjH* was considerably suppressed by the addition of supernatant from a 24 hour stationary-phase STM culture suggesting that it belongs to a new sensing and signalling regulatory pathway in STM. Moreover strong down-regulation was observed in an *fli*M mutant strain, which suggests some connection of *yhjH* to chemotaxis.

P. Švastová, Z. Jaglić, K. Nedbalcová, I. Pavlik, M. Bartoš (Veterinary Research Institute, Brno, Czech Republic): **Differentiation of *Actinobacillus pleuropneumoniae* by PCR-REA based on the variability of the *apx* IVA gene sequence.**

Actinobacillus pleuropneumoniae, a Gram-negative bacterium of the family *Pasteurellaceae*, is the aetiological agent of porcine pleuropneumonias and is responsible for considerable economic losses throughout the world. Serotyping is one of the most important and most frequently used tools in epizootology and sanitation programs to control porcine pleuropneumonia. Several serological methods, such as indirect fluorescence, indirect haemagglutination, complement fixation or coagglutination tests are available for the identification.

There have been observed significant differences in virulence among the different serotypes. It is most likely that the degree of virulence of the serotypes is for the most part associated with the exotoxins expressed by the different strains. Four different exotoxins, the strongly haemolytic Apx I, the weakly haemolytic Apx II, the nonhaemolytic Apx III, and Apx IV, have been identified. The genetic determinants for *apx* operons were described and methods based on analysis of the *apx* nucleotide sequences were developed for more accurate identification.

We have developed the method PCR-REA for simple and rapid differentiation of *Actinobacillus pleuropneumoniae* strains by restriction digestion of the amplification product, part of the gene for toxin Apx IV. The primers were developed from the position from 1 792 to 5 320 bp, according to the sequence published in the Gene Bank Acc. No. AF021919. The size of amplicon was estimated to be 3 529 bp. From the size differences of the amplification products it was possible to split the twelve-serotype collection strains into two groups. It means that the gene for Apx IV has a different nucleotide structure in different serotypes. The amplicons were hereafter digested by a total of 35 restriction endonucleases for a more detailed differentiation. After the comparison of these restriction profiles, two restriction endonucleases, Cfo I and Hpa I, were selected, which enable distinguishing the serotype strains, apart from serotype strains 9 and 11, in two steps. After digestion by the restriction enzyme Cfo I the serotypes 1, 2, 3, 4, 5, 6, 7, and 12 could be differentiated. The pair of the serotypes 8/10 and 9/11 could not be differentiated by Cfo I. After digestion by restriction endonuclease Hpa I it was possible to differentiate the serotypes 8 and 10. The serotypes 9 and 11 could not be differentiated after digestion by 35 selected restriction endonucleases. The PCR-REA results were confirmed on a selected collection and field strains by serotyping.

This work was supported by the grant No. QC0195 of the National Agency for Agricultural Research, the Czech Ministry of Agriculture.

*K. Štouračová, P. Alexa, J. Hamřík, I. Rychlík (*Veterinary Research Institute, Brno, Czech Republic): **Subtyping of colonization factor K88 (F4) in enterotoxigenic *Escherichia coli* by PCR.**

Enterotoxigenic *E. coli* is a frequent cause of diarrhoeal disorders in post weaning pigs. Production of enterotoxins and expression of fimbrial adhesins are considered as major virulence factors. One of the frequent adhesins is the K88 (F4) fimbriae that can appear in 3 distinct subtypes – K88ab, K88ac, and K88ad.

In the years 1995–2000, more than 4000 field strains of *E. coli* were isolated in our laboratory from the pigs with diarrhoea. Strains for subsequent K88 subtyping (n = 283) were selected from those giving positive reaction in (i) serological testing for the presence of K88 fimbriae, or (ii) in adhesion to brush border enterocytes. All the 283 strains were analysed using K88 specific PCRs.

Three primer pairs were designed. The first allowed amplification of DNA in strains harbouring either K88ab or K88ac genes. Strains positive in this PCR were subsequently analysed using the second PCR specific to the K88ab gene variant only. Strains positive in the second PCR were classified as belonging to the group of K88ab strains. Strains positive in the first PCR but negative in the second PCR were classified as K88ac. Negative strains from the first PCR were finally tested by K88ad specific PCR. Those resulting in positive amplification were classified as belonging among K88ad strains, and the negative strains were characterised as free of any type of K88 fimbriae.

In 237 strains analysed, at least one of the PCR reactions was positive. Subtype K88ac was detected in 232 strains belonging mainly to serological groups O149, O8, and O147. Subtype K88ab was identified in 2 strains originating from the same farm and belonging to the O8 serological group. The last 3 strains of the serological group O8, originating from another farm, expressed K88ad fimbriae.

The study showed that the K88ac antigenic variant of K88 fimbriae is the most predominant among strains isolated from the farms in the Czech Republic. This finding might be useful in design and composition of vaccines against enterotoxigenic *E. coli* used in the Czech Republic.

This work has been supported by the grant of the Czech Ministry of Agriculture, No. M03-99-01.

