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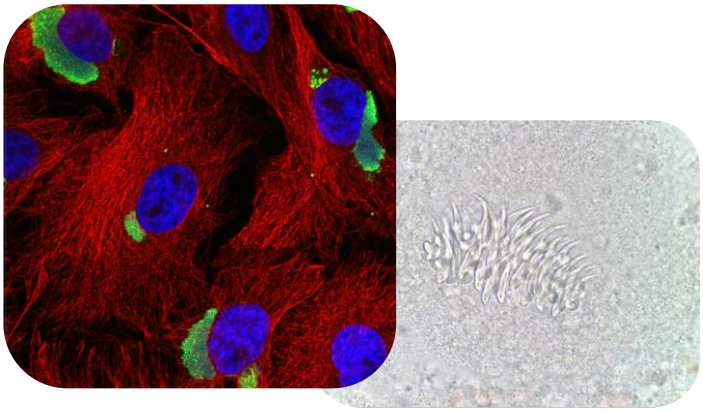
ABSTRACTS BOOK



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ABSTRACTS TALKS

O-6

DECIPHERING MECHANISMS OF (HETERO) RESISTANCE TO COLISTIN IN ACINETOBACTER BAUMANNII

Y. CHARRETIER[1], S. M. DIENE[1], D. BAUD[1], S. CHATELLIER[2], E. SANTIAGO-ALLEXANT[3], A. VAN BELKUM[4], G. GUIGON[3], J. SCHRENZEL[1]

[1]Genomic Research Laboratory, Service of Infectious Diseases, Geneva University Hospitals, Geneva Switzerland, [2]Innovation Unit, bioMérieux SA, La Balme Les Grottes, France, [3]Innovation Unit, bioMérieux SA, Marcy l'Etoile, France, [4]R&D Microbiology Unit, bioMérieux SA, La Balme Les Grottes, France

Multidrug-resistant Acinetobacter baumannii infection has recently emerged as a worldwide clinical problem and colistin is increasingly being used as a last-resort therapy. Despite its favorable bacterial killing property, resistance and heteroresistance to colistin has been described. The purpose of this study was to identify resistance mechanisms selected during short antimicrobial exposure and to evaluate the in vitro fate of colistin resistance after withdrawal of antibiotic pressure using global epidemic strains. Three clinical isolates representing global clones were selected and exposed to colistin at the MIC or above. Four resistant mutants were isolated in a one-step liquid-based culture. A second screening of the mutants with or without colistin in the culture medium helped to select phenotypic revertants. Targeted sequencing of the pmrCAB operon was performed to correlate phenotypic and genotypic resistance markers. Whole genome sequencing, population analysis profiling and quantitative RT-PCR were performed on a subset of strains. Use of high and low colistin concentrations allowed the selection of mutants resistant or heteroresistant to colistin. Each mutant showed at least one genetic change in either pmrA or pmrB. Population analysis profiling revealed heteroresistance. Whole genome sequencing demonstrated that mutations were exclusively found in pmrCAB. Quantitative RT-PCR demonstrated the diversity in pmrCAB regulation. Reversion by compensatory mutations was observed only in a single mutant through ISAba1 mobilization. Colistin resistance is easily and rapidly acquired in vitro and its fate appeared to be at least strain-dependent and probably clone-specific. This report demonstrates the involvement of mutations in pmrAB resulting in colistin (hetero)resistance.

O-7

Clinical Microbiology, Session 2: Bacterial Genomics / A-1

OUTBREAK INVESTIGATION FOR TOXIGENIC CORYNEBACTERIUM DIPHTHERIAE WOUND INFECTIONS IN REFUGEES FROM EAST AFRICA IN SWITZERLAND AND GERMANY BY WGS

DM. MEINEL[1,2], R. KUEHL[3], R. ZBINDEN[4], V. BOSKOVA[5], C. GARZONI[6], M. DOLINA[7], B. BLÜMEL[8], S. TSCHUDIN[3], AF. WIDMER[3], JA. BIELICKI[9], U. HEININGER[9], R. KONRAD[2], A. BERGER[2], V. HINIC[1], D. GOLDENBERGER[1], A. BLAICH[1], T. STADLER[5], M. BATTEGAY[3], A. SING[2], A. EGLI[1]

[1]Clinical Microbiology, University Hospital Basel, Switzerland, [2]National Consiliary Laboratory on Diphtheria, Oberschleissheim, Germany, [3]Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Switzerland, [4]Institute for Medical Microbiology, University Zurich, Switzerland, [5]Computational Evolution, ETH Zurich, Switzerland, [6]Computational Evolution, ETH Zurich, Switzerland, 6Department of Internal medicine and Infectious Diseases, Clinica Luganese, Switzerland, [7]Clinical Microbiology, EOLAB, Bellinzona, Switzerland , [8]Institute of Medical Microbiology and Hygiene, University Medical Centre Freiburg, Germany, [9]9 Paediatric Infectious Diseases, University of Basel Children's Hospital, Switzerland

Background: Toxigenic Corynebacterium diphtheriae is an important and potential fatal threat to patients and public health. During the current dramatic influx of refugees in Europe, we used whole genome sequencing to characterise a suspected outbreak of C. diphtheriae among refugees.
Methods: After conventional culture, we identified C. diphtheriae isolates using MALDI-TOF mass-spectrometry. Whole genome sequencing was performed on a MiSeq Illumina platform with >70x coverage, 2x250bp read length.
Results: Twenty cases of cutaneous C. diphtheriae in refugees from East African countries and Syria identified between April and August 2015 were included. Patients presented with wound infections shortly after arrival in Switzerland and Germany. Toxin production was detected in 11/20 (55%) isolates. Whole genome sequencing-based typing revealed the relatedness between isolates using Neighbour joining algorithms. We detected three separate clusters among these epidemiologically related refugees. Although the isolates within a cluster showed strong relatedness, they were not absolutely identical.
Conclusions: Toxigenic C. diphtheriae associated wound infections may currently be observed more frequently in Europe, perhaps due to refugees travelling under poor hygienic conditions. Close genetic relatedness of C. diphtheriae isolates from 20 refugees with wound infections indicates likely transmission between affected patients. However, the diversity within each cluster and the phylogenic time-tree analysis suggest that transmission happened several months ago, most likely outside Europe. Whole genome sequencing offers the potential to describe outbreaks in very high resolution and is a helpful tool in infection tracking and identification of transmission routes

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Clinical Microbiology, Session 2: Bacterial Genomics / A-3

TOWARDS RAPID BACTERIAL DETECTION DIRECTLY FROM CLINICAL SAMPLES USING NEXT-GENERATION SEQUENCING.

