ECCO XXXVI

European Culture Collections' Organisation Annual Meeting

Let's meet, cooperate, improve

Brno, Czech Republic
13-15 September, 2017

Prepared by *Czech Collection of Microorganisms* (Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, Building A25, Brno 625 00, Czech Republic).

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XXXVI Annual Meeting of the European Culture Collections' Organisation (ECCO 2017)

13-15 September, 2017

Brno, Czech Republic

Organised by the Czech Collection of Microorganisms on behalf of the European Culture Collections' Organisation

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Masaryk University Campus
Building A11
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Academic Canteen
B1
B2
Campus Resid. Area
Univerzitní kampus, sever
60
Studentská
A38
A26
A25
sklenik
A34
Univerzitní kampus
60, N90
A33
kor. UKB sever
A32
A31
sklenik
A24
A29
NRIT
Biology park
Kamenice
61, 69
Univerzitní kampus
25, 37, 50, N90
Univerzitní kampus
61, 69, 82
Welcome drink
LK
Kamenice
Kamenice
61, 69
Academic restaurant
Univerzitní kampus
25, 37, 50, 60, 61
69, 82, N90
A14
A15
A12
A13
A10
A5
A4
A3
A2
A1, b2
A1, b1
VH1
kor. UKB střed
Main Entrance
A22
A17
A11
A9
A7
A6
kor. UKB jih
Z
A21
A20
A19
A18
A16
A8
Campus square
Netroufalky
Nemocnice Bohunice
25, 37, 50, 60, 61, 69, 82,
N90, 405, 406
FN Bohunice
Vypracoval:
Odd. facility managementu SLNB.
Brno, duben 2016.

XXXVI ECCO Meeting Partners

The organisers of the XXXVI ECCO meeting are grateful for the support from the following entities:

Czechoslovak Society for Microbiology
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Contents

Programme

Wednesday, 13 September 2017

14:00 - 15:00 Registration and Posters mounting

15:00 - 15:10 Welcome address by Ivo Sedláček (ECCO 2017 Meeting Chairman) and Nelson Lima (ECCO President)

15:10 - 17:00 Session 1 New Trends in Microbial Taxonomy

Chairpersons: Antonio Martínez-Murcia and Ivo Sedláček

15:10 S1.1 Antonio Martínez-Murcia: Promoting the phylogenetic analysis for the identification of isolates and genetic detection methods in laboratories of routine control

15:40 S1.2 Hans-Jürgen Busse: Chemotaxonomy of *Arthrobacter* - possibilities and limits

16:10 S1.3 Petra Vídeňská: Metagenomics – does exist the easy way how to study the microbiome?

16:35 S1.4 Ondrej Šedo: MALDI-TOF MS profiling of bacteria: how far can we go?

17:00 - 18:00 Coffee break and Poster session

17:30 - 19:30 MIRRI - closed meeting

18:00 - 19:30 Visit of the Czech Collection of Microorganisms (groups of about 10 people who will be interested in visiting CCM)

19:30 - 21:00 Welcome drink (Masaryk University Campus, Na Lávce café, located between buildings A10 and A36)

08:00 - 08:30 Registration and Poster session

08:30 - 10:20 Session 2 Environmental Microbiology

Chairpersons: Petr Baldrian and Monika Laichmanová

8:30 S2.1 Petr Baldrian: Environmental microbiology coming back to strain isolation and characterization: the future role of culture collections

9:00 S2.2 Laura Selbmann: Black yeasts in the Culture Collection of Fungi from Extreme Environments (CCFEE), phylogeny and ecology

9:30 S2.3 Daniel Nývlt: Potential of Antarctic habitats for microbial research

9:55 S2.4 Christiane Baschien: Isolation and metabarcoding of aquatic mycodiversity of lakes along a humic substance gradient

10:20 - 11:00 Coffee break and Poster session

11:00 - 12:45 Session 3 Biotechnology and Microbial Preservation

Chairpersons: Mark Liles and Stanislava Králová

11:00 S3.1 Mark Liles: Application of soil-derived bacterial culture and metagenome collections for disease control

11:30 S3.2 Alica Chroňáková: Environmental microorganisms – a source of unexpected treasure? Story of soil actinomycetes collection

11:55 S3.3 Lukáš Chrást: Engineering bacterial strains degrading environmental pollutants by synthetic biology

12:20 S3.4 Giovanna Cristina Varese: Mycoviruses: are they an important issue for the quality control of a fungal collection?

12:45 - 14:00 Lunch

14:00 - 15:45 Session 4 Clinical Microbiology

Chairpersons: Edward Moore and Pavel Švec

14:00 S4.1 Edward Moore: Diagnostics of infectious bacteria is dependent upon a reliable and comprehensive identification and taxonomy framework

14:30 S4.2 Charles M. A. P. Franz: Foodborne pathogens and antibiotic-resistant enterobacteria from fresh produce in Germany: potential of bacteriophages for biocontrol

14:55 S4.3 Oto Melter: Classification of *Bartonella* species and pathogenesis of infections

15:20 S4.4 Eva Krejčí: Do we need precise bacterial taxonomy in clinical laboratory?

16:15 Departute to the Punkva Caves and Congress dinner

09:00 - 10:30 Session 5 Legal & Standards Challenges for Culture Collections

Chairpersons: Philippe Desmeth and Dana Nováková

9:00 S5.1 Philippe Desmeth: The Nagoya Protocol the EU Regulation and ECCO; Culture Collections looking for cost effective solutions

9:30 S5.2 Jörg Overmann: Novel challenges and predicted trends for culture collections in the age of the Nagoya protocol

10:00 S5.3 Eliška Rolfová: Implementation of the Nagoya Protocol and related legislation in the Czech Republic

10:30 - 11:15 Coffee break and Poster session

11:15 - 12:30 Session 5 Legal & Standards Challenges for Culture Collections (cont.)

11:15 S5.4 Anabela Martins: Proactive approaches to quality management

11:35 S5.5 Christoph Brochhausen: BRoTHER, a biobank cooperation between Regensburg, Pilsen and Brno

11:50 S5.6 Christoph Brochhausen: SmartFreezer®, a fully automated robotic system for biomaterial storage at -196°C

12:05 S5.7 Alexander Vasilenko: Data integration with Life Science databases: the technical aspects

12:30 - 13:45 Lunch

13:45 - 15:45 ECCO Annual General Meeting

Chairperson: Nelson Lima

ECCO board agenda, presentation of new ECCO membership applicants, next ECCO meeting venue presentation, closure of the meeting

Oral Presentations

S1.1 Promoting the phylogenetic analysis for the identification of isolates and genetic detection methods in laboratories of routine control

Antonio Martinez-Murcia

Genetic PCR Solutions™ and University Miguel Hernández, Spain

Actual capacity to describe phylogenetic diversity had opened a hopeful scenario in bacterial systematics still, in our view, to be exploited for both main objectives: i, identification of bacterial species isolated as pure cultures; and ii, rapid detection of specific pathogens directly on samples. Although genetic information (intrinsically informative of natural relationships, stable and non-subjective) is today easy and rapid to obtain, developed methods have not yet been adopted for use in routine laboratory procedures for species identification. Multi-Locus Phylogenetic Analysis (MLPA), inferred from a few housekeeping genes, has shown a level of evolutionary concert that seems supported by the genomic sequences currently compiled. The MLPA framework available constitutes a stable and "reasonable" criterion for species delineation, which accepts new descriptions and diversity without interfering the current phylogeny. The arrival of Whole Genome Sequencing (WGS) provides a new chance to assess total DNA similarities (now *in silico*) and offers the ultimate primary information to perform MLPA. Nevertheless, several difficulties make this approach too tedious, impractical for standard laboratories of microbial control. Accumulated data, the mean active of the WGS generation, still should be carefully catalogued to inherit a reliable database. On the other hand, the implementation of species-specific detection tests based on the strategy of "polymerase chain reaction" (PCR; originally described in 1988), requires rigorous validation exercises.

Microbiologists should be encouraged to implement genetically based practices as an additional alternative to the current phenotypic methods extensively used for species identification and detection. Drawbacks of genetic technology together with the major hopes, taking into account the microbiologist end-user point of view, were explored in contrast to these of research with taxonomical purposes. Major needs found for applied markets of microbial control were simplicity, robustness, suitability, and competitiveness, all intrinsic to a concept as desired as little used: "pragmatic".

Within last three years, our laboratory has developed and produced quantitative PCR (qPCR) reagents specific to more than 200 taxa-specific genetic targets of bacteria, viruses, and parasites. Specificity *in silico*, a critical key highly dependent of published data, and chemical-structure considerations (% GC,

dimers, 3'-ends, etc.) remain the highest commitments to ensure optimized thermodynamics. The GPS™ dtec-qPCR kits for the detection of pathogenic bacteria selected under the European project AQUAVALENS have undergone validation following the guidelines of the UNE/EN ISO/IEC 17025:2005 and French Standard NF T90-471:2010. Validation terms included *in vitro* specificity (inclusivity/exclusivity), the quantitative phase analysis using the standard curve calibration (against ten-fold serial dilutions of 10-10^6 standard DNA copies), reliability (repeatability/reproducibility) and sensitivity (detection/quantification limits). Our criteria of acceptance requires 90% correct results for a minimum of 10 repetitions. Additionally, a format called "MONODOSE" (specific pathogen, dried single-dose PCR tubes, ready to add the sample) and fast methods were developed and validated. Innovations made so far provided pragmatic advantages, making the PCR approach a simple, reliable, and quick task, becoming a considerably help to promote the transfer of this technology. For instance, a fully developed assay allowed foodborne pathogen detection in one day: pre-enrichment incubation time was reduced to 8 hours in food samples (spinach and chicken) spiked with low cell solutions of *Salmonella enterica* and *Escherichia coli* O157: H7, while *Listeria monocytogenes* (ca. <10 CFU) only required incubation for 12 hours.

GPS™ is now projecting a more pragmatic MLPA approach for extensive screening of isolates (single colonies) to be used as a routine protocol for species identification. This simple, universalized and automated protocol, packaged in an easy-to-use box, may be relatively accessible to most laboratories and should be cost effective. Relatively "universal" primers, inclusive to relevant bacterial groups, to amplify and sequence a portion of the most used protein-coding housekeeping genes (*gyrB, recA, rpoB*) are under development. Selected targets were the most common pathogens species belonging to more than 40 genera. The strategy will allow massive identification of colonies isolated on cultures with different types of rich media without restrictions. This window perhaps was not fully opened as classically may has been bypassed by the use of, for instance, selective pre-enrichments. Although the contribution of the MLPA approach to taxonomy has already surpassed any expectation, everything seems to indicate that a vast description of phylogenetic diversity is still to come. MLPA implemented for routinely practices would feedback with enormous amounts of data to enrich taxonomic frames.

The research leading to these results has received funding from the European Union Seventh Framework Programme [FP7/2007-2011] under Grant agreement no: 311846.