K. Tomanová-Hejcmanová, J. Smola, V. Celer (Institute of Microbiology and Immunology, Faculty of Veterinary Medicine, Veterinary and Pharmaceutical University, Brno, Czech Republic): **Detection of specific DNA sequences of *Lawsonia intracellularis* in various animals.**

L. intracellularis is a Gram-negative, obligate intracellular bacterium, which causes a disease of the digestive system in the pig, generally called proliferative enteropathy. Except the pig, a similar disease was described in other animals as horse, deer, ostrich, rabbit, hamster, and monkey. In these cases, the presence of the microorganism was confirmed by PCR with subsequent sequencing of PCR products. In our study, samples from various animal species were investigated by the nested PCR method. A specific reaction was detected in domestic and wild pig, horse, deer, roebuck, wolf, fox, dog, hamster, and pigeon. DNA sequences of PCR fragments were compared with a reference strain NCTC 12656^{T} of porcine provenience. The investigated strains showed 100 % reciprocal genetic homology and 99 % homology when compared with a reference strain of the bacterium. The presented study shows that DNA fragments from the Czech strains of *L. intracellularis* differed from appropriate genomic regions of a reference strain of the bacterium and from the sequence of *L. intracellularis* isolated from other animal species.

8. Microbiology in Epidemiological and Epizootic Context

A. Liptáková, L. Siegfried, L. Podracká, M. Uher, E. Kraková, M. Kmeťová, E. Bogyiová, J. Rosocha, H. Sehnalková, D. Kotulová (Institute of Medical Microbiology, Medical Faculty, Šafárik University, Košice, Hospital for Sick Children, Medical Faculty, Šafárik University, Košice, Epidemiological Unit of the State Health Department, Poprad, Associated Tissue Bank, Medical Faculty, Šafárik University, Košice, Institute of Microbiology, Medical Faculty, Comenius University, Bratislava, Slovakia): **Familiar incidence of the haemolytic-uremic syndrome caused by shiga toxin producing *Escherichia coli* O157.**

Selective cultivation, immunomagnetic separation, RPLA, and multiplex PCR were used for the detection of shiga toxin-producing *E. coli* (STEC) O157 in November 2001. The strains were isolated from stool samples of three children suffering from the haemolytic uremic syndrome. The children were admitted at the Clinic for Sick Children in Košice. Consequently, stool samples of all of the family members were collected. STEC O157 was confirmed in stool samples of four adults (parents) and two children with bloody diarrhoea. After epidemiological and microbiological investigation unpasteurised milk from a private farm was found as the source of infection.

H. Jelínková, P. Pazdiora, R. Bícová (Institute of Epidemiology, Faculty of Medicine, Charles University, Plzeň, Czech Republic): **Experience with vaccination against pneumococcal infections.**

Pneumonias are an important health problem. One of the most common pathogens is *Streptococcus pneumoniae*. An effective prevention is enabled by anti-pneumococcal vaccination.

Starting from the year 1998, the elderly pension inhabitants and hemodialysation unit patients in the former Western Bohemia Region were gradually vaccinated with the Pneumo 23 vaccine. Our work shows antibody titres against pneumococci and their gradual decrease during several years.

At the same time, we observed the pneumonia ratio on the total mortality in two elderly boarding houses of Plzeň. In the years 1998–2001, 329 persons died here, 40 of them due to pneumonia. Among them, two had been vaccinated.

L. Mejzlíková, R. Tejkalová, J. Svoboda (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic): **Strains of *Pseudomonas aeruginosa* as causative agents of hospital infections.**

In large hospitals, especially in those with a rich program including care of immunocompromised patients and patients after transplantations, hospital infections present a serious risk. One of the very important causative agents is *Pseudomonas aeruginosa* – the situation is usually worsened by its poor antibiotic susceptibility. That is why surveillance here is very important to prevent outbreaks that could be dangerous for patients.

The work describes the collecting of *Pseudomonas aeruginosa* strains for assessment of their possible migration during transfer of patients between various wards in our hospital. The aim is to enhance the monitoring of the epidemiological situation of hospital infections.

A. Kohutová[1], O. Zahradníček[1], J. Kaustová[2] ([1]Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, [2]Laboratory for Mycobacterial Diagnostics, Regional Office of Public Health, Ostrava, Czech Republic): **Characteristics of mycobacterial infections in refugees and in common patients.**

Tuberculosis and other mycobacterial infections belong to very important diseases. Their incidence in the Czech Republic seems to stagnate, but the ratio of foreigners in the incidence is on the increase (9.92 % in 2000). The study focuses on tuberculosis, but also on other infections caused by mycobacteria.

Patients of the "Specialized Medical Institute for Tuberculosis and Respiratory Diseases" (SMITRD) in Jablunkov, Czech Republic, were investigated. Out of a total of 107 persons, almost a half (44 persons) were refugee status seekers from the admission camp of the Ministry of Interior of the Czech Republic in Vyšní Lhoty. The aim of this study was a comparison of the population of refugee status seekers with common patients of SMITRD Jablunkov.

Out of 107 patients there were 44 refugee status seekers (41 %), coming in 14 cases from Ukraine, 9 from Vietnam, further from Romania, Georgia, Moldova, but even two patients from Slovakia appeared. Concerning the age, in Czech citizens mycobacterial infections were most common in the older age groups with a maximum lying between 65 and 74 years, unlike the refugees, where the age ranged mostly between 25 and 44 years.