S LEO[1], N GAIA[1], V LAZAREVIC[1], E RUPPE[1], J SCHRENZEL[1]

[1]Genomic Research Lab, Service of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland

We report the metagenomic analyses of Illumina shot-gun sequencing libraries prepared from the broncho-alveolar lavage (BAL) of a severely immunocompromised patient developing a nosocomial pneumonia. To enrich for microbial sequences, aliquots of the BAL sample have been separately treated with two methyl-directed restriction enzymes (GlaI and McrBC) for human DNA depletion prior to sequencing. Illumina paired reads were processed, mapped to reference genomes with the two classifiers, Clark (1) and Kraken (2) and the results were correlated with culture and molecular data obtained by routine diagnostic procedures performed by the bacteriology laboratory of Geneva University Hospitals. In accordance with culture data, in the untreated aliquot the two reported bacterial pathogens Mycobacterium abscessus and Corynebacterium jeikeium as well as filamentous fungi were the most abundant organisms identified. McrBC and GlaI treatments decreased the number of human-mapped reads by 10% and 20% respectively, but with a comparable increment of unclassified reads. Most microbial sequences mapped to Burkholderia pseudomallei and Torque teno viruses, undetected by culture analyses, while the read counts for C. jeikeium and, to a lesser extent, for M. abscessus were dramatically reduced, probably because of an extensive enzymatic digestion of the samples. Therefore, further optimization of these procedures for human DNA removal is required. Overall, we report here a metagenomic approach with its bioinformatic pipeline as a possible tool for faster identification of micro-organisms in a BAL, an achievement that further supports the development of culture-independent methods in clinical routine diagnosis.

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2. D E Wood, S L Salzberg. Genome Biol. 2014; 15(3): R46.

O-15

Prokaryotic Biology, Session 3: Cell biology – seeing is believing / A-4

CAN BIOFILM BE EFFICIENTLY REMOVED FROM ENDOSCOPES? DEVELOPMENT AND EVALUATION PROCESS OF AN ENZYMATIC CLEANER

P STIEFEL[1], S MAUERHOFER[2], S ALTENRIED[1], J SCHNEIDER[1], K MANIURA-WEBER[1], U ROSENBERG[2], Q REN[1]

[1]Laboratory for Biointerfaces, Swiss Federal Laboratories for Materials Science and Technology (Empa), Lerchenfeldstrasse 5, CH-9014 St. Gallen, Switzerland, [2]Borer Chemie AG, Gewerbestrasse 13, 4528 Zuchwil, Switzerland

Efficient removal of biofilm from medical devices is a big challenge in healthcare to avoid hospital acquired infections, especially from delicate devices like flexible endoscopes which cannot be reprocessed using harsh chemicals or high temperatures. Therefore, milder solutions such as enzymatic cleaners have to be used, which need to be carefully developed to ensure efficacious performance. Our newly developed in vitro biofilm model system in 96-well plate [1] was used to select and optimize the formulation of enzymatic cleaners. Removal of the biofilm was quantified by Crystal Violet staining while the disinfecting properties were evaluated by BacTiter-Glo assay. Biofilm removal efficacy of the selected cleaner was further tested using the European standard for endoscope cleaning EN ISO 15883 and removal of artificial blood soil was investigated by treating TOSI® cleaning indicators. Using the established process a novel enzymatic endoscope cleaner was developed which removed 95% of Staphylococcus aureus and 90% of Pseudomonas aeruginosa biofilm in the 96-well plate system. With a >99% reduction of colony forming units and >90% reduction of extracellular polymeric substances this cleaner enabled subsequent complete disinfection and fulfilled acceptance criteria of EN ISO 15883. Furthermore, it efficiently removed blood soil and significantly outperformed comparable commercial products. The cleaning performance was stable even after storage of the cleaner for 6 months. It was demonstrated that incorporation of appropriate enzymes into the cleaner enhanced the performance significantly.

[1] Stiefel P, Rosenberg U, Schneider J, Mauerhofer S, Maniura-Weber K, Ren Q. 2016. Is biofilm removal properly assessed? Comparison of different quantification methods in a 96-well plate system. Appl Microbiol Biotechnol DOI: 10.1007/s00253-016-7396-9 [accepted].

O-20

SSM Annual Meeting 2016, Session 3: Host Responses to Microbes / A-1

DISORDERED NASAL MICROBIOTA WITHIN THE FIRST YEAR OF LIFE IN INFANTS WITH CYSTIC FIBROSIS

M MIKA[1], I KORTEN[2], W QI[3], N REGAMEY[2], U FREY[4], C CASAULTA[2], P LATZIN[2], M HILTY[1], SCILD STUDY GROUP[5]

[1]Institute for Infectious Diseases, University of Bern, Bern, Switzerland, [2]Division of Respiratory Medicine, Department of Pediatrics, Inselspital and University of Bern, Bern, Switzerland, [3]Functional Genomics Center, Swiss Federal Institute of Technology Zurich/ University of Zurich, Zurich, Switzerland, [4]University Children`s Hospital (UKBB), Basel, Switzerland, [5]SCILD Study Group, Switzerland

Background: Respiratory tract infections and subsequent airway inflammation occur early in the life of infants with cystic fibrosis (CF). However, there is a lack of information regarding the microbial composition of the respiratory tract in infants with CF. Our aim was to perform an in-depth characterization of the upper respiratory tract microbiota in infants with CF, who were followed throughout their first year of life.
Methods: Microbiota characterization was performed using 16S rRNA pyrosequencing of nasal swabs (n=461) collected biweekly from 30 infants with CF. Data was compared to an age-matched cohort of 47 healthy infants (n=872 samples). Additionally, the influence of antibiotic therapy on the microbiota of infants with CF was investigated.
Results: The microbiota of infants with CF differed compositionally from the microbiota of healthy infants (permutational MANOVA, P=0.001). This was mainly, but not solely, due to an overall increase in the mean relative abundance of Staphylococcaceae, which persisted during antibiotic therapy. The mean relative abundance of Moraxellaceae decreased significantly, while a spectrum of various bacterial families emerged (multivariable linear regression model, P=0.03 and <0.001, respectively). There was also an increase in bacterial diversity (Shannon Diversity Index) upon antibiotic administration (P=0.02).
Conclusions: This study describes compositional differences in the microbiota of CF infants and healthy controls, and the disordering of the microbiota upon antibiotic administration. These findings improve our understanding of the microbiota and its manipulation in the prevention of early disease progression in infants with CF.