S1.2 Chemotaxonomy of *Arthrobacter* - possibilities and limits

Hans-Jürgen Busse

Institut of Microbiology, Veterinary University Vienna, Austria

The genus *Arthrobacter* was proposed by Conn & Dimmick (1947). In the following decades new species were assigned to the genus as long as they were Gram-positive, aerobic, non-spore forming organisms with a rod-coccus growth cycle and L-lysine as the characteristic diamino acid of the peptidoglycan. Already in the 1980s it became clear that the genus is phylogenetically and chemotaxonomically heterogeneous. Ignoring this heterogeneity, still new species were assigned to the genus if, based on 16S rRNA gene sequences, a species of the genus *Arthrobacter* was identified as the next relative. As a result the genus was composed of species showing more than 15 different peptidoglycan types, five quinone systems and at least two polar lipid profiles. Furthermore, it was shown that several *Arthrobacter* species are phylogenetically more closely related to species of other genera of the family *Micrococcaceae* than they are to the type species of the genus *Arthrobacter globiformis*. Considering phylogenetic and chemotaxonomic homogeneity numerous *Arthrobacter* species have been recently reclassified in novel genera, namely *Sinomonas, Paenarthrobacter, Pseudarthrobacter, Glutamicibacter, Paeniglutamicibacter, Pseudoglutamicibacter* and *Haematomicrobium*. Even after these reclassifications the genus *Arthrobacter* harbors numerous species which are only distantly related to *Arthrobacter globiformis* but lack of defining chemotaxonomic traits are hampering their transfer to novel genera.

Conn, H. J. & Dimmick, I. (1947). Soil bacteria similar in morphology to *Mycobacterium* and *Corynebacterium*. *J Bacteriol* 54, 291–303.

S1.3 Metagenomics – does exist the easy way how to study the microbiome?

Petra Videnska[1], Barbora Zwinsova[1], Kristyna Smerkova[1], Lenka Micenkova[1], Karel Sedlar[2], Eva Budinska[1]

[1]Research Centre for Toxic Compounds in the Environment, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic; [2]Department of Biomedical Engineering, Brno University of Technology, Technicka 12, Brno, Czech Republic

The significance of studying microbial communities has increased in the past years. Great progress was made especially in the area of investigating uncultured bacteria, which constitute the main part of microbiome and which were previously difficult to characterize with techniques like cloning, Sanger sequencing or DGGE. It is mainly the next generation sequencing (NGS), that allows detailed study of the microbiome.

The major pitfall of this technology is the inconsistency of approaches employed by different microbiome studies [1]. The final microbial composition as detected by sequencing is heavily influenced by a multitude of factors: sampling method [2], sample storage conditions [3], DNA extraction [4], primers design [5] and bioinformatic analysis [6]. This may lead to misinterpretation of results and complicates their direct comparison between individual studies.

In our work, we focused on the effects of sampling and DNA extraction on the composition of gut microbiome. We compared multiple sampling (cotton swab, FloqSwab and stool container) and DNA isolation kits (Powerlyzer PowerSoil DNA isolation Kit - PS and QIAamp DNA Stool MINI Kit - QS) in order to provide guidelines for microbiome stool sampling in cohort studies.

	DNA yield	DNA purity	DNA quality	Presence of inhibitors	Presence of human DNA	Observed species	G+ bacteria isolation
PS	**â**	**á**	**á**	**â**	**á**	**á**	**á**
QS	**á**	**á**	**â**	**â**	**á**	**á**	**â**

Based on the results we selected stool container as the best sampling kit for healthy volunteers and PS kit as more suitable isolation kit providing the

best trade-off between the performance and efficiency in lysis of the G+ cell walls.

[1] Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. Diversity, stability and resilience of the human gut microbiota. *Nature* 489, 220–30 (2012).

[2] Loftfield, E. *et al.* Comparison of collection methods for fecal samples for discovery metabolomics in epidemiologic studies. *Cancer Epidemiol. Biomarkers Prev.* 25, 1483–1490 (2016).

[3] Cardona. Storage conditions of intestinal microbiota matter in metagenomic analysis. *BMC Microbiol.* 12, 158 (2012).

[4] Mackenzie, B. W., Waite, D. W. & Taylor, M. W. Evaluating variation in human gut microbiota profiles due to DNA extraction method and inter-subject differences. *Front. Microbiol.* 6, 130 (2015).

[5] Walker, A. W. *et al.* 16S rRNA gene-based profiling of the human infant gut microbiota is strongly influenced by sample processing and PCR primer choice. *Microbiome* 3, 26 (2015).

[6] Schloss, P. D., Gevers, D. & Westcott, S. L. Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies. *PLoS One* 6, e27310 (2011).

S1.4 MALDI-TOF MS profiling of bacteria: how far can we go?

Ondrej Šedo

CEITEC, Masaryk University, Brno, Czech Republic

Matrix-Assisted Laser Desorption-Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) has become the first method of choice for bacterial identification in clinical diagnostics. The identification is based on profiling of proteins from unknown bacterial isolates and their comparison to database entries obtained by analysis of known strains carried out under equivalent conditions. The method provides analysis outcome within minutes and the discriminatory power of the method reaches beyond the species level. In the present paper, we examined various fields of applied microbiology, where, in addition to clinical diagnostics, MALDI-TOF MS is capable of providing relevant outcomes. The extension of currently available databases of reference mass spectra represents the only and easily fulfillable requirement. In particular cases, we reached the limits of the discriminatory power of the method, related usually to distinguishing between closely related species. Therefore, the reference databases should always be carefully updated and evaluated to avoid misidentifications. To improve the performance of the method, we proposed simple methodical variants of MALDI-TOF MS profiling modulating its discriminatory power. We defined conditions permitting reliable discrimination between selected strains of the same species, however, universal identification of individual strains seems to be out of the scope of the method.

Acknowledgement: This work was carried out with the support of the Proteomics Core Facility of CEITEC – Central European Institute of Technology under CIISB project, ID number LM2015043, funded by the Ministry of Education, Youth and Sports of the Czech Republic, and by Czech Science Foundation (project no. P206/12/G151).

S2.1 Environmental microbiology coming back to strain isolation and characterization: the future role of culture collections

Petr Baldrian

Institute of Microbiology of the CAS, Prague, Czech Republic

Here we have performed a comprehensive isolation effort to obtain multiple dominant bacterial taxa from a *Picea abies* forest soil and provide their physiological characterization. This information allows us to link ecological traits with groups of microorganisms. In the study, conventional culture techniques at acidic pH and low-nutrient content led to the recovery of 299 bacterial isolates. The isolates represented operational taxonomic units (OTUs) that contained 20% and 32% of all bacterial genomes detected in the litter and soil by 16S amplicon analysis, including some of those bacterial strains representing the most abundant and active OTUs. These included also several isolates of the still underexplored phylum of the *Acidobacteria*, all of them belonging to the subdivision 1 of the phylum. Acidobacterial isolates produced the widest range of enzymes among all isolates and highest enzyme activities in acidic conditions. Moreover, members of the *Acidobacteria* represented more than 50% of the isolates able to grow on disaccharides produced during the breakdown of cellulose, chitin and starch. Our results indicate that *Acidobacteria* may play an important ecological role by degrading polysaccharides of plant and fungal origin in the important ecosystems of acidic coniferous forests.

S2.2 Black yeasts in the Culture Collection of Fungi from Extreme Environments (CCFEE), phylogeny and ecology

Laura Selbmann

Department of Ecological and BiologicalSciences (DEB), University of Tuscia, Largo dell'Università, Viterbo 01100, Italy

Black yeasts, or black meristematic fungi, are peculiar for their bewildering ability to spread in the extremes; they have already been known by the end of the nineteenth century but, since recently, only few workers were familiar with them. Now that the amplitude of their biodiversity, stunning ecologies and potential applications have become apparent, they are becoming an ever-expanding fields of study spanning from microbial ecophysiology, evolution and adaptation to extremes, human pathogenicity, bioremediation, biodeterioration, and astrobiology. The CCFEE (Culture Collection of Fungi From Extreme Environments) in the Thematic Section of Mycology of the Italian National Antarctic Museum, University of Tuscia, Viterbo, Italy, includes black fungi from extreme locations worldwide as salty, hydrocarbon-contaminated, and acidic sites, stone monuments, hot and cold deserts and mountain tops. The large majority of the strains comes from Antarctic ice-free areas were black meristematic fungi live associated with rocks, tacking part of microbial endolithic communities. This is the largest collection of fungi from these communities in the world, obtained over three decades of researches, in the frame of the Italian National Program for Research in Antarctica, and a number donated by I.E. Friedmann. At present, it counts more than 1300 fungal strains, among which 851 are black yeasts, with a high incidence of still unknown species. Environmental pressure and isolation promote adaptive radiation; to date, studies based on molecular phylogeny led to the description of 20 new genera and 50 new species. Black yeasts in the CCFEE are mainly distributed in two classes: Dothideomycetes (specially Capnodiales) overrepresented in remote, icy locations and Eurotiomycetes (specially Chaetothyriales) more frequent in hot and polluted conditions. The skill in adapting cold stress in Dothideomycetes is probably due to their phylogenetic roots, since they diversified in the Silurian–Devonian period, when temperatures were much cooler than today. Survival in the extremes is a clear class-wide tendency with a number of lineages purely constituted of black-specialized extremophiles. Some species, belonging to this class, preserved in the CCFEE, show a rather restricted distribution as the Antarctic endemic genus and species *Friedmanniomyces endolithicus*; others are spanning both hemispheres but, with few exceptions, occur specifically in the cold. Chaetothyriales have evolved after the Permian-Triassic mass extinction

and an expansion of arid landmasses, this correlate with their tolerance to higher temperatures; they have a larger spectrum of assimilative abilities including toxic organic compounds that, coupled with extremotolerance, promote shifting to opportunism, the main evolutionary tendency within the order. Species in the CCFEE found on monuments, urban environments, and hydrocarbon rich substrates fall mainly in this order.

S2.3 Potential of Antarctic habitats for microbial research

Daniel Nývlt

Head of the Czech Antarctic Research Programme, Department of Geography, Masaryk University, Brno, Czechia

The J. G. Mendel Czech Antarctic Station is located on the northern coast of Ulu Peninsula, James Ross Island, which represents the largest ice-free area in the Antarctic Peninsula region. The gradual deglaciation of the area since the Late Pleistocene was locally interrupted by neoglacial mid- to late-Holocene advances of local glaciers. The long-lasting evolution of deglaciated landscape led to the origin of diverse habitats, such as lakes, streams, seepages, wet walls, permafrost and its active layer, bare ground, soils, surrounding of decaying seal carcasses any many more. Especially the aquatic environments have been identified as spots of microbial and cryptogam diversity in the marginal part of Antarctica. In spite of tens new species of lichens, algae, cyanobacteria, fungi and bacteria recently described from this area, Antarctica still has great potential for future microbial research.