Among mycobacterial taxons in the two compared populations, *Mycobacterium tuberculosis* was the most important. In refugee status seekers, *M. gordonae* and some non-identifiable strains appeared too; in the common population sample we detected eight more mycobacterial taxons, and in one patient a rare combination of *M. avium* and *M. fortuitum* was present.

I had supposed that due to incomplete or interrupted treatment a higher resistance ration in refugee status seekers would be found. Surprisingly, the results showed that in 54 % refugee status seekers the resistance to the five-antituberculotic combination tested was zero, while in Czech patients it was only 38 %.

V. Holá[1], J. Schlegelová[2] ([1] Faculty of Environment, Jan Evangelista Purkyně University, Ústí nad Labem, [2]Veterinary Research Institute, Brno, Czech Republic): **An ecological study in herds with high incidence of strains of *Staphylococcus* spp.**

Mastitis is the most common and costly production disease affecting dairy cows worldwide. The importance of the problem of coagulase-negative staphylococci, as causative agents of subclinical as well as clinical mastitis, has been increasing during the last few years.

This work is concerned with ecology of the species of the genus *Staphylococcus* in herds with high prevalence of mastitis, caused by these pathogens, and in the identification and demonstration of vectors of infection transmission in the herd. Flies and waste milk (quarter milk samples) fed to the calves or pigs were considered as possible vectors of infection transmission.

The samples of smear from the nares of calves, cows and pigs, smear of cows' udders, quarter milk samples, and samples of flies were collected in four farms concerned. After a microbiological examination of the samples the data were used for modelling of paths and interactions between the

factors affecting the situation in the farms. A direct redundancy analysis from the group of generalised linear models in the Canoco version 4.02 programme was used for these models.

From the 23 recorded species of the genus *Staphylococcus* the most common of all types of samples from all the four farms were the species *S. carnosus*, *S. chromogenes*, *S. piscifermentans*, *S. simulans*, *S. warneri,* and *S. xylosus*. The flies were contaminated with up to 20 species of the *Staphylococcus* genus and the quarter milk samples were contaminated with up to 15 species of the genus *Staphylococcus*. The species *S. muscae* and *S. pulvereri* were isolated only from quarter milk samples and the species *S. cohnii* ssp. *cohnii*, *S. felis*, *S. gallinarum* and *S. pasteuri* were isolated only from flies.

Both vectors of transmission were significant at a probability level of 95 % ($p = 0{,}018$; $F = 1{,}892$) and additional factors conductive to the transmission of infection were recognized. These additional factors comprise the localisation of the farm (farms situated in the area of villages have a larger spectrum of the species recorded), the possibility of contact of flies with waste milk (while being fed to calves or pigs), or the number and availability of sites for the growth of fly population (manure reservoirs, waste from sheds, etc.).

The study was financially supported by the fund MZE-M03-99-01 of the Ministry of Agriculture, Czech Republic, and by the grant QC0196/2000 of the National Agency for Agricultural Research of the Ministry of Agriculture, Czech Republic.

9. Microbiology in Clinical Context

J. Zahradníčková, M. Muchová, J. Audová (Department of Neonatology, Faculty Hospital Brno, Czech Republic): **Adnate neonatal sepsis and meningitis, caused by *Escherichia coli* – a case report**.

We present a case of peracute neonatal sepsis and meningitis. Symptoms (fever, convulsions, elevated oxygen demands, metabolic acidosis, anaemia, thrombocytopenia, and haematuria) have appeared in the course of the first 12 hours after delivery. *E. coli* with wide susceptibility to all the tested antibiotics was found in blood culture. The infant was treated by ampicillin with amikacin for 12 days, the first 2 days together with gentamicin, but fever and hypertonus of extremities did not withdraw, although the second blood culture and the cerebrospinal fluid were sterile after a 4 days' therapy. However, the cerebrospinal fluid revealed severe failure of the hematoencephalic barrier. Only after meropenem (6 days) therapy the infant improved. The neurology testing at discharge found a mild central hypotonic syndrome and recommended development rehabilitation and monitoring at the department for infants with perinatal risks.

O. Kubová[1], J. Skřičková[1], Z. Merta[1], D. Dvořáková[2], M. Votava[3] ([1]Dept. of Respiratory Diseases and Tuberculosis, Faculty of Medicine, Masaryk University, University Hospital Brno-Bohunice, [2]Dept. of Internal Medicine – Haemato-oncology, Faculty of Medicine, Masaryk University, University Hospital Brno-Bohunice, [3]Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic): **Pneumocystis pneumonia in the year 2001.**

The diagnosis of pulmonary infection can only be established by proving the presence of the microorganism in pulmonary secretions, or directly in the pulmonary tissue. Induced sputum, bronchoalveolar fluid (BAF), material from transbronchial biopsy or brushing, or material obtained via open lung biopsy can all be used. The immunofluorescent method using monoclonal antibodies and the polymerase chain reaction (PCR) method have been used most widely in recent years to prove the presence of the microorganism. Despite huge effort of a number of laboratories throughout the world, no method of culturing *Pneumocystis carinii* has been successful yet. Trimethoprim-sulfamethox azole is used as the antipneumocystis drug of choice in patients with *P. carinii* infection.

We evaluated clinical findings and indications for the commencement of antipneumocystis treatment in patients with *P. carinii* found in BAF.