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Clinical Microbiology, Session 3: Molecular Diagnostics and Serology / A-3

COMPARISON OF DIFFERENT SEROLOGIC TESTING SYSTEMS FOR T. PALLIDUM INFECTION

P BITTEL[1], N JEANNET[1], D MAYOR[1], F M SUTER-RINIKER[1]

[1]Clinical Microbiology, Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, CH-3001 Bern

Aim of this study was to review and compare serologic results of different treponemal specific tests (Architect Syphilis TP and SERODIA-TPPA) in order to determine diagnostic performance with a special focus on T. pallidum reinfections. Architect Syphilis TP, a chemiluminescent microparticle immunoassay, is widely used as a primary screening test due to its high degree of automation and high sensitivity. We reviewed samples from patients received between 2005 and 2015 (n=905) with parallel Architect and TPPA results. Upon detection of Architect and TPPA discordant results (Architect positive and TPPA negative) we analyzed the temporal characteristics of the agreement between Architect and TPPA. Additionally, we analyzed 32 samples from HIV positive patients with a T. pallidum reinfection, which is defined as at least four-fold increase in TPPA titers. Thereby we assessed whether the (qualitative) Architect Syphilis TP assay can be used to detect T. pallidum reinfections. Architect and TP•PA results plotted against each other showed that TPPA titers from 10'240 up to 327'680 can no longer be grouped into statistically significantly different groups according to the respective values obtained by Architect when performing multiple comparison one-way ANOVA. In HIV and syphilis co-infected patients, TPPA titers are likely to remain elevated despite appropriate antimicrobial treatment. In the T. pallidum reinfection dataset, ten out of 32 HIV co-infected patients had a long- term residual TPPA titer of ≥10'240 prior to reinfection. Reinfection with T. pallidum in these patients is accordingly characterized by a non-significant increase of the corresponding Architect result. Additionally, a total of 21 patients had positive Architect and negative TPPA results combined with antecedent positive TPPA results. We conclude that the Architect Syphilis TP as a screening tool for the detection of T. pallidum reinfections should be used with caution, particularly when HIV co-infected patient samples are analyzed. However, Architect Syphilis TP has a higher sensitivity when compared to the SERODIA-TPPA especially for the detection of residual antibody titers against T. pallidum.

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Clinical Microbiology, Session 2: Bacterial Genomics / A-4

SUSCEPTIBILITY OF MULTIDRUG-RESISTANT E.COLI STRAINS TO COMMERCIAL BACTERIOPHAGE COCKTAILS AND MONOPHAGE

N KARUMIDZE[1], O.J. BERNASCONI[1], D JGENTI[2], M GODERDZISHVILI[2], A ENDIMIANI[1]

[1]Institute for Infectious Diseases, University of Bern, Bern, Switzerland, [2]Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia

Background: Infections due to MDR E. coli represent a public health concern. These pathogens are resistant to the last-generation cephalosporins due to the production of extended-spectrum β-lactamases (ESBLs) and/or plasmid-mediated AmpCs (pAmpCs), leaving carbapenems as last options. However, also these drugs can be hydrolyzed by carbapenemases. One of the alternative ways to treat infections caused by these MDR pathogens could be the use of bacteriophages.
Methods: The spot test susceptibility method was used to evaluate two commercially available bacteriophage cocktails (Pyophage and Intestiphage; EliavaBiopreparations, Tbilisi, Georgia) and the vB_GEC_E. coli_AI monophage (George Eliava Collection) against 60 previously characterized non-clonal E. coli strains isolated from humans and animals. The collection included ESBL (n=26; of which 16 CTX-M-15-like), pAmpC (n=15; of which 12 CMY-2-like), carbapenemase producers (n=7; of which 5 NDM) and controls. Strains were grown in 5ml BHI broth for 4 hrs at 37°C. Then, they were transferred on BHI agar plates to form lanes. Ten µl of phages were subsequently spotted on each bacterial lane. Plates were incubated for 18 hrs. Results were defined as “resistant” when no phage(s) activity was observed.
Results: Overall, susceptibility to Pyophage, Intestiphage, and monophage was 85%, 82% and 66%, respectively. In particular, 96%, 92% and 81% of ESBL producers were susceptible to Pyophage, Intestiphage and monophage, respectively. With regard to pAmpC producers, 86.6% were susceptible to either Pyophage or Intestiphage, whereas 54% were susceptible to the monophage. Overall, only 8% of the strains (of which 2/7 carbapenemase producers) tested resistant to all three phage compounds. We did not recorded differences between the strains collected from humans and animals.
Conclusion: Our data indicates that bacteriophages may be considered as a possible option to treat infections due to MDR E. coli, including those pan-drug resistant due to production of carbapenemases. However, strains should undergo appropriate phage susceptibility tests before implementation.
This work was supported by SNF grant No. 153377 to AE

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Clinical Microbiology, Session 4 / A-2

A RANDOMIZED CONTROLLED TRIAL TO DETERMINE THE IMPACT OF MALDI-TOF BASED IDENTIFICATION DIRECTLY FROM POSITIVE BLOOD CULTURES ON PATIENT MANAGEMENT

M OSTHOFF[1], N GÜRTLER[1], S BASSETTI[2], GM BALESTRA[3], S MARSCH[3], H PARGGER[4], M WEISSER[1], A EGLI[5]

[1]Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, [2]Internal Medicine, University Hospital Basel, Basel, [3]Medical Intensive Care Unit, University Hospital Basel, Basel, [4]Surgical Intensive Care Unit, University Hospital Basel, Basel, [5]Clincial Microbiology, University Hospital Basel, Basel

Background: Rapid identification of pathogens directly from positive blood cultures (BCs) has been associated with improved antibiotic treatment and patient outcomes in combination with an antimicrobial stewardship program (ASP). Hence, we aimed to quantify the impact of rapid identification in a hospital setting with low resistance rates and an already established ASP.
Material/methods: 425 patients with positive BCs during a one-year period underwent randomization by weekday into two arms: (i) rapid identification by MALDI-TOF (Microflex, Bruker) directly from positive BCs (BactAlert FA/FN plus, bioMérieux) using the Sepsityper Kit (Bruker) versus (ii) conventional processing including subculture and subsequent identification. 57 patients were excluded from analysis (lost to follow-up). Episodes of bloodstream infections (BSI) (n=242) and contaminants (n=126) were analyzed separately.

Results: Median time to organism identification was significantly shorter in the MALDI-TOF group (23.5h vs. 46.0h, p<0.001). We could not observe a significant difference in the duration of intravenous antimicrobial therapy (median 8.6d vs. 10.7d, p=0.7) or length of hospital stay (11d vs. 13d, p=0.5) between the groups. We observed a trend towards a reduced 30-day mortality in the MALDI-TOF group (HR 0.5, 95% CI 0.2-1.1, p=0.09). Importantly, effective treatment within 2h after identification was administered more frequently in the MALDI-TOF group (97.4 vs. 90.6%, p=0.03). Rapid identification resulted in improved optimal treatment at 48h only in the subgroup of BSIs (100/242 episodes) caused by AmpC-producing and non-fermenting organisms, S. aureus and Streptococcus spp. (57 vs. 38%, p=0.06), whereas it had no impact on E. coli or Klebsiella spp. BSI (98/242). In patients with contaminations rapid identification was associated with a shorter median duration of antimicrobial therapy (7.0d vs. 9.3d, p=0.04).