The Czech Antarctic Research Infrastructure provides full support to all national and international research activities in James Ross Archipelago at the margin of Antarctica.

S2.4 Isolation and metabarcoding of aquatic mycodiversity of lakes along a humic substance gradient

Christiane Baschien, Josephina Papathanasiou, Andrey Yurkov, Jörg Overmann

Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Fungi in aquatic environments degrade organic matter and thus transfer nutrients to other trophic levels. They comprise a phylogenetically heterogeneous group of fungi adapted to life in aquatic habitats. Biodiversity assessments and living fungal cultures from lakes are both scarce. We investigated the fungal diversity in three different lakes displaying a gradient in organic carbon (OC) content and quality. Cultivation and meta-genomic barcoding were employed to study freshwater fungi in littoral water, fine particulate organic matter (FPOM), sediment and coarse organic matter (CPOM) such as leaves and wood. Furthermore, isolates were tested for ability of humic matter degradation. Three isolation methods applied to four substrates of three lakes revealed 262 cultures. CPOM showed the highest diversity among analyzed substrates. Compared to other cultivation approaches, multi-well methods were least successful. The taxonomic diversity was dominated by Ascomycetes, most of which are known as plant pathogens or saprobes. The study yielded about 20 potential new species. The production of laccases and peroxidases was observed in 25 % of all isolated species. The metabarcoding approach yielded 90,772 ITS2 sequences. The water samples yielded highest numbers of sequences. The taxonomic assignment was manually curated. The final dataset contained 572 OTUs of which 74.5 % were classified on species level. Metabarcoding showed communities to be highly variable among lakes and substrates. Community structure correlated with the quality of carbon, the temperature and available nitrogen. Almost 90 % of isolate sequences were retrieved in metabarcoding substrate samples. The majority of fungal species usually reported from terrestrial habitats were detected in both, the metabarcoding and isolation approach.

S3.1 Application of soil-derived bacterial culture and metagenome collections for disease control

Mark Liles[1,2], Alinne Pereira[1], Jinglie Zhou[1], Scott Monsma[3], ChengCang Wu[3], David Mead[2]

[1]Auburn University, Department of Biological Sciences; [2]Varigen Biosciences Corporation; [3]Lucigen Corporation

Cultured microorganisms have historically been a rich resource for bioactive natural products, yet many environmental microorganisms are recalcitrant to laboratory cultivation. Two research projects will be discussed that include (1) Obtaining novel rhizobacteria cultures that produce bioactive metabolites and (2) Identifying and expressing biosynthetic gene clusters from a soil metagenomic library. (1) To isolate rhizobacteria, soil microorganisms were cultivated for 3 months using a low nutrient medium supplemented with a root or soil extract. Among the over 500 unique bacterial isolates obtained, two *Actinobacteria* isolates produced metabolites that inhibited methicillin-resistant *Staphylococcus aureus* (MRSA) growth. The complete genome sequence of each *Actinobacteria* isolate was determined and was predicted to encode 32 and 28 biosynthetic gene clusters, respectively. Biochemical and bioassay data indicate that one *Streptomyces* spp. isolate encodes novel antibacterial and antifungal compounds. (2) Using a culture-independent approach, a large-insert metagenomic library was constructed from an agricultural soil in a broad host range bacterial artificial chromosome (BAC) vector. Identification of biosynthetic gene clusters was conducted using DNA hybridization, PCR and next-generation sequencing. A conserved domain of Type I polyketide synthases (PKS) was identified by macroarray hybridization or PCR, resulting in 12 and 110 pathway-containing clones, respectively. In addition, a strategy in which plates, rows and columns were separately pooled and sequenced using Illumina HiSeq allowed identification of 593 clones that contained a PKS and/or non-ribosomal peptide synthetase pathway among 1,516 total biosynthetic pathways identified. BAC clones containing a PKS pathway were conjugally transferred into Streptomyces coelicolor strain M1154 and screened for MRSA growth inhibition. We identified 19 BAC clones that expressed anti-MRSA activity and in silico analyses indicate that these heterologously expressed clones encode pathways highly divergent from known PKS pathways. These studies illustrate the potential of both culture-dependent and -independent approaches to identify bioactive metabolites encoded by soil microorganisms.

S3.2 Environmental microorganisms – a source of unexpected treasure? Story of soil actinomycetes collection

Alica Chroňáková[1], Tomáš Chrudimský[1], Kateřina Petříčková[2], Miroslav Petříček[2], Václav Krištůfek[1]

[1]Biology Centre CAS, Institute of Soil Biology; [2]University of South Bohemia in České Budějovice, Faculty of Sciences

Actinomycetes are known as potent producers of bioactive compounds of innumerable structures and activities. This is also one of the main reasons why we collect the wild strains from diverse terrestrial habitats in the Culture Collection of Soil Actinomycetes in České Budějovice (CCASCB) and screen their potential to produce secondary metabolites (SMs) since 2007. Microbial natural products represent the important resource for discovery of novel drugs. As the traditional activity-based screening techniques turned ineffective with numerous re-discoveries of characterized metabolites, novel approaches are needed to get an access to rarely produced structures or products of silent genetic information. Genomic data suggest that only a minority of actinomycetes biosynthetic potential is operational under laboratory conditions and most of it remains hidden for phenotype-based screening. The development of strategies to activate orphan biosynthetic machineries is one of the major challenges in current natural product research. To achieve the successful discovery of new producers of bioactive molecules, our group is focused mainly on following approaches: i) isolation of wild strains from unexplored and rare habitats; ii) metagenomic and genomic screening for genes involved in SMs biosynthesis; iii) phylogenetic analyses; iv) heterologous expression and comparative metabolomics; v) co-culturing of microorganism. With help of gene screening we have already isolated new bioactive molecule colabomycin E, with promising biological activities. Recently, we have demonstrated that prediction of gene clusters producing novel compounds is possible based on phylogenetic reconstruction of key enzyme evolution, which was showed on the example of cyclizing 5-aminolevulinate synthases (cALAS). As an analogy to cALAS model we constructed the phylogenetic tree from newly described category of chain length factors associated with polyketide synthases II. Recent progress will be discussed.

This work was supported by the project No. 17-30091S (Czech National Funds).

S3.3 Engineering bacterial strains degrading environmental pollutants by synthetic biology

Lukáš Chrást[1], Pavel Dvořák[1,2], Radka Chaloupková[1] and Jiří Damborský[1]

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Synthetic biology and metabolic engineering provide a broad range of powerful tools for improvement of microorganisms for the production of valuable chemicals, expression of pharmaceutically important proteins or biodegradation of harmful chemicals. Our work is focused on the construction of synthetic metabolic pathway for degradation of anthropogenic pollutant 1,2,3-trichloropropane (TCP), which is a by-product of epichlorohydrin synthesis [1]. Until today, no natural strain capable of degrading TCP has been discovered. We have constructed several strains of degraders derived from *Escherichia coli* BL21(DE3) [2] and *Pseudomonas putida* KT2440 carrying a synthetic metabolic pathway for complete degradation of toxic TCP. Constructed strains vary mainly in the expression of the pathway enzymes and thus the biodegradation capability. In terms of overall efficiency of the pathway, *E. coli* derived degraders are better under optimal conditions than those derived from *P. putida*.

The viability of the cells in the presence of a toxic compound is essential for their future practical application. While the growth of *P. putida* was inhibited in the presence of 4 mM TCP or the metabolite epichlorohydrin, *E. coli* was partially inhibited at 4 mM TCP and fully inhibited even in presence of 1 mM epichlorohydrin. Overall viability of *P. putida* degraders was higher than those of *E. coli*. Most of the *P. putida* strains maintained over 85 % relative viability after 5 hrs incubation with 2 mM TCP. Best survival and the lowest cellular stress was observed with *P. putida* strain containing TCP operon carried out by the chromosome. Flow cytometry showed the membrane depolarization and reactive oxygen species formation to be the main stress causes. In summary, we have shown that the construction of the synthetic metabolic pathway can be a solution for non-biodegradable chemicals. *P. putida* seems to be a better host for such applications compared to *E. coli*, due to its natural robustness leading to a better fitness and viability.

[1] Dvořák P. et al. (2014) Environ. Sci. Technol. 48: 6859-6866.

[2] Kurumbang N.P. *et al.* (2014) ACS Synth. Biol. 3: 172-181.

S3.4 Mycoviruses: are they an important issue for the quality control of a fungal collection?

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Mycoviruses, widespread in all the major fungal family, are viruses able to replicate in fungal cells. In recent years a high number of new viral species were reported mainly from plant pathogenic fungi. This is due to the interest in finding viruses able to cause hypovirulence in their phytopathogenic fungal hosts, or other interesting feature with potential biotechnological applications, such as metabolic variation. We performed an in depth screening of about 200 marine fungi preserved at Mycotheca Universitatis Taurinesis, isolated from different marine matrices searching any kind of mycovirus (encapsidated or capsidless, dsRNA, ssRNA (+/-) and circular ssDNA). About 15% of the fungal strains resulted infected by one or more viruses belonging to different viral families of dsRNA, (+) ssRNA and (-) ssRNA lineages. In specific, one of the fungal isolate, Penicillium aurantiogriseum (MUT4330), hosts six different viruses which we demonstrated to be actively replicating in a single fungal cell. The impact of viruses on the morphological and/or chemical features of the fungal hosts will be shown comparing the results of wild types and semi-cured and or cured strains.

S4.1 Diagnostics of infectious bacteria is dependent upon a reliable and comprehensive identification and taxonomy framework

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The global expansion of anti-microbial resistance (AMR) in bacteria, including human pathogens, presents difficult challenges for treatment and the prevention of the spread of infection. The World Health Organisation (WHO) has predicted the advent of infectious diseases for which no antibiotic treatment will be available [1]. With such escalation of AMR, combined with continuing decline in new antibiotic discovery, development of innovative, reliable, rapid and cost-efficient analytical techniques for effective characterisations and diagnostics of infectious microorganisms is increasingly essential to confront rising mortality and costs associated with AMR infections. However, the routine methodologies used for diagnosing infectious disease in most cases depend upon methods requiring prior cultivation of pathogenic bacteria from clinical samples. Faced with patients exhibiting symptoms of severe infection, physicians often resort to prescribing broad-spectrum antibiotics while they may wait days or weeks for results from the laboratory. Importantly, whole-genome sequence-based protocols are becoming increasingly relevant and MS-based proteomics also have increasingly been applied to biological studies. Proteomic analyses of bacterial cells may be considered to be indirect analyses of the genomes. The 'proteome' comprises the entire set of proteins expressed by a cell, an organism or a biological system. 'Proteotyping' [2], using state-of-the-art LC-MS/MS analyses of generated cellular peptides, enables identifications of bacterial species, as well as sub-species-level discrimination, AMR- and virulence-factors, from single MS analyses. Comprehensive and accurate genome sequence data is key for accurate peptide matching and to be able to discriminate the most closely related species.