During the year 2001, there was a suspicion of pneumocystis pneumonia (PCP) in 104 patients; bronchoalveolar lavage (BAL) was therefore indicated in all of them and BAF was examined. In 36 of these 104 patients, haemato-oncological disease was present, and 67 patients had long-lasting pulmonary disease (interstitial pulmonary process, asthma) or pulmonary signs with no unequivocal diagnosis yet made.

BAF was evaluated microbiologically and cytologically (stressing particularly the differential cell count) in all patients with suspect PCP. To identify *P. carinii*, the immunofluorescent method using monoclonal antibodies (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital) and PCR were used (Laboratory of Molecular Genetics, Dept. of Internal Medicine – Haemato-oncology). In order to make the diagnosis more accurate, an analysis of symptoms and signs, chest x-rays, laboratory findings and (if feasible) a complex lung function investigation were performed.

PC was proved in BAF in 40 (38.5%) of 104 patients where the suspicion of PCP had been voiced. Out of these 40 patients, 8 (20%) had a haemato-oncological disease. In 32 (80%) patients, lung disease was present (interstitial lung process, sarcoidosis, asthma). In 16 (40% of 40), PC was proved using PCR, in 20 (50% of 40) using monoclonal antibodies, and in 4 (10% of 40) by both methods. After the evaluation of clinical findings (discussed further in this paper), antipneumocystis treatment was started in 12 (30% of 40) patients, whereas in 28 patients the finding of PC in BAT was considered to be mere colonization.

In patients with an immunocompromising condition, the proof of PC in BAT is considered as PCP, and antipneumocystis treatment has started . However, in patients without unequivocally proven immunodeficiency, the final decision is made according to clinical symptoms and signs, laboratory results, and imaging methods. Some findings may be assessed as mere airway colonization.

Z. Kunová[1], I. Komendová[2] ([1]Institute of Preventive and Clinical Medicine, Bratislava; [2]Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia*):* ***Chaetomium* spp. as indoor fungi.**

Microscopic filamentous fungi in indoor environments are unaesthetic, produce volatile compounds with unpleasant smell, and moreover they represent a serious health risk for people living in affected buildings. An epidemiological relation between nonspecific health problems (allergies, acute and chronic respiratory tract illnesses, etc.) of predisposed people and microscopic fungi and their toxic secondary metabolites has been proved. The genus *Chaetomium* is one of the relatively frequent contaminants of indoor places. The aim of our work was an *in vitro* study of ciliostatic activity of endo- and exometabolites of *Chaetomium* sp. isolated from wall scrapes.

A chloroform extract of metabolites from a biomass of mycelia and spores of *Chaetomium* sp. and of a liquid medium with yeast extract (2%) and sucrose (10 %), cultivated at 25 °C for 10 days, was prepared. Its ciliostatic activity was investigated in 1 day old chickens' tracheas *in vitro* after 24, 48, and 72 hours.

The endometabolites of 17 isolates of *Chaetomium* spp. were ciliostatically active (stopping the cilia beating). After 24 h endometabolites of 7 isolates (41.2%), 48 h – 9 isolates (52.9%), and after 72 h – 10 extracts (58.8%) stopped the movement of tracheal cilia. In the case of exometabolites, the extracts of 17 species of *Chaetomium* spp. were ciliostatically active: after 24 h – exometabolites of 2 isolates (11.8%), 48 h – 5 isolates (29.4%), and after 72 h – 6 isolates (35.3%) of *Chaetomium* spp.

Based on the data presented, *Chaetomium* spp. as indoor fungi may be considered to contribute to the sick building syndrome or other health troubles in occupants of mouldy damp flats.

Z. Šándorčínová[1], D. Heinová[2], V. Takáčová[3], A. Studená[4], L. Siegfried[1] ([1]Institute of Medical Microbiology, Faculty of Medicine, P. J. Šafárik University, Košice, [2]Department of Biochemistry, University of Veterinary Medicine, Košice, [3]Department of Clinical Microbiology, University Hospital, Košice, [4]1st Clinic of Anaesthesiology and Intensive Care Medicine, University Hospital Košice, Slovakia): ***Enterobacteriaceae*, extended-spectrum beta-lactamases and intensive care unit patients.**

A long-term hospital stay, admission at an intensive care unit, central-venous or urinary catheter, and extended-spectrum beta-lactam therapy represent specific risk factors described in patients colonized or infected by extended-spectrum beta-lactamase (ESBL) producing bacteria.

69 clinical bacterial strains (members of the family *Enterobacteriaceae*) were isolated from patients hospitalized at the First Clinic of Anaesthesiology and Intensive Care Medicine (1st CAICM) during a three months' period, and the production of AmpC, TEM, and SHV type beta-lactamases, including ESBL were investigated. A three-dimensional test (AmpC, ESBL), a double-disk synergy test, Etest

(ESBL), and PCR (TEM, SHV) were used for the detection of the enzymes mentioned. Minimum inhibitory concentrations to selected cephalosporins and aminoglycosides were determined by the agar plate dilution method in all clinical strains.