Conclusions: Rapid identification by MALDI-TOF directly from positive BCs provided fast and reliable microbiological results and improved treatment quality in case of contaminated BCs and BSIs not caused by E. coli and Klebsiella spp in a setting with an established ASP.

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Clinical Microbiology, Session 4 / A-1

THE REPRODUCIBILITY OF MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME OF FLIGHT MASS SPECTROMETRY BASED TYPING: EMPLOYMENT OF BIOINFORMATICS

M. OBERLE[1], N. WOHLWEND[2], D. JONAS[3], F. MAURER[4], G. JOST[5], K. VRANCKX[6], A. EGLI[7]

[1]Medizinische Mikrobiologie, Institut für Labormedizin, Kantonsspital Aarau, Aarau, Schweiz, [2]Labormedizinisches Zentrum Dr. Risch AG, Liebefeld-Bern, Schweiz, [3]Institute for Environmental Medicine, University of Freiburg, Germany, [4]Institute for Medical Microbiology, University of Zurich, Switzerland, [5]Clinical Microbiology, Dianalabs, Geneva, Switzerland, [6]Applied Maths NV, Sint-Martens-Latem, Belgium, [7]Clinical Microbiology, University Hospital Basel, Basel, Switzerland

Due to the wide access to MALDI-TOF, an increasing number of studies show reliable typing results. This 'new' method holds promise for an easy access typing method compared to conventional approaches. However, the technical, biological and center reproducibility of such typing data has not yet been explored. The aim of this study is to compare typing data from a multi-center study employing bioinformatics.
Seven different center, tested a set of 12 extended spectrum beta-lactamase (ESBL)-producing Escherichia coli isolates – including two outbreak and one non-related cluster. Every center used a Microflex MALDI-TOF system (Bruker, Bremen, Germany) and was instructed to use a previously evaluated standard operating procedure. Each center recorded four spectra from each isolate (technical replicate) and repeated the procedure at three different days (biological replicate). The raw data was used to calculate the technical and biological reproducibility in each center and between the centers using the software Bionumerics (Applied Maths, Belgium).
The median values of the technical reproducibility ranged between 96.8 % and 99.4% for each center. The median values of the biological reproducibility ranged between 47.6 % and 94.4 %. The clustering among identical isolates was reproducible between centers, however a more complex bioinformatics approach was necessary:
We used a discriminant analysis to better separate the three clusters. For this, we defined a reference center who's spectra were used to identify 18 discriminating peaks using an Anova analysis. With this approach, the identification of the cluster was 100% correct.
Detection of specific clusters is possible from spectra obtained from different centers. However, bioinformatic techniques are required to make the analysis robust and reliable. The closer related different clusters are to each other, the less reliable their separation is. Technical reproducibility is a key element to implement MALDI-TOF based typing. Further examinations are needed to study which elements during the preparation and the acquirement of spectra contribute most to the technical variability.

O-34

VON WILLEBRAND BINDING PROTEIN, BUT NOT STAPHYLOCOAGULASE, IS INVOLVED IN THE INITIATION OF STAPHYLOCOCCUS AUREUS EXPERIMENTAL INFECTIVE ENDOCARDITIS

S. MANCINI[1], F. OECHSLIN[1], C. MENZI[1], Y.A. QUE[1], T.R. VELOSO[2], P. MOREILLON[1], J.M. ENTENZA[1]

[1]Department of Fundamental Microbiology, UNIL, Lausanne, Switzerland, [2]Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium

Staphylococcus aureus is the most frequent causative agent of infective endocarditis (IE). S. aureus possesses several cell-wall associated proteins, such as the fibrinogen-binding protein ClfA, and secreted plasma-clotting factors such as staphylocoagulase (Coa) and von Willebrand factor binding protein (vWbp). The critical role of ClfA in promoting IE has been already demonstrated (1). The function of Coa and vWbp in IE pathogenesis, however, has not yet been elucidated. Here we used recombinant Lactococcus lactis expressing S. aureus CoA and vWbp, individually (L. lactis Coa and L. lactis vWbp) or together (L. lactis Coa/vWbp), and a rat model of IE, to investigate the role of Coa and vWbp in the initiation of IE. L. lactis pIL253 (parent) was used as control. Rats with catheter-induced aortic vegetations were inoculated with 106 CFU of each L. lactis. Vegetation infection was assessed 24h later. Like the parent L. lactis pIL253 (0% infected vegetations), L. lactis Coa was unable to promote IE (13% infected vegetations). By contrast, L. lactis vWbp increased the incidence of IE (67% infected vegetations; P= 0.007 vs L. lactis Coa). The association of both Coa and vWbp did not further enhanced valve infection (62%). To further investigate the role of vWbp in IE in its natural context, i.e., in S. aureus possessing ClfA, animals were inoculated with 104 CFU of S. aureus Newman harboring both ClfA and vWbp (ClfA+/vWbp+) or isogenic mutants lacking either ClfA (ClfA-/vWbp+) or vWbp (ClfA+/ vWb–). While no significant reduction in IE incidence was observed with S. aureus lacking vWbp (64% infected vegetations) as compared with the ClfA+/vWbp+ strain (82% infected vegetations), the absence of ClfA led to a significant decrease in S. aureus infectivity (42% infected vegetations; P= 0.02 vs ClfA+/vWbp+). Taken together these results suggest that: (i) vWbp but not Coa supports S. aureus IE development; (ii) ClfA but not vWBP is essential to initiate this infection; (iii) vWbp in association with ClfA may further promote the emergence of IE.

(1) Moreillon P, Entenza JM, Francioli P, Mc Devitt D, Foster TJ, François P, Vaudaux P. Infect. Immun. 1995;63: 4738-4743.

O-37

Clinical Microbiology, Session 1: Meta Genomics / A-3

NEW STRAIN OF NON-TOXIGENIC CORYNEBACTERIUM DIPHTHERIAE FOUND IN A PATIENT WITH TRACHEO-BRONCHITIS: UNPRECEDENTED GENOMIC FEATURES.