Characterisations and identifications of responsible agents of infectious disease have relied heavily upon established systematic frameworks and documented features of well-described microbial taxa. As new methodologies, such as genomics and proteomics are developed to enable more comprehensive, detailed and complex analyses, comprehensive databases linked with a reliable systematic framework are essential for reliable diagnostics.

[1] World Health Organization. 2014. Antimicrobial Resistance: Global Report on Surveillance. ISBN: 978-92-4-15674-8.

[2] Karlsson et al. 2015. Proteotyping: Proteomic characterization, classification and identification of microorganisms – A prospectus. Syst Appl Microbiol 38:246-257.

S4.2 Foodborne pathogens and antibiotic-resistant enterobacteria from fresh produce in Germany: potential of bacteriophages for biocontrol

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Although fresh vegetables are generally regarded as safe and nutritious foods, there have been some outbreaks with foodborne pathogens associated with these. The most notable was the 2017 EHEC outbreak in Germany, which was linked to the consumption of sprouts. The markets are experiencing increased demands for processed vegetables, such as ready-to-eat salads and this underlines the importance that these products must be safe for the consumers.

A total of 288 vegetable samples from retail were obtained and investigated microbiologically using classical and molecular biological techniques. Thus, the bacterial loads on leafy salads, cucumbers, herbs, carrots, ready-to-eat salads and sprouts were quantitatively determined. The presence of *Salmonella* serovars, *Listeria monocytogenes*, and shigatoxin-producing *E. coli* (STEC) were qualitatively determined. High microbial loads occurred in ready-to-eat, mixed salads and sprouts (mesophilic counts of 10^7-10^9 CFU/g). Only one *Salmonella* serovar (0.35%) and two STEC (0.7 % of samples, each) could be isolated, while *Listeria monocytogenes* was isolated from three vegetable samples (1.04%). Four samples contained *Staphylococcus aureus* (1.4 %). The study showed that although the vegetables showed high microbial loads, the incidences of foodborne pathogens was low. Tetracycline-resistant enterobacteria (*Citrobacter, Enterobacter*, *Klebsiella, Serratia* and *E. coli*) could be isolated from all product groups, in many cases these also showed resistances to other antibiotics. Multi-locus sequence typing was used for identification, and isolates were identified as *C. freundii*, *E. ludwigii*, *E. cloacae*, *S. marcescens* and *E. coli*. A potentially novel *Klebsiella* species provisionally characterized as *Klebsiella kielensis* was isolated from carrots and sprouts. High-level cefotaxime-resistant strains with extended spectrum β Lactamase (ESBL) genes were identified, especially from sprouts. The genomes of 20 isolates were sequenced and the resistance genes were bioinformatically determined.

More than 40 lytic bacteriophages with different activity spectra against antibiotic-resistant, opportunistic pathogens or pathogens such as STEC, *Salmonella* and *Klebsiella*, could be isolated. The genomes of >20 phages were completely sequenced to characterize the phages which are intended to be used as potential biocontrol microorganisms in further studies.

S4.3 Classification of *Bartonella* species and pathogenesis of infections

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Taxonomically, bartonellae belong to the order of *Rhizobiales*, which includes mainly symbiotic plant bacteria. However, because of pathogenesis, symptomatology, diagnosis and treatment, bartonellae are still associated with the *Rickettsiales*, to which they have been historically classified for decades. The genus *Bartonella* currently comprises 35 species including 4 subspecies. Bartonellae are hemotropic intracellular bacteria. *B. bacilliformis* and *B. quintana*, can cause severe infections of a person with high lethality. However, *B. bacilliformis* infection is only endemic in the Andean region of Peru. The source of these two infections is exclusively man and the vector are ectoparasites. In addition to *B. bacilliformis*, other species are called modern bartonellae, and according to genomic studies they originated from the adaptation of *B. bacilliforms* to different hosts with the reduction of its genome. The hosts are domestic or wild animals, especially mammals, including small mammals and even insects (*B. apis*). Bartonellae notoriously violate one of Koch's postulates that the blood and tissues of healthy animals and humans are sterile. For example, *B. henselae* can be detected in venous blood of asymptomatic cats up to 10 000 000 / ml. Infections caused by modern bartonellae are transmitted by ectoparasites or by accidental injury or manipulation by the animal. Modern bartonellae may be agents of fever illnesses with varied clinical symptoms including systemic infections such as endocarditis. Infection is commonly prevalent in Europe and in our country and is underdiagnosed. Diagnosis in microbiological laboratories is based mainly on the detection of specific IgM and IgG antibodies and can be complemented by direct PCR detection from the affected tissue in a specialized workplace. Bartonellae are well susceptible to antibiotics, most probably tetracyclines, macrolides or fluoroquinolones. Treatment should be long-lasting, and systemic infections accompanied by monitoring of the structure or function of the affected organs.

S4.4 Do we need precise bacterial taxonomy in clinical laboratory?

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From the very beginning, the clinical microbiology was connected with Koch's postulates. Still, in the clinical samples, we are looking for the bacteria causing an infection. The following treatment of the infection is initiated according to the tested susceptibility to the antibiotics of the potentially pathogenic species.

During the last decade, the methods used in clinical bacteriology has been changed (MALDI-TOF MS, sequencing) and the number of bacteria species recognized in the routine laboratory significantly increased. Thus the final examination reports can easily contain the name of bacterial species, which was not detected in the clinical material until then. In some cases, it is unknown, if these species could be pathogenic or not or if they are a part of the normal bacterial flora. Also, the guidance how to test antimicrobial susceptibility or the data about the intrinsic resistance to routinely used antibiotics were not established yet.

On the other hand, the pressure to provide clinical examinations rapidly and with low-cost or point-of-care tests is leading to the simplifaction of the cultivation techniques. This situation narrows the chance to isolate some uneasily cultivable bacteria and thus influences the knowledge about the human microbiota. In the time the conventional tube-based biochemical reactions have been used and their results were compared to charts of expected biochemical reactions the decision if it is necessary to initiate the treatment or not, was more clear. Nowadays we are routinely faced with the new species with unknown pathogenic potential. Bacterial species isolated from clinical samples could be easily overestimated or underestimated.

S5.1 The Nagoya Protocol the EU Regulation and ECCO; Culture Collections looking for cost effective solutions

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What is it about?

The Convention on Biological Diversity (CBD) has three objectives: first to preserve the world's biodiversity, then to exploit it in a sustainable way and thirdly to redistribute fairly the socio-economic profits generated by the use of biodiversity in R&D. This third objective called the Access and Benefit Sharing (ABS) principle is the very purpose of the Nagoya Protocol (NP)[i] which takes source in CBD article 15. The Protocol deals with the use of biodiversity in Research & Development where considerable investments in research may lead to significant added value. Yet, like any other legitimate principle, the Protocol must be transposed into applicable regulations or it would become just another counterproductive impediment.

In 1997, the Belgian Coordinated Collection of Microorganisms (BCCM)[ii] launched the Micro-Organisms Sustainable use and Access regulation International Code of Conduct project (MOSAICC) funded by the Directorate General Research of the European Commission, to work on the implementation in microbiology of articles 15 et seq of the CBD.

The first version of MOSAICC was issued in 1999, three years before the 2002 Bonn Guidelines[iii] that were the first achievement of the Parties of the CBD towards practical solutions for managing ABS. Although designed by different groups of protagonists and in different times, MOSAICC and the Bonn Guidelines are fully compatible because both are common sense driven.

In 2005, ECCO exploited the MOSAICC experience to develop the ECCO Core MTA[iv] approved in 2009.

In 2016, with twenty years of experience, BCCM and the World Federation for Culture Collections (WFCC) are proposing an integrated system to implement the Nagoya Protocol.

Anticipation and proactive management

The Nagoya Protocol (NP) entered into force in October 2014. It is a global agreement on how to administrate the access to biological raw material for R&D and the redistribution of the profit based on research outcomes. Several NP provisions were anticipated in MOSAICC, such as the special considerations of article 8, where upstream -basic- research would be facilitated, and emergencies dealt with in an expedite way not to jeopardize public health. Also, using Globally Unique Identifier GUID to streamline the tracking of biological material was included earlier on in MOSAICC.

EU implementation of the Nagoya Protocol

The EU regulation implementing the Nagoya Protocol is designed with well-defined distribution of competence between the member states and the EU level.

The EU regulation focuses on the compliance by the users of the law of the countries where the biological material originates. It leaves the access regulation in the hand of the EU member states who effectively exercise their sovereign rights (CBD Article 15.1[v]) on their natural resources.

The corner stone of the EU legislation is regulation EU 511/2014[vi]. It is complemented by the EU Commission implementing regulation EU 2015/1966[vii]. Next to these legal texts, a consultative body, the ABS consultative Forum has been put in place and several guidance documents, one horizontal Commission notice[viii] has been published and nine other sectorial guidance documents are prepared in collaboration with all players of the civil society.

Cost effective, attractive solution

While MOSAICC is a code of conduct, the microbiologists need a system that facilitates their daily life. The active contributors to MOSAICC decided to upgrade it into TRUST, TRansparent User-friendly System of Transfer, because an efficient, profitmaking ABS system must be transparent. It must provide reliable data with legal certainty. It must be user-friendly, cost-effective and automated to facilitate transfers of biological material and related data and to foster international collaboration.

The WFCC Data Centre (the World Data Centre for Microorganisms - WDCM) is developing the Global Catalogue of Microorganisms to get a global web-based data management connected to the official ABS website of the CBD, to grease the wheels of communication between sources and users of micro-biodiversity.

Next developments

For 20 years MOSAICC and now TRUST reconcile the expectations of all protagonists and incorporates their business model into one system. Fortunately, that is also what the Directorate General Environment of the European Commission is doing through the optimizing of the implementation of EU Regulation 511/2014.

Responsible stakeholders like all the protagonists teaming with BCCM are convinced of the relevance of the NP objectives. But many fear that big guns are used to kill flies, in other words that significant amounts allocated to the actual research may be diverted from their goal and used only to set up a new bureaucracy, where marginal costs exceed profit it may generates.

A good system doesn't need to be complex or coercive; it must facilitate the daily work of all protagonists in such way that using the system is more profitable than bypassing it. The Nagoya Protocol is meant to be set at national level. At the source, the countries that will organize an attractive web connected system will yield better socio-economic profits when R&D will be conducted in cooperation with their nationals. On the other end of the R&D chain, for the users, securing legitimate access to biodiversity and securing their investments is as strategic as securing access to energy for prospering states.

In short, the Nagoya Protocol is an evolution in the framework of the CBD aiming at regulating the exploitation of the world's biodiversity, it's not a revolution. The rules set by the Protocol need to be implemented cost-effectively to enable profit-making and subsequent benefit sharing. Such goal may be difficult to reach when the implementation is a burden, not a help for economic agents and costs more than the profits actually reaped.