18 strains of *Escherichia coli*, 11 strains of *Enterobacter spp.*, 5 strains of *Serratia spp.,* and 35 strains of *Klebsiella spp.* were isolated. Six strains of *Escherichia coli* (33.3 %), 5 strains of *Enterobacter ssp*. (45.5 %), and 3 strains of *Serratia spp*. (60 %) were positive for the production of AmpC. These strains were either full susceptible to beta-lactams or resistant to all the tested antimicrobial agents, except meropenem. Production of ESBL was determined in 1 *Escherichia coli* (5.6 %) and 11 *Klebsiella spp.* (31.4 %). The presence of bla_{SHV} genes was determined in 10 ESBL-producing strains. All the strains were susceptible only to meropenem and amikacin. bla_{SHV} genes were detected in non-ESBL *Klebsiella spp.* Specific fragments of bla_{TEM} genes were amplified in 6 *Escherichia coli* (33.3 %) without ESBL activity. According to the non-ESBL phenotype (DDST, Etest, three-dimensional test, and MIC) these could be probably genes encoding SHV-1, TEM-1 or TEM-2. ESBL-positive *Enterobacteriaceae* were isolated from the respiratory tract of patients usually one week after their admission at 1st CAICM.

M. Střítecká, R. Tejkalová, O. Zahradníček (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic): **ESBL: yes or no?**

Nosocomial infections represent a problem for every hospital, especially if they are caused by pathogens producing ESBL as *Klebsiella pneumoniae*. Since the end of the year 2001, the occurrence of uroinfections caused by this *K. pneumoniae* has been steadily growing in St. Anne's Faculty Hospital in Brno. We have observed that many of these infections were hospital infections. The profile of the patients: men, 70 years old with hypertrophic prostate gland. We want to monitor these uroinfections. A first problem is the detection of ESBL. We used the double-disk synergy effect (recommended by the National Reference Laboratory) and the diffusion disk test using susceptibility difference between 3rd generation cephalosporin and the same cephalosporin together with clavulanate (recommended by NCCLS). We found the double-disk synergy test not to be a suitable test for *K. pneumoniae* in our hospital.

F. Prusík[1], O. Nyč[1], P. Dřevínek[2] ([1]Department of Medical Microbiology, Faculty Hospital Motol, Prague, [2]2nd Paediatric Clinic, Faculty Hospital Motol, Prague, Czech Republic): **Epidemiology and determination of in vitro susceptibility of strains of *Burkholderia cepacia* isolated from cystic fibrosis patients.**

Burkholderia cepacia belongs among Gram-negative nonfermenting rods. It gained its medical importance in previous decades as a frequent causative agent of hospital infections, especially in cystic fibrosis (CF) patients.

The application of methods of molecular biology has allowed a subdivision of *B. cepacia* into nine genomovars: I, II, IIIa, IIIb, IV, V, VI, VII, VIII, IX. Some of these genomovars have already acquired their species names: II – *B. multivorans*, IV- *B. stabilis*, V – B. *vietnamiensis*. And that is the reason why all members of this group are classified within the *Burkholderia cepacia* complex (BCC). According to the severity of the clinical course, epidemiological aspects, and a high level of resistance to antimicrobial drugs, the genomovar IIIa is of crucial importance.

The current evidenced BCC colonization of patients of the CF Centre in FH Motol varies around 20 %. In 96 % of the colonized patients it was IIIa genomovar that has been diagnosed unlike the genomovars of BCC strains isolated from non-CF patients. Multiple resistance or even panresistance of IIIa genomovar strains limits the possibility of choosing an efficient combination of antimicrobial drugs. (Monotherapy is administered only occasionally in CF patients.)

Testing the synergistic effect of various antimicrobial drugs in vitro gives rise to new possible options of treatment. The E–test is an optimal method in our laboratory conditions. It has allowed visualization of synergy mainly in the following antibiotic combinations: meropenem + ceftazidime (54 %), ceftazidime + cotrimoxazol (36 %). A synergy between meropenem and cotrimoxazol (24 %) or ceftazidime and netilmicin (18 %) could be seen less often.

The project was supported by the grant IGA NM/ 6568-3.

I. Švantnerová, A. Žáková, P. Milosovič (HPL Laboratories, Bratislava, Slovakia): **Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction.**

Hospital infections caused by methicillin-resistant *Staphylococcus aureus* pose a serious problem in many countries. In the period of study, 11 580 strains of *S. aureus* were isolated. Ninety-seven strains of *S. aureus* were resistant to oxacillin. Methicillin-resistant *S. aureus* produces a low-affinity penicillin-binding protein (PBP 2` or PBP 2a) in addition to the usual PBPs. The structural gene of this PBP (*mec*A) is present in the resistant strains but not in the susceptible ones. In the present study, the polymerase chain reaction (PCR) was used to detect the methicillin resistance determinant by amplifying a 533-bp region of the *mec*A gene. The detection of the *mec*A gene by the polymerase chain reaction (PCR) was studied in strains resistant to oxalic acid in the disk-diffusion test.