F TAGINI[1], A KOUTSOKERA[2], T PILLONEL[1], A LOVIS[2], G GREUB[1]

[1]Institute of Microbiology, Department of Laboratory, University of Lausanne and University Hospital Center, Lausanne, Switzerland, [2]Service of Pneumology, Department of Medicine, University Hospital Center Lausanne, Switzerland

Corynebacterium diphtheriae is the causal agent of diphtheria. Classical diphtheria is due to the production of a prophage encoded toxin (tox), against which potent vaccines have shown efficacy since 1923. However, there is a recent increase of non-toxigenic C. diphtheriae infections. We performed here a genomic analysis of a strain isolated in a bronchoalveolar lavage taken from a twenty-two year-old immunocompetent male with tracheo-bronchitis. Genome assembly was done using SPAdes Genome Assembler 3.6.2. 174 contigs of more than 1000 base pairs (bp) were obtained for a total length of 2.89 megabases (Mb) and a GC content of 53.76%. In comparison, genomes sizes of the other 14 complete genomes available on NCBI range from 2.395 Mb to 2.535 Mb. Moreover, the Average Nucleotide Identity (ANI) between our strain and the other complete genomes range from 95.15% to 95.36% whereas the ANI of the reference genome (NCTC13129) compared to the others range from 98.24% to 98.56%. Ten prophages sequences were predicted using PHAST and could partially explain the higher amount of DNA found in the genome. Interestingly, an incomplete prophage, which does not bear the toxin gene, is present at the same locus where the usual tox+ corynephage inserts. We found only 1 putative Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) region with 3 direct repeats and 2 spacers. C. diphtheriae is usually associated with 2 CRISPR regions of a larger size, which could be a reason of the elevated number of predicted prophages. After BLASTing the Virulence Factor Database on our assembly, we found 39 hits with > 75.0% identity and an e-value < 10^-5. Thirty-one of them are involved in the iron-uptake or siderophore system and we noted the presence of a conserved diphtheria toxin regulator (dtxR) gene. Two interesting genomic islands were found with IslandViewer 3 and one of them bears 8 genes coding for the siderophore system. In conclusion, thanks to sequencing, we were able (i) to determine the absence of the toxin quickly, which had a major impact on patient care and (ii) to characterize this very divergent pathogenic strain holding an unusual high number of prophages.

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SSM Annual Meeting 2016, Session 1: Microbe evolution / A-1

ROLE OF PROPHAGES IN GENOME PLASTICITY, VIRULENCE AND HOST TROPISM: CONDITIONING OF STPAHYLOCOCCUS AUREUS CC398 HUMAN-ADAPTED STRAINS

F LAUMAY[1], AR CORVAGLIA[1], SM DIENE[1], N VAN DER MEE-MARQUET[2], P FRANCOIS[1]

[1]Genomic Research Lab, Department of Infectious Diseases Geneva University Hospital Rue Gabrielle-Perret-Gentil 4 CH-1211 GENEVA 14 SWITZERLAND, [2]CHRU de Tours, Département de Microbiologie et Hygiène, Hôpital Trousseau, Réseau des Hygiénistes du Centre RHC, UMR 1282, Infectiologie Santé Publique, Université de Tours, 37044 TOURS cedex

Staphylococcus aureus is a ubiquitous bacterium, frequently colonizing human skin but also a highly pathogenic microorganism. Part of its virulence is related to the acquisition of mobile genetic elements (MGEs).Lysogenic bacteriophages are a type of MGEs known to play an important role in S. aureus pathogenicity. Recently, Uhlemann et al. (2012) described the emergence of S. aureus CC398 strains initially colonizing animals and now able to infect and spread in humans living in animal-free environments. We analysed 21 isolates of CC398 by whole genome sequencing, from cases of colonization and infections in animals and humans; it appears that their prophages content provides them important new features contributing to their genome plasticity and virulence. Indeed, human-adapted strains are resistant to transduction and are 4-fold more prone to invade non-phagocytic cells than the positive control. However, the exact molecular mechanisms are unknown. In order to investigate the role of prophages in genome plasticity and virulence of CC398 human-adapted strains, we have selected: [i] the human-adapted S1 strain, which possesses two prophages; [ii] the prophage-free and non-human-adapted strain S123; [iii] the strain S123sa2, derived from prophage-free S123 and transduced with prophages from S1. We noticed that S123sa2 has acquired some important features related to virulence, like adhesion to human extracellular matrix proteins ability; moreover, S123 and S123sa2 display different gene expression profiles. Interestingly, numerous open reading frames (ORFs) of S1 prophages encode for hypothetical protein with unknowm function. Therefore, we plan to perform deletions of prophages' ORFs in S124sa2 and to examine phenotypic features related to genome plasticity or virulence of mutants, in parallel with RNA-seq experiments. Elucidating the molecular mechanisms triggered by prophages harboured represents an original field of research, poorly explored to date in our settings. Providing new insights related to acquisition of genetic material is a prerequisite to identify new targets and to understand epidemiological and virulence evolution.

O-42

Prokaryotic Biology, Session 2: The 3 Cs – cytoplasm, chromatin and chaperones / A-3

IDENTIFICATION AND CHARACTERIZATION OF A W. CHONDROPHILA EFFECTOR SECRETED IN THE HOST CYTOPLASM

C KEBBI-BEGHDADI[1], L PILLOUX[1], G GREUB[1]

[1]Center for Research on Intracellular Bacteria (CRIB), Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland

Waddlia chondrophila is a strict intracellular bacterium causing adverse pregnancy outcomes in humans and abortion in ruminants. Similarly to classical Chlamydiae responsible for some severe human and animal diseases, W. chondrophila belongs to the Chlamydiales order and harbors a biphasic life cycle. Like for every obligate intracellular bacteria, it is crucial for W. chondrophila to modulate its environment to establish a replicative niche, acquire nutrients and escape the host defense mechanisms. These bacteria-host interactions are triggered by bacterial effector proteins that are actively secreted, mainly by the Type 3 Secretion System (T3SS), in the membrane of the bacteria-containing vacuole or in the host cytoplasm.
The hypothetical protein WcT3SS_001 was previously identified as an immunological protein (Kebbi-Beghdadi et al, 2012) and is predicted by several in silico algorithms to be a T3SS effector. We demonstrated by immunofluorescence and confocal microscopy that this putative T3SS effector is secreted in the host cell cytoplasm. Analysis of the mRNA and protein expression levels during the bacterial replication cycle by RT-qPCR and western blot indicated that the WcT3SS_001 gene is transcribed during the early phase of the cycle and that the protein is produced and secreted 16 to 24 hours post infection. This time point corresponds to the beginning of the replication phase, which could suggest that this effector plays a role in the establishment of favorable conditions to sustain an exponential multiplication of the bacteria.
To further demonstrate that WcT3SS_001 is indeed secreted by the T3SS and since Chlamydiales bacteria are only poorly amenable to genetic modifications, we expressed the protein in a heterologous system (Yersinia enterocolitica) where type three secretion can be selectively blocked or induced. In addition, we will also express this protein, fused to GFP, in human epithelial cells and identify its eukaryotic target(s) by co-immunoprecipitation. These results will provide insights into the function of this hypothetical protein and potentially open novel perspectives for the development of new drugs active against chlamydiae.