The latest developments in the negotiation for the interpretation of the Nagoya Protocol go towards regulating the sequencing of genomes and the use of these data. When looking at the explosive increase of sequencing data and the ensuing struggle to process and manage such overwhelming data amount, the obsessive will to regulate some basic activities in life sciences seems a heresy in terms of cost/benefit ratio as well as in direct contradiction with the two first objectives of the CBD.

Should the NP parties jeopardize the conservation and the sustainable use of the earth biome for short term economic misperception? Scientist must remain alert and advocate sound integrated management of the raw material and related data for R&D.

ECCO has a major role to play by dialoguing with the European Union authorities as well as with every countries of the European continent, these countries being either or not EU member. ECCO has also a role to play with WFCC at global level.

[i] Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity, adopted on the 10th meeting of the Conference of the Parties on 29 October 2010, in Nagoya, Japan. http://www.cbd.int/abs/text/

[ii] BCCM is a public support programme of the Belgian Science Policy Office that funds and coordinates a consortium of microbial collections since 1983. BCCM is ranked in the world top ten centres providing for microbiological material, information and services.

[iii] Bonn Guidelines on Access to Genetic Resources and Fair and Equitable Sharing of the Benefits Arising out of their Utilization.

[iv] https://www.eccosite.org/ecco-core-mta/

[v] CBD Art 1. Recognizing the sovereign rights of States over their natural resources, the authority to determine access to genetic resources rests with the national governments and is subject to national legislation.

[vi] Regulation (EU) No 511/2014 of the European Parliament and of the Council of 16 April 2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union.

[vii] Commission implementing regulation EU 2015/1966 of 13 October 2015 laying down detailed rules for the implementation of Regulation EU 511/2014 of the European Parliament and of the Council as regards the register of collections, monitoring user compliance and best practices.

[viii] Commission notice - guidance document on the scope of application and core obligations of Regulation (EU) N° 511/2014 of the European Parliament and of the Council on the compliance measures for users from the Nagoya protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation in the Union.

S5.2 Novel challenges and predicted trends for culture collections in the age of the Nagoya protocol

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The Convention on Biological Diversity (CBD) acknowledges the right of each state to exploit the biological resources under its jurisdiction. The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (short, "Nagoya Protocol") to the CBD has been adopted to provide legal certainty for the actors involved in the use and international exchange of genetic resources and specifies means to share benefits gained from the use of genetic materials. Motives of the Nagoya Protocol were to (i) prevent misappropriation of biological resources and traditional knowledge that fall under the sovereignty of a provider state, (ii) contribute to the conservation and the sustainable use of biological diversity, and (iii) develop scientific capabilities in developing countries through international scientific cooperation in biodiversity research. The Nagoya Protocol entered into force on October 12, 2014, and within the EU is implemented through regulation 511/2014.

According to the Nagoya Protocol "use" of biological resources also includes non-commercial, basic research activities. The definition of "genetic resources" not only extends to microbial strains as such, but also their biochemical compounds, DNA/RNA; even inclusion of the information on nucleic or protein sequences is currently being discussed. Compliance with the new legislation requires (1) proof of legal acquisition of any microbial resource, (2) documentation of this proof and (3) inspections of users by the national authorities. Particular features of microorganisms that are relevant for the complicance with the Nagoya Protocol are the frequent lack of biogeography and of latitudinal diversity gradients.

Against this background, the presentation will focus on the far-reaching implications of the Nagoya Protocol for the practical work and management of microbiological culture collections. Real world examples will be used to illustrate the challenges and consequences of the new obligations and also to deduce possible future trends.

S5.3 Implementation of the Nagoya Protocol and related legislation in the Czech Republic

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ABS NFP, Ministry of the Environment of the Czech Republic

The Nagoya Protocol [1] to the Convention on Biological Diversity provides legal framework for the implementation of one of the objectives of the Convention as well as of its Article 15, in which the sovereign rights of States over their natural resources are recognised together with their right to regulate access to their genetic resources (any material of plant, animal, microbial or other origin containing functional units of heredity, of actual or potential value). The Protocol entered into force on 12 October 2014, including for the EU and its Member States. It rests on 3 key pillars: Access to genetic resources, Benefit sharing and Compliance.

Within the EU, Member States decide themselves on the implementation of ABS measures. On the other hand, user compliance measures are implemented on EU level, by Regulation (EU) No 511/2014 [2] and Commission Implementing Regulation (EU) 2015/1866 [3]. In brief, the main obligation for EU users (i.e. those who conduct research and development on the genetic or biochemical composition of genetic resources) is to exercise due diligence, that is to seek, keep and transfer to subsequent users information demonstrating that the genetic resources they utilise have been accessed in accordance with applicable ABS legislation or regulatory requirements, and that benefits are fairly and equitably shared upon mutually agreed terms. Next, users have to make a due diligence declaration at the stage of research funding / of final development of a product. In addition, the EU Regulation provides for collections of genetic resources to become registered if they fulfil certain criteria. A user obtaining a genetic resource from such a collection is considered compliant with the due diligence obligation of seeking information. Collections that do not perform research and development are not seen as users. However, general good practice for the collection holders is to exercise due diligence, too.

In order to provide users with guidance on how to comply with these obligations, the European Commission is preparing guidance documents. First document, on the scope and main obligations of the EU Regulation, was adopted in 2016 [4]. Further guidance for users from specific sectors (including collection holders) is under development.

In the Czech Republic, draft legislation implementing the EU Regulation is awaiting its approval by the Parliament. As regards possible ABS measures, the Czech Republic does not intend to regulate access to its genetic resources. Thus, no prior informed consent or mutually agreed terms are required.

[1] Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity.

[2] Regulation (EU) No 511/2014 of the European Parliament and of the Council of 16 April 2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union.

[3] Commission Implementing Regulation (EU) 2015/1866 of 13 October 2015 laying down detailed rules for the implementation of Regulation (EU) No 511/2014 of the European Parliament and of the Council as regards the register of collections, monitoring user compliance and best practices.

[4] Guidance document on the scope of application and core obligations of Regulation (EU) No 511/2014 of the European Parliament and of the Council on the compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation in the Union.

S5.4 Proactive approaches to quality management

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The publication of the *ISO 20387 - Biotechnology - Biobanking - General requirements for biobanking* standard – planned for the end of 2018 - can broadly impact the manner of culture collections - also known as biobanks from microbial domain (mBb) - work and interact with each other and society. It is currently going through its public consultation stage providing mBb with an (almost unique) opportunity to comment and improve the standard's appropriateness to the mBb needs. ISO 20387 is intended to ensure competence and a consistent biobanking operation by conveying general provisions including quality control requirements.

Quality control is related to Quality by Testing, the current paradigm to manage quality (concerning the microorganism testing) in mBb. It is a reactive approach where consistency relies on extensive testing throughout the *microorganism preservation lifecycle*.

Through process design, qualification and continual improvement - using proactive and knowledge based approaches such as Quality by Design and Knowledge Management, consistency can be efficiently achieved and process capability improved. Besides microorganism testing for viability, identity and purity, process validation can ensure consistency in preserving the microorganisms for the long term.

S5.5 BRoTHER, a biobank cooperation between Regensburg, Pilsen and Brno

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Building networks of biobanks represent a crucial element to enable large multicentric clinical trials. On the European level the "Biobanking and Biomolecular Resources Research Infrastructure – European Research Infrastructure Consortium" (BBMRI-ERIC) is an important network for biobanks. BBMRI is a European leader in biobanking, and is crucially involved in further developments of the biobank-idea. Beside this network, regional networks could significantly improve the translational and basic research within the connected regions. To enable an optimal collaboration of regional biobanks, web based tools for data exchanges are mandatory to guarantee a long-term success.

The aim is to create a prototype of a digital pathology framework in which secondary consultations regarding biobank-specimens could be conducted remotely. Therefore, we believe that employing the mature enough Whole Slide Imaging (WSI) and Virtual Microscopy (VM) technologies in a regional network of tissue biobanks will not only provide access to such expertise, but will also create new research-projects within that network. Furthermore, by comparing the produced histological slides of the stored tissue samples between the connected biobanks an interactive discussion regarding tissue storage and pre-analytics will be promoted. Therefore, our framework will include the possibility of interactive standard operation procedure (SOP) - development. Thus, the network will promote future collaboration-projects due to harmonized SOP's. Finally, a so-called minimal-information set for the stores specimens will be established. To train the handling of the digital pathology framework and the application of the SOP's within the network, a training-program will be established for biobank-staff. In addition, the consortium will inform the public in the participating regions regarding the need and the function of biobanking. Thus, BRoTHER integrates both, the interregional cooperation on the scientific but also on the public level.

S5.6 SmartFreezer®, a fully automated robotic system for biomaterial storage at -196°C

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Biobanking is of growing relevance in translational and basic science. In this context, the biological quality of biobank specimens is crucial for its potential use. However, lack of quality and un-reproducible research data are well-known problems of many biobanks [1]. A national survey from US-biobanks clearly demonstrated that not the arsenal of modern molecular-biological techniques but the biological quality of cryopreserved biobank specimens and the corresponding clinical data represent the bottleneck for the feasibility of research projects with biobank specimens and for the value of the data produced with biobank-specimens [2]. Thus, excellent biological quality, the monitoring of the biobank processes and a proper correlation to clinical data is crucial for modern biobanking.

Based on an automatic robotic biobank system (Smartfreezer®) we developed a data management structure, which enable not only an exact monitoring of the storing environment but also the acquisition of further data to every single specimen [3].

To shed light on the influence of different storage temperature after long-term storage on biobank specimens, we analysed the vitality, the proliferation behaviour and the cellular integrity of different cell lines after a storing period of 10 years at -80°C and -196°C. After thawing vitality of cells was assessed. For proliferation assays, cells were cultured for at least 72 hours in a two-dimensional cell culture system. The structural cellular integrity was analysed by scanning (SEM) and transmission electron microscopy (TEM).

Our results revealed a significant higher vitality of cells after storage at the gas phase of nitrogen compared to storage at -80°C. The proliferation of cells was significant higher after storage in the gas phase of nitrogen with a good colonization of the underground and the formation of tubular structures in case of adenocarcinoma cell lines. Ultrastructurally, these cells showed intact membranes and intact cell organelles, whereas cells after 10 year storage at -80 degrees were characterized by huge cell damages and widely dissociated cytoplasm with damaged cell organelles.

A potential reason for the positive effect given by storage in the gas phase of nitrogen could be the lack of ice-crystal formation within the cells – a well-known cause for membrane damages. Our results imply, that the fully automated storage at the gas phase of nitrogen preserve not only the functional but also the morphological integrity of biological specimens.

[1] Baker M. Nature 2012; 486: 141–146.

[2] Massett HA, Atkinson NL, Weber D, Myles R, Ryan C, Grady M, et al. JNCI Monogr 2011; 42: 8–15.