M. Dubničková, M. Molnárová, M. Bukovský (Department of Cellular and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia): **A study of lipid A from strains of *E. coli* adapted to ammonium salts and amine oxide on human nonspecific immune activities.**

The nonspecific immunomodulatory properties of lipid A isolated from a sensitive strain (C) and from a strain adapted to ammonium salt (LBr) and amine oxide (LNO) of *E. coli* were investigated. The lipid A analogues differed in their immunostimulatory and immunosuppressive effects on human monocytes/macrophages in the phagocytic, microbicidal and metabolic activity. At concentrations of 1 and 5 μg/ml, analogues C, LNO and LBr were stimulatory, whereas at higher concentrations the C sample had an immunosuppressive effect on the phagocytic activity. The LBr analogue exhibited a significant immunosuppressive potential for the activation of human cells at concentrations over 5 μg/ml. The immunostimulatory and immunosuppressive activity of lipid A analogues is dose-dependent and depends on the character of the immunomodulatory activities tested.

M. Pravcová, M. Faldynová, F. Šišák, H. Havlíčková, I. Rychlík (Veterinary Research Institute, Brno, Czech Republic): **Characterisation of multiresistant strains of *Salmonella* Typhimurium.**

Within the last 5 years an increase in multidrug-resistant strains of *Salmonella* Typhimurium, frequently belonging to phage type DT104, has been recorded in Western Europe. Consequently, multidrug-resistant *S.* Typhimurium strains start to appear also in the Czech Republic. Such strains are characterised by resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide, and tetracycline, and are therefore described as ACSSuT. This was the reason why we have started to monitor antibiotic resistance in strains in the Czech Republic against 13 antibiotics (ampicillin, amoxycillin, apramycin, colistin, sulphametoxazol, cefotaxime, enrofloxacin, gentamycin, neomycin, streptomycin, tetracycline, chloramphenicol, and nalidixic acid) using the disk diffusion method NCCLS.

Altogether, 26 resistant and 3 control sensitive strains were tested. Resistance identified by the disk diffusion method was further confirmed by gene specific PCR for the detection of *aadA2* (aminoglycoside adenylate transferase, resistance to streptomycin), *sul1* (resistance to sulphonamide), *oxa1* (resistance to ampicillin and oxacillin), and *tetA* (resistance to tetracycline).

As expected, all the 3 sensitive strains were negative in all kinds of PCR. Thirteen pentaresistant strains of *S.* Typhimurium DT104 were positive in PCR for the detection of *aadA2* and *sul1* genes but negative in *oxa1* and *tetA* specific PCR. The remaining 14 resistant strains of *S.* Typhimurium were however negative in all four PCRs. In this group of strains, those resistant to streptomycin and sulphonamide were predominant.

We propose that pentaresistant ACSSuT *S.* Typhimurium DT104 strains are quite stable and of clonal origin. The same clone was isolated both from human and animal sources. However, strains with other resistances belonging to different phage types are quite heterogenous and the resistance genes present in them seem to be quite unstable.

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I. Holko[1], A. Nováčková[1], J. Urbanová[1], V. Kmet[2] ([1]State Veterinary Institute, Dolný Kubín, [2]Institute of Animal Physiology of the Slovak Academy of Sciences, Košice, Slovakia): **Identification of virulence factor genes in *Escherichia coli* strains isolated from non-pasteurised sheep milk cheese.**

In the year 2001 we examined 95 strains of *E. coli* isolated from two kinds of sheep cheese – one is solid sheep cheese and the other is the traditional Slovak soft cheese called *bryndza*. We tested the strains of *E. coli* using the PCR method. Our goal was to determine some of 11 genes of virulence factors VT1, VT2, VT2e, CNF1, CNF2, STI, STII, LTI, eaeA, Einv, Eagg. The result of our testing was the proof of at least one of the virulence factor genes in 9 strains (9.72 %). We proved neither the factors VT1 and VT2, nor Einv and Eagg in any strains. We determined the VT2e virulence factor gene in one strain isolated from solid sheep cheese. The most frequently determined genes were cnf_1 (3.09 %) and *eaeA* (3.09 %).

Our testing demonstrated the perspective possibility of using the PCR method regarding food safety control of the products from non-pasteurised sheep milk that are not examined by this type of investigation. Our results show that there is not only the need of quantification but also typing of *E. coli* isolated from food products because we have determined a relatively high ratio of virulent types of this potential pathogen (9.72 %).

E. Bogyiová[1], M. Kmeťová[1], E. Biroš[2], L. Siegfried[1] ([1]Institute of Medical Microbiology, Šafárik University, Košice, Slovakia, [2]Laboratory of Genetic Ecotoxicology, Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic): **PCR detection of urovirulence factors among α-haemolytic *Escherichia coli* strains isolated from various clinical materials.**

In this study we detected the genes responsible for the expression of α-haemolysin (hly), cytotoxic necrotizing factor type 1 (cnf1), P-fimbriae (pap) and S/F1C-fimbriae (sfa/foc), aerobactin (aer), and afimbrial adhesin (afaI) among 127 haemolytic *E. coli* strains isolated from various clinical material by the PCR method. We found that all gynaecological α-haemolytic strains were positive for cnf1. There was a significant difference when compared to 78 % of urine strains ($p < 0.001$), and 61 % of faecal strains ($p < 0.0001$). Similarly, sfa/foc specific DNA sequences were found in 100 % of gynaecological isolates, also significantly different when compared to 85 % of urine strains ($p = 0.01$), and 83 % of faecal strains ($p = 0.005$). From this point of view the female genital tract seems to represent a reservoir of *Escherichia coli* strains with extraintestinal pathogenic capacity.