O-46

Clinical Microbiology, Session 1: Meta Genomics / A-2

IMPACT OF DIFFERENT FARMING SYSTEMS ON THE ABUNDANCE AND DIVERSITY OF ANTIMICROBIAL RESISTANCE DETERMINANTS AND BACTERIAL COMMUNITY

E. GOMEZ-SANZ[1], S. JAENICKE[2], R. WITTWER[3], A. GOESMANN[2], M.G.A. VAN DER HEIJDEN[3], B. DUFFY[1], T.H.M. SMITS[1]

[1]Environmental Genomics and Systems Biology Research Group, ZHAW, Wädenswil, Switzerland, [2]Bioinformatics and Systems Biology, Justus-Liebig-University, Giessen, Germany, [3]Plant Soil Interactions, Agroscope, Zürich, Switzerland

This study examines soil management systems for abundance, diversity and dynamics of microbial communities and antimicrobial resistance (AMR) genes in a Swiss experimental farming trial with four different management systems: organic production with ploughing or reduced tillage and conventional production with ploughing or no tillage. The organic treatments were fertilized with cattle slurry from organic dairy farms; the conventional systems with mineral fertilizer. A combination of amplicon sequencing and total DNA metagenomics approaches was used to generate an overview of the complete microbial and AMR diversity. Actinobacteria and Alphaproteobacteria were most abundant in all systems. Beta- and Gammaproteobacteria, and Sphingobacteria were also abundant in diverse counts in the management systems. Based on the ARG-ANNOT database, AMR genes related to most clinically relevant families were only detected in low counts, with the fluoroquinolone and tetracycline resistance genes as the most abundant in all systems. Currently, we are examining the temporal dynamics of a number of clinically relevant AMR genes in the four soil management systems by qPCR. Temporal increase in bacterial 16S rRNA gene copies was observed in all systems a few days after fertilization. Resistance to tetracyclines and sulfonamides was detected in quantifiable amounts only in both organic systems and showed a temporal increase after slurry addition, harboring relatively high counts of those genes per 16S rRNA gene copy. Gene abundance decreased again after manure addition but the baseline remained higher than in conventional systems along the sampling period. We are also developing new qPCR primer sets for AMR genes of interest, including some identified by the metagenomics approach. Based on the current data, we conclude that long-term fertilization with slurry influences the abundance and diversity of the antimicrobial resistome in the investigated farming soil systems, despite the organic farm origin of the amended slurry.

O-48

Clinical Microbiology, Session 3: Molecular Diagnostics and Serology / A-1

LABORATORY DIAGNOSIS OF ACUTE NEUROBORRELIOSIS – HOW AN UNSPECIFIC MARKER MIGHT GIVE A MORE SPECIFIC ANSWER.

R. LIENHARD[1], L. VON ALLMEN[2], L. BERTAIOLA-MONNERAT[3], A. DUMOULIN[4], M.L. TRITTEN[1], H.H. SIEGRIST[1]

[1]ADMED Microbiology, and Laboratory, National Reference Centre for Tick-borne Diseases (NRZK), La Chaus-de-Fonds, Switzerland, [2]ADMED Microbiology, La Chaux-de-Fonds, Switzerland, [3]Laboratory of Microbiology, Hôpital Cantonal, Fribourg, Switzerland. , [4]Institut Central des Hôpitaux, Hôpital du Valais, Sion, Switzerland

Purpose. Cytokine CXCL13 was quantified in CSF to evaluate its diagnostic potential for neuroborreliosis. Material and Method. One hundred and sixty-two CSF samples were quantified for cytokine CXCL13 on the CXCL 13 ELISA (Euroimmun, Lübeck, Germany). A selection of 55 patients presenting neuroborreliosis with production of specific intrathecal antibodies (SIA) was used to test sensitivity. The determination of SIA in IgG and IgM was made using the IDEIA Lyme Neuroborreliosis test (Oxoid, Basel Switzerland). Specificity was tested on 77 CSF samples of patients presenting central nervous system disorder with known aetiology. A further 30 CSF samples coming from suspected neuroborreliosis with a negative SIA on our test, but a positive SIA determined in the primary laboratory were analysed. The recently defined cut-off value of 250 pg/ml was used. Results and Discussion. We obtained a sensitivity for CXCL13 of 85.5% and a specificity of 94.8%. In the group with suspected neuroborreliosis, 10 (33.3%) CSF were positive. CXCL13 chemokine secretion is not stimulated by any specific Borrelia burgdorferi s.l. antigen. This unspecific marker showed, however, a very highly specific answer for acute neuroborreliosis. Furthermore most unspecific reactions can be easily identified. Sensitivity was rather low, it showed a better correlation with IgM SIA than IgG SIA. A delay of antibiotic therapy is essential to consider testing CXCL13 as its level drops quickly after the first dose. This frequently unavailable information might be a reason for lower sensitivity. Conclusion. Quantitative CXCL13 in CSF should be implemented in neuroborreliosis diagnosis as SIA determination harbours some pitfalls and PCR sensitivity is not satisfactory.

O-50

Clinical Microbiology, Session 4 / A-3

TIME-KILL EXPERIMENTS WITH PENICILLIN, CEFTRIAXONE, GENTAMICIN AND DAPTOMYCIN ALONE AND IN COMBINATION AGAINST AEROCOCCUS URINAE

C HIRZEL[1], L HIRZBERGER[1], H FURRER[1], A ENDIMIANI[2]

[1]Department of Infectious Diseases, Bern University Hospital, University of Bern, Bern, Switzerland, [2]Institute for Infectious Diseases, University of Bern, Bern, Switzerland