[3] Winther HB, Brochhausen C, Brochhausen M, Topaloglu U, Kirkpatrick CJ. Virchows Arch 2014; 465(Suppl 1): 295.

S5.7 Data integration with Life Science databases: the technical aspects

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In this research we are looking for efficient solution schema for the tasks:

1. to make mBRC data visible and accessible from Life Science databases,

2. to make Life Science database records visible and accessible from mBRC aggregated catalogue.

For this goal we inspected most of the databases available in Internet, presented in Biosharing, MetaBase, BioMedBrigeds and ELIXIR lists, in ExPASy, NAR and in majority of other sources that we found. Each database was characterised by the name, acronym, the year of the last correction, URL, area of practical use (health system, agriculture, etc.), presence of microbial data, database producer. Databases with microbial data were inspected in more detail: the lists of the partner databases, the lists of ontologies used, the access format from computer programs, keywords - chemistry, diseases, genes, proteomics, taxonomy, etc.

2658 Life Science databases collected in our new metabase, 1112 databases with microbial data. Using databases interconnection system constructed in this research we calculated data integration level indicators. With these indicators we found the minimum number of integration contracts sufficient for desirable level of interconnection with Life Science databases.

Our report presents characteristics of the metabase as well as technology of access to the key database infrustructures, such as NCBI, EMBL-EBI, UNIPROT.

Poster presentations

P01 The Culture Collection of Soil Actinomycetes in České Budějovice

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The Culture Collection of Soil Actinomycetes České Budějovice (CCSACB) was founded in the Institute of Soil Biology, Biology Centre CAS, v. v. i. in 2007. The CCSACB became a member of Biology Centre Collection of Organisms (BCCO) in 2015. The CCSACB serves as a depository for cultures of soil actinomycetes, used mainly for screening of strains producing important secondary metabolites. Moreover, the CCSACB isolates can be used for research, industrial applications, education, and general scientific interest. Most of the cultures belong to the family *Streptomycetaceae* (genera *Streptomyces* and *Kitasatospora*), however some other families are also represented (*Pseudonocardiaceae*: gg. *Letznea*, *Amycolatopsis*, *Saccharothrix*, and *Nocardiaceae*: g. *Nocardia*). The major activity of the CCSACB is preserving actinomycetes which were isolated from soil and sediments of some unique habitats (agricultural fields, gardens, native prairies, savannas, temporal forest, tropical rainforest, grassland, bat guano, caves, glacier fore-fields, Miocene lacustrine sediment, iron ore mining sediment (technozem), brown coal colliery spoil, dead bees, ambrosia beetle galleries, etc.). We are opened to cooperate with research institutes, universities and pharmaceutical companies on research of substances with antibiotic or anti-inflammatory effect. Moreover, members of genus *Streptomyces* are recognised as the producers of many bioactive metabolites that are useful: i) in medicine as antifungals, antivirals, antithrombotics, immunomodifiers, antitumor drugs and enzyme inhibitors; ii) in agriculture as insecticides, herbicides, fungicides and growth promoting substances for plants and animals.

This work was supported by Strategy AV21 - program: Diversity of Life and Health of Ecosystems, activity: The development, presentation and popularisation of the biological collections from institutes of The Czech Academy of Sciences.

P02 *Fomes fomentarius* lineages in the Mediterranean biogeographical region

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Fomes fomentarius is a common and economically important wood-rotting fungus in deciduous forests and prefers different main hosts depending on the region. The fungus often arrives at dead wood relatively early or colonizes already a living tree as a parasite but contrary to other early arriving fungi it exhibits better combative abilities and is not easily replaced (Větrovský et al., 2011). It is distributed within the northern hemisphere in Europe, North Africa, North America, China and Japan. Until recently, *F. fomentarius* had been considered a homogeneous species but the existence of distinct ITS lineages/sublineages among its strains has been established. Studies were based on ITS rDNA sequence analysis and host preference evaluation. Firstly there were lineages A and B, after more detailed investigation the lineage A was separated into sublineage A1 (consisting of strains isolated from North America) and sublineage A2 (consisting of strains only from Europe). The lineage B consists of strains from Europe and Asia (Gáper et al., 2016). There is little information available about this polypore sequences originating from Portugal so the aim of the present study was to evaluate genetic variability in *F. fomentarius* in southwestern part of Europe and enrich so far known data in GenBank and biodiversity databases. Results showed that the lineage that occurs in Portugal, and generally in Mediterranean region, is the lineage B. We can conclude that *Fomes fomentarius* is a non-homogenous medicinal mushroom and its lineages correlate with geographical distribution and host preference.

Větrovský T, Voříšková J, Snajdr J, Gabriel J, Baldrian P., 211.Ecology of coarse wood decomposition by the saprotrophic fungus *Fomes fomentarius*. Biodegradation, 22(4):709-18

Gáper J., Gáperová S., Pristaš P., Náplavová K. 2016. Medicinal value and taxonomy of the Tinder polypore, *Fomes fomentarius* (*Agaricomycetes*): A Review. International Journal of Medicinal Mushrooms 18 (10): 851-859.

P03 Impact of new bio-fertilizers on soil microbial populations in mini-field vegetation experiments with rape and barley

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The aim of the present study was to investigate effect of two biological preparations (BFs) on soil microbial genetic material concentrations and species composition. BFs were tested in vegetation experiments: a) BF-P – soil microbial consortium (*Bacillus* spp., *Burkholderia cepacia*, *Pseudomonas putida*, *Trichoderma* sp.) with peat as a carrier; b) BF-S – consortium of cellulolytic microorganisms with hemp straw as a carrier. 75-day vegetation experiment was conducted in loamy soil. DNA was extracted using the PowerSoil® DNA Isolation Kit. 16S rDNA region of bacterial DNA was amplified with universal primers ForB and RevB. Fungal DNA was estimated by qPCR with the primer sets ITS1F and ITS4. The DNA was digested with restriction endonuclease *Bsu*RI for the estimation of the Shannon-Weaver diversity index. Real-time quantitative PCR was used to quantify fungal genes in soil samples. The 16S rDNA restriction profiles were grouped in software R using hierarchical cluster analysis and Euclidean distance matrix. It was found that the communities of microscopic fungi of rhizosphere were influenced by the plant species and type of the BF tested.The predominant genera of fungi in barley soil without BFs were *Acremonium*, *Penicillium*, *Verticillium*, *Mucor* and *Fusarium*. The predominant genus was *Aspergillus* reaching 90%, while low concentration of *Mucor* and *Penicillium* was observed. New BFs influenced concentration of all studied groups of microorganisms in barley and oilseed rape soil and changed species composition of rhizosphere microbiota in different ways. BF-P increased the concentration of bacteria in barley rhizosphere and concentration of fungi in rape rhizosphere. Fungal diversity did not differ significantly between the BFs treated and untreated barley soils, but BF-P increased fungal diversity in rape rhizosphere.

This study was supported by the National Research Programme of Latvia “ResProd” and by the project of University of Latvia "Climate change and sustainable use of natural resources" (No. AAP2017/B04).

P04 Verification of yeast species originated from water and trees of southwest Slovakia

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Microorganisms, among them yeasts, are intensely utilized in biotechnology, medical treatment and research. Proper identification of microbial cultures is crucial step of: classifying them into the risk groups; publishing valid results related and defining specific properties typical of individual species; finding genetic differences among them; and discovering their potential in science and bioindustry (Groenewald et al. 2012; Smith et al. 2014). Since 2016, the verification of yeast strains, isolated from natural sources located in Southwest Slovakia (lake, river and fish-pond waters; blossoms, fruits, leaves of fruit trees; leaves and needles of forest trees), using sequence analysis of the D1/D2 domains of the 26S rRNA gene, has been started. For many yeast strains, proper identification, based on their physiological and morphological properties, have been confirmed by molecular methods, whereas for others, inaccurate identification and more diverse yeast species have been found. *Hannaella* (*Bullera*) *coprosmaensis*, *Vishniacozyma* (*Cryptococcus*) *foliicola* (both species identified as *Bullera alba*), *Papiliotrema* (*Cryptococcus*) *laurentii*, *Solicoccozyma aeria* (*Cryptococcus aerius*), *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* were associated with the water environment and leaves of forest and fruit trees. The other basidiomycetous species *Kwonniela pini* (identified as *Cryptococcus magnus*) was found in a fresh-water lake, whereas *Cystofilobasidium* (*Cryptococcus*) *macerans* (identified as *Cystofilobasidium infirmominiatum*) was isolated from the fish-pond water. Ascomycetous yeasts *Saccharomyces paradoxus* (identified as *Saccharomyces cerevisiae*), *Hanseniaspora osmophila* (identified as *Hanseniaspora vineae*) and *Diutina catenulata* were isolated from leaves of forest trees, whereas *Candida magnoliae* (identified as *Debaryomyces hansenii*), *Starmerella bombicola* and *Barnettozyma californica* were associated with different plant organs of fruit trees.

This work was supported by the grant from the Slovak Research and Development Agency (APVV-15-0744) and VEGA 2/0023/14.

Groenewald, M. et al. 2012. Int J Syst Evol Microbiol 62: 3072-3080. Smith, D. et al. 2014. Springerplus 3: 81.

P05 Czech National Programme for Conservation and Utilization of Microbial Genetic Resources Important for Food and Agriculture

Zedek Vlastimil, Křížková Iva

Ministry of Agriculture of the Czech Republic

The task of the protection of biodiversity arises from the international treaty Convention on Biological Diversity (CBD) and international and national policies. The measures for the conservation and sustainable use of genetic resources for food and agriculture are considered integral parts of Czech National Biodiversity Strategy for the period of 2016-2025 and Czech Ministry of Agriculture Strategy towards 2030. Czech National Programme on Plant, Animal and Microbial Genetic Resources Important for Food and Agriculture serves as a basis for the long-term protection, conservation and use of genetic resources. Its part, the National Programme on Microbial Genetic Resources comprises twenty culture collections that hold more than 7,500 accessions belonging to 1,200 species or lower taxonomic units. Culture strains of microorganisms kept in collections are extremely heterogeneous. Numerous group of strains represents viruses, bacteria and fungi pathogenic to plant and bacteria and viruses of veterinary relevance. Several collections hold beneficial microorganisms such as brewery yeasts, lactic acid bacteria and yeasts involved in fermenting dairy products, fungi from basidiomycetes group utilizable for bioremediation of soil, ethanol fermentation yeasts, baker's yeasts and other yeasts and bacteria used for special production in food industry. Many strains serve as test, control and bioassay strains. Most of strains are characterized and can therefore meet the needs of both R & D and commercial activities.