V. Durmanová[1], J. Košovský[1], J. Rajčáni[2] ([1]Institute of Virology, Slovak Academy of Sciences, Bratislava, [2]Department of Microbiology, Jessenius Medical Faculty, Martin, Slovakia): **Detection of immediate early and early herpes simplex virus 1 (HSV-1) proteins during productive infection and reactivation .**

The expression of HSV-1 genome shows a cascade pattern followed by the synthesis of immediate early (IE), early (E), and late (L) proteins at productive infection. At virus reactivation, the kinetics of synthesis of HSV-1 proteins might be different due to the absence of the viral transactivation factor (α-TIF/VP16). We studied the kinetics of appearance of non-structural IE and E HSV-1 proteins in infected Vero cells and in explanted trigeminal ganglion fragments coming from rabbits with established latency. The appearance of IE63 (ICP27), TK (thymidine kinase), RR1 (ribonucleotide reductase, large subunit), and UL9 (OBP, *ori* binding protein) polypeptides was assessed by indirect immunofluorescence using immune mouse sera against the corresponding recombinant proteins. The fusion (biotin–tagged) proteins were expressed in *E. coli* transformed with recombinant Pin Point Xa-1 plasmid and then purified using avidin conjugated resin in the access of biotin. By immunofluorescence, the IE polypeptide IE63 (ICP27) appeared from 3 h post inoculation (p. i.), the E proteins RR1and UL9 from 4 h p. i, while E protein TK was detected from 6 h p. i. in infected Vero cells (during productive replication). The IE63, TK and UL9 proteins were seen mainly in the nuclei of infected cells, while RR1 was found only in the cytoplasm. In explanted trigeminal ganglion fragments (after virus reactivation), the IE63 antigen was detected from day 1 in culture, while the TK

from day 2 in culture suggesting a similar order of appearance of IE and E proteins during productive replication and reactivation. Both proteins were found not only in neurons, but also in the satellite cells surrounding them; this suggests that reactivation of the latent viral genome may frequently occur in non-neural ganglion cells.

A. Vargová, M. Stará, S. Bopegamage (Department of Virology, Institute of Preventive and Clinical Medicine, Bratislava, Slovakia): **In vitro cytokine induction by coxsackievirus B3 (CVB3) .**

The potential of any virus to induce cytokines significantly contributes to the pathological outcome of viral infections. Coxsackie viruses are implicated in the pathogenesis of chronic diseases such as type 1 diabetes and myocarditis. Cytokines play an important role in the pathology of these diseases. In vitro cytokine response was induced in the cells of Peyer's patches, axillary lymph nodes, bone marrow, spleen, thymus, and peritoneal exudates by CVB3 (Nancy) 0.1 multiplicity of infection (M.O.I). The cells were separated into adherent and non-adherent. Interferons (IFN) alpha/beta (α/β) and gamma (γ), and tumour necrosis factor alpha (TNF-α) were measured at 24, 48, and 72 hours after induction. The splenocytes induced the highest amounts of cytokines α/β at 24 h (1024U), TNF-α (343.6 pg/ml) and IFN-γ at 48h (181 pg/ml), in virus stimulated non-adherent cells. PHA induction enhanced the stimulation of IFNs α/β and γ but not TNF-α.

Our results show that the CVB3 (Nancy) strain is a potential cytokine inducer.

M. Stará, A. Vargová, S. Bopegamage (Department of Virology, Institute of Preventive and Clinical Medicine, Bratislava, Slovakia): **Comparison of the viral kinetics and serological factors during the intraperitoneal and oral routes of murine experimental coxsackievirus B3 (CVB3) infection.**

An experimental mouse model is often used to study the pathogenesis of coxsackieviral (CV) infections. CVB belong to the genus *Enterovirus*, family *Picornaviridae;* these viruses infect the human host via the faecal oral route. Host genetics, immunological status, sex, age, viral genetic factors, and the route of infection of the virus may influence the pathogenesis and outcome of infection.

We studied the outcome of the oral and intraperitoneal (i. p.) CVB3 (Nancy) infection in immunocompetent, Swiss Albino (ICR) mice. We studied the viral kinetics in the heart, pancreas, thymus, spleen, small and large intestines, and serum viremia and virus neutralising antibodies (VNA). A longer duration of the viremic phase and replicating virus in the spleen, late appearance of VNA, and the presence of the replicating virus in the small intestine were found by day 28 p.i. in orally infected mice. The viral neutralisation test indicated an earlier presence of neutralising antibodies in the i.p. route (day 5 p.i.) and the highest level was reached on day 8 p.i., whereas the oral route showed the presence of antibodies from day 6 p.i. and highest at days 9 p.i. and 10 p.i.

We conclude that the gut associated immunity can modulate viral replication and the outcome of the infectious process by coxsackievirus B infections.