Introduction: A. urinae may cause severe infections (bacteremia and endocarditis) associated with high mortality. However, data on bactericidal and synergistic activity for clinically implemented antibiotics is scarce.
Methods: We performed time-kill (TK) analyses on 2 clinical isolates (AU1 and AU2) and ATCC700306 for penicillin (PG), ceftriaxone (CRO), gentamicin (GEN), daptomycin (DAP) and their combinations. TK experiments were performed in TH broth (supplemented with 50 µg/ml calcium for experiments with DAP). All experiments were performed at least 3 times at antibiotic concentrations of 0.5x, 1x, 2x, and 4x the MICs. Using the EUCAST criteria for viridans streptococci AU1 and AU2 were CRO-resistant (MICs, 2 µg/ml). ATCC was GEN high-level resistant (MIC, 512 µg/ml), whereas all strains were PG- and DAP-susceptible (MICs <0.125 and ≤1 µg/ml, respectively). CFU/ml count was determined at 0, 2, 4, 6, 8, 24, 30, and 48 hrs. Bactericidal activity was defined as a ≥3-log10 decrease in CFU/ml compared to the initial inoculum at 8 and 24 hrs. Synergism was defined as a ≥2-log10 decrease in CFU/ml at 8 and 24 hrs comparing the antimicrobial combination to the most active single antimicrobial drug.
Results: PG or CRO alone were not bactericidal for all strains, whereas DAP exhibited bactericidal activity at all MICs for AU2 and ATCC. The combination of PG or CRO with GEN was bactericidal for AU1 and AU2 at concentrations ≥1x MICs. Bactericidal synergism was detected for PG or CRO combined with GEN in the 2 clinical isolates. PG plus CRO showed non-bactericidal synergism for ATCC. DAP with GEN was synergistic at 1x MICs for AU1, whereas the killing activity of DAP was too pronounced to detect potential synergism in AU2.
Discussion: We demonstrated that the combination of PG or CRO with GEN is synergistic and bactericidal. Moreover, our data suggests that DAP may represent a potential bactericidal treatment alternative against A. urinae. This finding could be important for the treatment of patients with a ?-lactam allergy or renal insufficiency.
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O-51

TRENDS IN ANTIMICROBIAL SUSCEPTIBILITY AND CLONALITY OF NEISSERIA GONORRHOEAE ISOLATES COLLECTED FROM 1989 TO 2015 IN SWITZERLAND

V DONÀ[1], S KASRAIAN[1], R ZBINDEN[2], B SAVIC[3], F IMERI[3], H FURRER[4], N LOW[5], A ENDIMIANI[1]

[1]Institute for Infectious Diseases, University of Bern, Bern, Switzerland, [2]Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland, [3]Laborgemeinschaft 1, Zurich, Switzerland, [4]Department of Infectious Diseases, Bern University Hospital, University of Bern, Bern, Switzerland, [5]Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

Background: Antimicrobial resistance in Neisseria gonorrhoeae is a major public health concern, requiring continuous surveillance to identify emerging resistance and monitor susceptibility trends.
Methods: Overall, 270 N. gonorrhoeae isolates collected between 1989 and 2015 (of which: 1989-91, n=57; 1999-2003, n=71; and 2011-2015, n=99) in Bern (n=86) and Zurich (n=184) were included in our study. Antimicrobial susceptibility tests for cefixime, ceftriaxone, ciprofloxacin, azithromycin and spectinomycin were assessed using the Etest method on GC agar to obtain MIC values. Results were interpreted according to the EUCAST 2015 criteria. Sequence type (ST) was obtained from NG multi-antigen sequence typing (NG-MAST) for 105 (38.9%) representative isolates (of which: 1989-91, n=12; 2001-2005, n=32; and 2011-2015, n=61).
Results: The following MIC50/90 (µg/ml) and % of susceptible isolates were recorded: cefixime (1989-1991: ≤0.016/0.023, 100%; 1999-2003: ≤0.016/,≤0.016, 100%; 2011-2015: ≤0.016/0.094, 96%), ceftriaxone (1989-1991: ≤0.016/≤0.016, 100%; 1999-2003: ≤0.016/0.023, 100%; 2011-2015: ≤0.016/0.032, 100%), ciprofloxacin (1989-1991: ≤0.016/≤0.016, 98%; 1999-2003: ≤0.016/4, 79%; 2011-2015: ≤0.016/?32, 53%), and azithromycin (1989-1991: 0.125/0.38, 67%; 1999-2003: 0.19/0.38, 69%; 2011-2015: 0.19/0.38, 75%). All N. gonorrhoeae isolates were fully susceptible to spectinomycin. The most common STs in each period were: 1989-91 (ST10856, 3/12); 1999-2003 (ST8672, 3/32); 2011-2015 (ST2992, 8/61; ST1407, 3/61).
Conclusions: In Switzerland, MIC creep for extended-spectrum cephalosporins is occurring (4/99, 4.0% cefixime resistant isolates in 2011-2015). Ciprofloxacin resistance rose from 2% (1989-1991) to 47% (2011-2015) over the study period; the highest prevalence (89%) was in 2012. Around 30% of isolates in all periods were non-susceptible to azithromycin. The presence of international hyperepidemic clones (ST2992 and ST1407) was observed. Our results are consistent with those globally observed.
This study was funded by the SwissTransMed (Rapid Diagnosis of Antibiotic Resistance in Gonorrhoea, RaDAR-Go).

O-56

Clinical Microbiology, Session 3: Molecular Diagnostics and Serology / A-2

Visceral leishmaniasis in a lung transplant recipient: usefulness of highly sensitive real-time polymerase chain reaction for pre-emptive diagnosis

O OPOTA[1], Z BALMPOUZIS[2], C BERUTTO[3], J KAISER-GUIGNARD[4], G GREUB[1], J-D AUBERT[2], G PROD'HOM[1], O MANUEL[5], K JATON[1]

[1]Institute of Microbiology, University Hospital of Lausanne, Lausanne, Switzerland, [2]Pneumology Service, University Hospital of Lausanne, Lausanne, Switzerland, [3]Transplantation Center, University Hospital of Lausanne, Lausanne, Switzerland, [4]Hematology Service, University Hospital of Lausanne, Lausanne, Switzerland, [5]Infectious Diseases Service, University Hospital of Lausanne, Lausanne, Switzerland

Background: Molecular diagnostic methods have significantly improved the diagnosis/management/treatments of viral infections in immunocompromised host. However, studies addressing their usefulness for the diagnostic of parasitic diseases, often delayed due to non-specific signs and symptoms, are lacking. We report the case of a lung transplant recipient in whom the diagnostic of visceral leishmaniasis (VL) could have been made months before symptoms appearance by the use of a Leishmania-specific PCR on peripheral blood (PB), suggesting a role of pre-emptive PCR-based diagnosis in transplant recipients.
Material/methods: We diagnosed a VL in a 61 y/o Swiss lung transplant recipient 12 months after the onset of chronic pancytopenia; parasite load already reached 8.9E+3 parasites per ml. We retrospectively tested the patients' PB DNA samples collected after transplantation using a Leishmania specific real-time PCR targeting the 10,000 copies kinetoplastic DNA making it extremely sensitive (detection limit < 5.0E-4 parasites per ml).
Results: The first positive PCR (890 DNA copies/ml corresponding to less than 1 parasite/ml) occurred in a PB sample collected more than 6 months before the onset of pancytopenia, and 18 months before the diagnostic of VL. The parasite load regularly increased until the diagnostic of VL which occurred at a very high parasitic load (8.9E+7 DNA copies per ml; 8.9E+3 parasites per ml). The myelogram obtained 5 weeks before and initially negative by microscopy for parasites detection, was also positive by PCR (7.2E+5 DNA copies per ml). A 2 log decrease in DNA was observed 7 days after the initiation of the liposomal amphotericin B therapy and a monthly follow-up by PCR did not detect any parasite DNA at 6 months after discontinuation of amphotericin B.
Conclusions: This analysis demonstrates that using a Leishmania-specific real-time PCR on PB, parasite DNA can be detected long before the appearance of symptoms associated with VL in immunocompromised patients. This suggests the usefulness of pre-emptive PCR-based diagnosis on PB in SOT recipients at risk for developing VL, such as patients travelling and/or living in endemic areas.