P06 Culture collection of autotrophic organisms (CCALA) – past, present, and future

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Culture collection of autotrophic organisms (CCALA) was established in 1960 at the Institute of Microbiology of the Czechoslovak Academy of Sciences in Třeboň. Founders of this collection were two great personalities on the field of algology – Jiří Komárek and František Hindák. History of strains in our collection has been closely associated with other important algologists such as Václav Uhlíř, Silvestr Prát or Ernst Georg Pringsheim, who also placed their isolates to CCALA. Pringsheim's original strains have been still alive in the collection (e.g. *Aphanocapsa rivularis*, *Euglena gracilis*, *Interfilum paradoxum*, and several others). Currently, CCALA collection is composed of sub-collections focused on cyanobacteria (204 strains), algae (445 strains), and non-seed plants (39 strains). Among algae and cyanobacteria there are many strains serving as references to the type material. Strains originate from more than 50 countries and include organisms from various biotopes. In the collection there are strongly represented isolates connected with research of polar regions and subaerophytic habitats. In addition to keeping and cultivating the strains, the collection offers an option of maintaining patent strains with biotechnological potential, as well as cryopreservation of cyanobacteria and algae. Collection is constantly adding new isolates to its assortment. For the future we plan to popularize algae and cyanobacteria among the public by organizing workshops, lectures or exhibitions. Also, we still want to be well-liked source of material for science, biotechnology, applied science, and education.

P07 The Czech National Programme on Conservation and Utilization of Genetic Resources of Microorganisms Important for Agriculture

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The Czech National Programme on Genetic Resources of Microorganisms Important for Agriculture is directed by Czech Ministry of Agriculture. The aim: - conservation of genetic resources of microorganisms ex situ in collections, safe maintaining of the genetic resources and their biodiversity - record keeping and documentation of genetic resources, their evaluation on molecular level and possibility of utilization for agriculture - enhancing the international cooperation, exchange of genetic resources. The National Programme consist from 20 collections of genetic resources held in 12 different Institutes.

P08 Overview and current development in the CCF

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Charles University, Faculty of Science, Department of Botany, Prague, Czech Republic

Culture Collection of Fungi (CCF) is a microbial collection of the Department of Botany, Faculty of Science, Charles University, Prague (Czech Republic). Now it holds over 4000 microscopic saprotrophic fungi, mainly ascomycetes and zygomycetes, which are used for teaching and research purposes or serve as scientific documentation material. For preservation we use freeze-drying, mineral oil, agar slopes in refrigerator, alginate pellets, and partly also storing at -20 °C or in liquid nitrogen. Most of the strains were isolated from soil, human clinical isolates, food and feed, insect, and from plants. A significant proportion of the strains originates from the Czech Republic as a result of our research. An important part of preserved fungi is represented by the ex-type strains. Since 2006, a part of our fungal collection (over 300 strains of agriculture significance) is involved in the Czech National Programme of Protection of Genetic Resources of Economically Significant Microorganisms and Tiny Animals (NPPGR), which aims at the conservation and efficient use of these strains in teaching and research. At present, our research is focused mainly on taxonomy and ecology of clinically important fungi (e.g. *Aspergillus,* dermatophytes), indoor fungi, fungal pathogens of fishes, microfungi of acidic soils, microfungi of underground spaces and litter fungi. Currently, the identification of approximately 36 % of strains was verified by means of molecular methods. Some other properties of cultures (e.g. secondary metabolites production) are verified in collaboration with other research institutes. For example, aflatoxin production was tested at The National Institute of Public Health in Brno. Cooperation with Slovak University of Technology in Bratislava has led to discovery of zygomycete strains producing gamma-linolenic acid and beta-carotene; while cooperation with the Institute of Microbiology in Prague has resulted in the discovery of novel metabolites (e.g. in *Biatriospora* spp.).

P09 Culture collection of soil algae and cyanobacteria

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The Culture collection of soil algae and cyanobacteria of the Institute of Soil Biology, Biology Centre CAS, v.v.i. (ISBAL) was established as a working collection in 1986. It became a member of Biology Centre Collection of Organisms (BCCO) in 2015. The collection includes strains isolated from various biotopes and ecosystems of different climatic and geographic zones. The highest number of strains originates from the temperate zone (CR, Slovakia, Germany, Holland, Sicily, Rumania, Canada, USA), followed by strains from polar regions (Antarctica, Canadian Arctic, Svalbard, subarctic-Abisko, alpine zone -Austria, Norway, Sweden) and from tropical and subtropical regions (Egypt, Brazil, Kenya, Cuba, Mexico, Indonesia). Strains were isolated from soils, cave substrates, colliery spoils, post-volcanic ash, intestine tracts and excrements of soil invertebrates, air, etc. At present, more than 2000 strains of algae and cyanobacteria (unialgal cultures) are maintained by subculturing and/or by cryopreservation. Cyanobacteria cover about 20% of the total spectrum of maintained strains, filamentous species (*Nostocales*, *Oscillatoriales*) prevail. Eukaryotes are mainly represented by strains of *Chlorophyta* (*Chlorophyceae*, *Trebouxiophyceae*, *Ulvophyceae*), Streptophyta and straminopiles (*Xanthophyceae*, *Eustigmatophyceae*). The main function of the collection is to collect, maintain and supply cultures of microscopic algae and cyanobacteria important for basic and applied research, pharmaceutical and food industry, agriculture, medicine and teaching. Maintained algal isolates are broadly used for scientific and education purposes. They also represent an important resource of biologically active compounds with biotechnological potential (e.g. polyunsaturated fatty acids, carotenoids), heterocytous cyanobacteria are known to produce many bioactive metabolites (e.g. toxins, enzyme inhibitors, antifungal and antitumor substances), some isolates have a potential as soil bioconditioners. Strains are available for any collaborative research.

This work was supported by Strategy AV21 - program: Diversity of Life and Health of Ecosystems, activity: The development, presentation and popularization of the biological collections from institutes of the Czech Academy of Sciences.

P10 Collection of phytopathogenic and agricultural important fungi, Crop Research Institute Prague – introduction, goals, problems, research

Novotny David

The Czech National Programme on Conservation and Utilization of Microbial Genetic Resources Important for Agriculture, Ministry of Agriculture of the Czech Republic

The collection was established in the 1990-thies. The long term focus of the collection is to obtained strains of fungi important for agriculture and agricultural research in the Czech Republic. At present time the collection holds more than 400 strains of phytopathogenic and potentially phytopathogenic, mycotoxinogenic and potentially mycotoxinogenic (except biotrophic species), endophytic fungi and more than 120 strains of edible and medicinal mushrooms. Majority of strains holds in the collection belong to groups Ascomycota (e.g. *Fusarium*, *Botrytis*, *Neofabraea*, *Penicillium*, *Aspergillus*, *Colletotrichum*, *Trichoderma*, *Pyrenophora*, *Mycosphaerella*, *Alternaria*) and Basidiomycota (e.g. *Pleurotus*, *Ganoderma*, *Stropharia*, *Hericium*), but any strains belong to groups Zygomycota and Oomycota (mainly *Phytophthora*). All strains are preserved under paraffin oil on an agar slant. The strains started to be preserved in liquid nitrogen and some of them started to be freeze-dried in this year. Among the main problems at present time belongs optimization of cryopreservation and freeze-drying of the strains and protection of fungal culture against mites. The research is mainly focused on protection of crop against harmful fungi. The investigation of protection of *Pleurotus ostreatus* in oyster mushroom farm against *Trichoderma pleuroti* is an example of it.

P11 A culture collection information system connected with a web catalogue via reflective programming and relational databases

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Use of commercial laboratory information management systems for the purposes of culture collection (CC) is limited, because data associated with CC are represented by a system with a specific structure and are subject to unique requirements. An important aspect of managing CC data is the need for a live link between confidential and public data. Data are stored in a MySQL relational database, updated via scripts written in the PHP programming language and visualised on web pages developed using PHP and javascript. The web pages intended for public use are located within the website of the institution, the database and related web forms for managing of all that data are located on an internal NAS server. A relational database was developed consisting of 50 tables containing all available information concerning the strains, their identification, characterisation, storage form and release to recipients. The system enables the tasks of lyophilisation, viability testing and expedition alongside with smooth updating of the on-line catalogue and order tracking. A combination of MySQL and PHP was shown to be feasible for development of a customised culture collection information system as long as the developer was an integral part of the culture collection team.

P12 Collection of reference strains of viral and bacterial pathogens for diagnostics of important livestock diseases

Reichelová Markéta, Malenovská Hana, Motlová Jitka, Prodělalová Jana, Gelbíčová Tereza, Procházková Markéta, Moutelíková Romana, Koláčková Ivana, Karpíšková Renáta

Veterinary Research Institute, Brno, Czech Republic

Infectious diseases monitoring plays an important role in maintaining a favorable animal health situation, and in the case of suspected infectious disease, rapid diagnosis is essential. In both cases, reference material is required, which must meet the strict quality and availability requirements. The aim of the study is to create a collection and on-line catalogue of 120 highly characterized reference strains of viral and bacterial pathogens important for the diagnosis of infectious livestock diseases. The strains will be available as a freeze-dried cultures. The strains of pathogens selected for study (56 viral and 64 bacterial strains) are deposited in Collection of Animal Pathogenic Microorganisms. For individual viral cultures, contamination by mycoplasmas and other viruses (Bovine viral diarrhea virus and Porcine circovirus 1) was checked by PCR-based methods. Cell lines used for the cultivation of selected viruses were also tested for the presence of the contaminants. Elimination of mycoplasmas was performed with commercially available antibiotics. Quantification of the virus by titration on cell lines (determination of TCID50) and subsequent lyophilization is being performed currently. Sequencing and phylogenetic analysis of the relevant section of the viral genome will allow taxonomic classification of the virus. Phenotypic properties of bacterial cultures were evaluated using conventional methods. Photodocumentation was taken during the macroscopic and microscopic examination of the culture. To determine the biochemical activity, commercial testing kits (e.g. API) and other additional tests were used. Detection of antigenic structures (serotyping) and measurement of antimicrobial susceptibility (using disk diffusion and microdillution methods) were performed in selected bacterial species. Strains were also identified by mass spectrometry. All the information obtained, including photodocumentation, will be placed in a publicly available database.

The study was supported by the Ministry of Agriculture (projects QJ1630210 and RO0516) and the Ministry of Education, Youth and Sports of the Czech Republic (project LO1218).

P13 Clinically interesting bacterial isolates identified in the NRL for antibiotics and in the Czech National Collection of Type Culture (CNCTC) in 2011-2015.