P. Lovecká, I. Melenová, P. Kučerová, H. Nováková, M. Macková, T. Ruml, K. Demnerová (Dept. of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology, Prague, Czech Republic): **Using different biological systems for evaluation of ecotoxicity of PCB and their bacterial and plant degradation products.**

In the last decade much research effort has been devoted to finding alternatives for the use of classical remediation techniques based on physical and chemical methods. Different microbial and plant species may possess enzymatic properties capable of metabolizing certain environmentally persistent xenobiotics contaminating soil and water. It was shown that both bacteria and plants metabolize different organic molecules, even polyaromatic hydrocarbons and polychlorinated biphenyls. Not much is known about intermediates in plants and their toxicity and about the effect of such compounds on animals and other organisms existing in nature. Organic compounds are transformed to less phytotoxic ones, then conjugated with sugars, amino acids, etc., and deposited in vacuoles or lignin parts of the cell wall. Unfortunately, this fact does not mean that metabolites or products are also less toxic for other living systems existing in nature. Different systems for ecotoxicity

measurement have been evaluated during the last 15 years. Many of them are based on measurement of viability of different organisms and their ability to survive in the presence of different toxicants.

In our experiments we studied the metabolism of PCBs in bacteria and plants. These organisms are mainly involved in transformation of toxic compounds in nature and they are responsible for further fate of xenobiotics and their intermediates in the environment. The bacterial metabolism of PCBs is well known and has been described several times. Generally, plant metabolism was studied with less extent than bacterial or mammalian metabolism and only little information is available. In our studies we used as a model aseptic plant cells cultivated in vitro in liquid media containing PCBs and also normal plants cultivated in contaminated soil. Bacteria were isolated from contaminated soil in the Czech Republic. The decrease of PCB congeners after biotransformation was detected by gas chromatography with an EC detector. Intermediates and products were identified by GC-MS. Toxicity of identified bacterial and plant products was studied using different ecotoxicity systems including plant cells, microbial and mammalian cell systems. At first, the toxicity was evaluated using two different bacterial systems – luminescent bacteria and Bioscreen system. Genotoxicity was measured by the Ames test. As a plant model hairy root culture of *Solanum nigrum* was used. The growth and embranchment of hairy roots were monitored during several days. Furthermore, the toxicity to keratinocytes as another eukaryotic model was compared. The data have shown different susceptibilities of the chosen ecotoxicity models to toxic compounds; generally eukaryotic cells exhibited higher tolerance.

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M. Hrdinová, J. Mazurová (Department of Biological and Biochemical Science, University of Pardubice, Czech Republic): **Occurrence of *Enterococcus species* at dogs and their susceptibility to antibiotics.**

Enterococci are one of the microorganisms most often isolated from clinical specimens of humans and animals. They are significant agents causing diseases and their high resistance to external influences and increasing resistance to various antibiotics bring them to attention.

The frequency of occurrence of individual *Enterococcus* species in the clinical material taken from dogs and their susceptibility to the individual antibiotics was monitored in this study. We treated 786 swabs taken from different locations, from which we cultivated 331 strains. These microorganisms were isolated from the rectum (81.3 %), vagina (34.4 %), prepuce (13.6 %), and oral cavity (21.0 %).

The most frequently isolated species were *E. faecalis* (52.6 %) and *E. faecium* (22.4 %). Other species found in the specimens were *E. hirae* (3.9 %), *E. casseliflavus* (3.0 %), *E. durans* (2.4 %), and *E. mundtii* (1.5 %).

Susceptibility to antimicrobial agents was provided by the disc diffusion test, and the results were evaluated according to Urbášková (1998). The most effective drugs turned out to be amoxicillin/clavulanic acid (100 %), vancomycin (96.2 %), penicillin (95.5 %), and ampicillin (91.4 %). Resistance was found in the case of rifampicin (65.3 %). Although good susceptibility of enterococci to vancomycin was identified using the disc diffusion method, 42 strains of enterococci were found resistant to vancomycin medium (6 mg/l) by the search method. These were especially species *E. faecalis* and *E. faecium*.

O. Zahradníček (Institute of Microbiology, Faculty of Medicine, Masaryk University, and St. Anne's Faculty Hospital, Brno, Czech Republic): **The most common mistakes when sending an abstract to the "Tomášek Days" conference.**

When sending an abstract to a conference (not only Tomášek Days), the participants often make some mistakes. This poster intends to show some typical ones to prevent their occurrence in the future and to help young scientists to optimize the abstracts for any conference, not only the Tomášek Days.

Here we offer some major rules:

Follow the instructions of the organizers, or a pattern for the abstract. For example, do not use capitals for the title if the pattern does not use it; use the full first name, or the abbreviation only, according to the instructions.

Do not use complicated formatting of the text. The best way is to send the text unformatted – the organizers have to replace the original formatting by a new one, anyway.

Do not use tabulators or even spacer to format the text.

When using spaces, dots, etc., observe the rules recommended in the normatives (for the Czech Republic: Czech State Norm 01 6910).

Use italics for generic and species names of microbes, but not for serovars (a common mistake for Salmonella genus).

Do not use decimal points in the Czech/Slovak text and decimal commas in the English text.

Each abbreviation, except for some well known, should be written in full and then listed in parentheses before being used for the fist time.

Some of these rules are not valid for conferences that use copying the abstracts directly from the sheets of paper sent by the author. Nevertheless, the majority of the conferences – including Tomášek Days – use the electronic way of abstract book composition, where the graphics are unified and where one page for each abstract is unnecessary.

A more detailed explanation is to be found in the poster, or may be sent to anybody by the author via e-mail (zahradnicek@fnusa.cz).

Compiled and revised by M. Votava