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The Czech National Collection of Type Cultures (CNCTC), based on the results of MALDI-TOF and sequencing analysis, completed the identification of the 16S rRNA genes of nine isolates from field laboratories where standard biochemical methods are used and, therefore, further identification could not be carried out. Strains were isolated in clinical laboratories and they came to the NRL/CNCTC as secondary isolates. Five of them were obtained from blood (*Asaia lannensis*, *Capnocytophaga cynodegmi*, *Brevibacillus brevis*, *Gordonia otitidis*, *Corynebacterium imitans*), two from wound swab (*Advenella mimigardefordensis*, *Actinomyces funkei*), one from implant (*Campylobacter gracilis*) and one from fluid (*Mycoplasma hominis*). Strains belonged to either rare taxa or taxa uncommonly associated with given diagnosis. Strains were initially put under extended biochemical tests (API-Biomerieux) and identificated by MALDI-TOF MS (Bruker Microflex). Samples for API were prapared according instructions belonging to particular API sets, samples for MALDI-TOF were prepared by both direct and extract methods according to Bruker instructions. Partial (or failed) identifications of all strains were enclosed by 16S rDNA-Sequential Analysis.

P14 Yeasts and yeast-like organisms inhabiting the soil adjacent to fruit trees

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Soil is a basis of the whole biosphere which represents the most important complex interface in the global exchange of matter and energy. Microbial composition present in soil is influenced by many different factors such as soil structure, nutrient composition, plant diversity, as well as weather conditions. Yeasts present in soil play an important role in affecting microbial and plant growth, soil aggregate formation, mineralization of organic material and maintenance of soil structure (Botha 2011). In total, 62 species (equal number of ascomycetous and basidiomycetous species) were isolated from 200 samples, collected from the soil beneath to five fruit trees during four sampling periods in two localities (Malé Leváre and Malé Zálužie). The ascomycetous yeasts formed about 61,5% of the total yeast population. Two yeast-like species: *Tausonia* (*Trichosporon*) *pullulans* and *Galactomyces candidum* were the most abundant; each of them formed more than 10% of the total yeast community. *Barnettozyma californica* (family Wickerhamomycetaceae) was isolated in significant amounts from both localities observed and dominated the yeast microbiota of peach trees. The other member of this family, *Cyberlindnera saturnus* was exclusively associated with Malé Leváre, whereas *Cyb. misumaiensis*, *Clavispora reshetovae* and *Schwanniomyces capriottii* (family Debaryomycetaceae) with Malé Zálužie. The order Trichosporonales consisted of the *Apiotrichum* and *Trichosporon* species and formed about 12% of the total yeast community. The fruit-associated yeasts *M. pulcherrima* and *H. uvarum* probably entered the soil with fallen fruits. The yeasts commonly associated with phyloplane were mainly represented by the genera *Cystofilobasidium*, *Rhodotorula* and *Sporodiobolus*. Two species of the genus *Holtermanniella* were isolated from both localities only during the individual sampling in April.

This work was supported by the Slovak Grant Agency of Science (VEGA 2/0023/14, VEGA 2/0058/16 and APVV-15-0744).

Botha A. (2011). The importance and ecology of yeasts in soil. Soil Biol Biochem 43(1):S1-8.

P15 Biodiversity of yeasts in French Guyana

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We investigated the extent of the diversity of yeasts in tropical rain forest and from different environments in French Guiana. We collected 365 samples from various substrates such as plants, fruits and insects at 14 locations and employed enrichment methods to isolate yeasts. Sequence analysis of the D1/D2 domains of the large subunit rRNA gene of 279 isolates indicated that these strains belonged to three taxa with 233 Saccharomycotina, 44 Basidiomycota and 2 Pezizomycotina. This analysis allowed the identification of 199 yeast strains to the species level. These included 20 basidiomycetes, mainly belonging to the species *Pseudozyma hubeiensis* (5) and *Trichosporon asahii* (5). We observed a large diversity among the isolated yeasts, since the identified strains were found to belong to 70 Saccharomycotina species, mainly representatives of the genera *Candida* (59/233= 25.3%), *Hanseniaspora* (43/233 =18.5%) and *Pichia* (18/233 = 7.7%). The most commonly found species were *Hanseniaspora opuntiae* (25 isolates), *Candida natalensis* (13 isolates) and *Kodamaea ohmeri* (13 isolates). Among the 233 Saccharomycotina isolates, 56 could not be assigned to a known species. These belonged to 14 genera and should be studied further. This is the first report on a large-scale study aiming at evaluating the yeast species present in the French Guiana natural environment, which remains a territory where the biodiversity of yeasts is largely unexplored.

P16 Detection of expressed genomic clinical factors in infectious bacteria: Proteotyping, an LC-MS/MS-based proteo-genomics approach

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A global increase in the incidence of antimicrobial resistant bacteria causing infections has been observed in recent years. As a consequence, the number of patients with bacterial infections that do not respond to antibiotic treatments is rising and is expected to continue increasing. In order to address the problem, it is crucial to rapidly and correctly identify the bacteria causing infections and their resistance to antibiotics, facilitating the determination of appropriate treatment regimens for patients.

Extended-spectrum β-lactamase-producing *Escherichia coli* strains have been used to analyse the capability of LC-MS/MS 'proteotyping' to detect antibiotic resistance factors. Whole-genome sequences were determined for the strains and antibiotic susceptibility tests were done to determine their resistance profiles. For proteomic characterizations, the strains were grown with and without added antibiotics. *E. coli* strain K12 (CCUG 49263) was used as negative control. Strain biomass was disrupted, using bead beating, and cell proteins were digested with trypsin. The peptides were separated and analysed, using LC-MS/MS. Mass spectra were matched to reference peptide sequences and determined peptide sequences were searched against an in-house database of resistance genes and a curated genome database.

The LC-MS/MS proteotyping analyses detected expressed antibiotic resistance genes in samples grown with and without antibiotic. Besides, it correctly identified the bacteria at the species level. Higher numbers of discriminatory peptides matching genes encoding antibiotic resistance factors were detected in the samples grown under antibiotic selection pressure. Genes associated with known antibiotic resistance factors had previously been

identified by whole-genome sequence analyses. Some of the resistance factors identified by DNA sequencing were not detected by proteomic analysis, indicating that they were not expressed. No peptides matching antibiotic resistance genes were found in the negative controls (*E. coli* strain K12).

LC-MS/MS-based proteotyping correctly identifies clinically-relevant bacterial species and shows promise to simultaneously detect expression of antibiotic resistance genes. The method is being optimized for analysing clinical samples directly, enabling rapid, sensitive detection of antibiotic-resistant bacteria causing infection, without prior cultivation. This rapid method of infection diagnosis will help address the global spread of antibiotic resistance.

P17 Spanish Type Culture Collection (CECT): A Microbial Resource Centre (mBRC)

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The Spanish Type Culture Collection (CECT) is the only public Microbial Resource Centre (mBRC) in Spain serving as a repository and provider of bacteria, archaea, yeast and filamentous fungi.

It is housed at the University of Valencia as a Service provider, satisfying the requirements of the International Depositary Authority.

It holds the ISO 9001 certificate, complies with the OECD Best Practice Guidelines for BRCs and actively participates in National, European and International initiatives working together with mBRCs to share good practices and experience in order to meet the demands of academic and industrial communities (GBRCN, EMbaRC, MIRRI, REDESMI…).

CECT Research focus on taxonomy and biotechnological applications (mainly marine and lactic acid bacteria) and collaborates in R&D with other companies and organizations.

CECT Mission: To provide quality controlled microbial reference material and associated data; to offer services related to preservation, identification and characterization of microorganisms; to provide training, consultancy and advice on aspects related to the handling and use of microbial resources, including legal issues; to perform R&D on microbial resources; to maintain high-performance levels by following international recommendations and standards.

CECT Vision: The CECT aims to promote Spanish strategies for the preservation and exploitation of microbial resources, in line with recommendations for BRCs established by the Convention of Biological Diversity (CBD) and the Organisation for Economic Co-operation and Development (OECD). The CECT aspires to act as an interface connecting Spain with worldwide efforts in this field, and work together with other mBRCs to help boost European competitiveness in biotechnology.

CECT Services:

- Supply pure and authentic microbial resources accessible through the on-line catalogue www.cect.org
- Identificaction and characterization of microorganisms
- Training: Handling, preservation and control of microbial strains
- Expert advice in preservation and taxonomy

Current challenges:

- Identification of strains deposited as *T. harzianum* and evaluation of RAPD profiles and gene sequencing for intra-species differentiation
- Improve the accuracy and robustness of MALDI-TOF MS identification technique of drinking water associated bacteria
- Oligotyping of Atole's LAB species
- Taxonomy of marine bacteria
- Determination of Shiga-Toxin genes in *E. coli* strains
- Work towards MIRRI's implementation phase
- Construction of the MIRRI Spanish National Node (REDESMI and MicroBioSpain)

P18 Characterization of *Blastococcus goeteborgensis*, sp. nov., isolated from human peritoneal dialysis effluent

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A strain of *Blastococcus* sp., CCUG 61487, was isolated from human peritoneal effluent. This is, to our knowledge, the first clinically isolated strain in the genus *Blastococcus*.

The *Blastococcus* genus is comprised within the *Geodermatophilaceae* family together with the *Geodermatophilus* and *Modestobacter* genera (Normand 2006). There are currently six described *Blastococcus* species most of which were isolated from harsh rock locations

The aim of this study was to characterise the first known case of a strain of *Blastococcus* isolated from a human clinical sample.

The strain was characterised phenotypically as well as genotypically and it was also whole genome sequenced.

The 16S gene sequence was 98.4% similar to other species. And low levels of hybridization were observed between the strain and the other species type strains; 38.7%-52.9%

It does have some distinct phenotypic characteristics it reduces nitrate, can grow on elevated salt concentrations and it can ferment glucose

We compare our findings to the already described species of *Blastococcus* and propose species designation *Blastococcus goeteborgensis* sp. nov. for the strain CCUG 61487, which is also the proposed type strain of this novel species.

P19 Comparison of typing methods for classification of Antarctic *Flavobacterium* spp.

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Polar microbiology has recently received considerable scientific attention. Not only that microbiologists have had increasing access to the previously inaccessible polar areas, but also uniqueness of microbial communities and ecology of these environments are exciting objects of research.

Aim of this study was to compare three different typing methods for classification of gliding *Flavobacterium* spp. isolated from abiotic samples in Antarctica (J. G. Mendel Station, James Ross Island). Investigated *Flavobacterium* strains (n=76) were subjected to FAME analysis (fatty acid methyl esters analysis), MALDI-TOF MS (matrix assisted laser desorption/ionization with time of flight mass spectrometry) and repetitive PCR using $(GTG)_5$ primers.

Results of cluster analyses of all three methods showed allocation of investigated strains into seven phylogenetic groups. Only seven strains delineated into separate clusters based on results of all three methods. Compared to each other, cluster analysis of FAME profiles and MALDI-TOF MS spectra showed highly similar and comparable results. Repetitive PCR confirmed five out of seven phylogenetic clusters and showed discrepancies in two phylogenetic groups (I+II).

In conclusion, all three methods seemed to be able to separate investigated strains to similar phylogenetic groups. However, higher accuracy of chemotaxonomic methods was found regarding Antarctic *Flavobacterium* spp., what was also confirmed by 16S rRNA analysis.

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P20 Biodiversity of industrial yeasts

Misiewicz Anna

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