O-58

Prokaryotic Biology, Session 3: Cell biology – seeing is believing / A-3

BOVINE CATHELICIDINS HAVE POTENT BACTERICIDAL EFFECT AGAINST MASTITIS PATHOGENS AND MEDIATE SENSING OF BACTERIAL NUCLEIC ACIDS BY THE IMMUNE SYSTEM

MS KIENER[1], A BAUMANN[1], A SUMMERFIELD[1]

[1]Institute of Virology and Immunology, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland

Mastitis is an inflammation of the mammary gland usually caused by pathogens invading the lumen of the gland via the udder, and represents a major animal health concern of lactating cows. Antibiotic is the classical therapy but the massive use of such drugs have been associated with emergence of multi-resistant bacterial strains endangering both animal and human health. Cathelicidins represent a family of cationic peptides characterized by a strong antimicrobial activity and several effector functions in immunity. Understanding their induction and functions helps to identify new and sustainable antimicrobial strategies. Therefore, the antimicrobial activity of six synthetic bovine cathelicidins against mastitis pathogens was tested in vitro. The cathelicidin BMAP-27 was the most effective at killing bacteria, which was strictly dependent on its amphipathic structure. The modulatory functions were further investigated in an assay which consists to detect Mx expression after nucleic acids exposure in bovine turbinate cells. Interestingly, alpha-helical cathelicidins enhanced the sensing of very low concentration of nucleic acid. Strong bactericidal activity in the supernatants of stimulated neutrophils could be associated to bovine cathelicidins by western blot. Altogether, the present data support the role of cathelicidin in killing bacterial cells as well as helping the innate immune system in sensing of nucleic acids. These finding are relevant to develop future preventive strategies either as a potential alternative to antibiotics or as prophylactic strategy in animal health.

O-59

Clinical Microbiology, Session 1: Meta Genomics / A-1

COMPARISON OF 16SRRNA SEQUENCING DATA PROCESSING PIPELINES TO ASSESS CHANGES IN THE GUT MICROBIOTA INDUCED BY DAIRY INTERVENTIONS

M ROSIKIEWICZ[1], KJ BURTON[2], G PIMENTEL, U BUTIKOFER, U VON AH U[3], MJ VOIROL[2], S AEBY[1], G VERGÈRES[3], N VIONNET[2], FP PRALONG[2], G GREUB[1]

[1]Institute of microbiology of the University of Lausanne and University Hospital, Lausanne, Switzerland, [2]Service of Endocrinology, Diabetes and Metabolism, University Hospital, Lausanne, Switzerland, [3]Institute of Food Science, Agroscope, Federal Office of Agriculture, Berne, Switzerland

The intestinal microbiota has been described as a dynamic ecosystem that can influence both health and disease. Recent developments in technologies such as 16S sequencing provided fast methods to assess the changes in the intestinal microbiota. Different methods exist for the processing of such data including automated and user-defined pipelines. In this work we evaluate three data processing approaches and their impact on downstream analyses conducted in a dietary intervention study. A randomised, cross-over study design was used to assess the impact of a probiotic yoghurt and milk acidified with a prebiotic (gluconic acid) on the gut microbiota of fourteen healthy male volunteers. Each product was consumed on a daily basis (400g/day) for a two-week period, with a three-week wash-out period separating the two test phases. Faecal samples were taken at eight time points during the study. 16S rRNA sequencing was performed using the Illumina MiSeq. Three different pipelines were used to obtain taxonomic assignment of reads: automated Illumina Metagenomic Application with Illumina-customized Greengenes database, QIIME closed reference approach and de-novo clustering using UPARSE algorithm (USEARCH package) with SILVA database. Changes in the microbiota were assessed by differential analysis using DESeq2. In the pooled data, differences were observed in the total number of taxa identified depending on the data processing method used. Notably, total number of genera that were identified using the three processing techniques was highly divergent with 616 genera in the Illumina processed data, 303 genera in the QIIME processed data and 208 genera obtained in USEARCH analysed dataset. The differential analysis showed differences depending on the processing approach used but was robust with respect to the expected increases in Lactobacillus and Streptococcus after probiotic yoghurt intake and increased in Bifidobacteria after prebiotic acidified milk. The differences in taxa abundance associated with data processing methodology appears to be linked to the more stringent criteria and better pre-processing of the reads applied during analysis with customisable pipelines.

O-60

Clinical Microbiology, Session 2: Bacterial Genomics / A-2

IDENTIFICATION OF VIRULENCE FACTORS OF A STAPHYLOCOCCUS AUREUS STRAIN CAUSING SEPTIC SHOCK

T PILLONEL[1], C BERTELLI[1], J SCHRENZEL [2], G GREUB[1]

[1]Center for Research on Intracellular Bacteria, Institute of Microbiology, University Hospital Center and University of Lausanne, Switzerland, [2]Genomic Research Laboratory, Service of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland, [3]Service of Infectious Diseases, University Hospital Center, Lausanne, Switzerland

Variations of S. aureus virulence are attributed i) to the presence/absence of virulence factors (VFs) encoded on mobile genetic elements (MGE), but also ii) to changes in VFs encoding genes part of the core genome as well as iii) to changes in the expression of any of these VFs. Recently, a case of severe septic shock with major exfoliative rash and multi-organ dysfunction was due to a community-acquired S. aureus. This strain was isolated in pure culture and its genome was sequenced to investigate the genetic basis of its high virulence. Multilocus sequence typing analysis identified this isolate as Sequence Type 8. Two publicly available VFs databases were used to annotate the virulome: VirulenceFinder 1.5 and the Virulence Factor Database (VFDB). The presence/absence of known VF was investigated using tBLASTn against the genome contigs. In addition, the genome was independently sequenced and analysed by the company Biomérieux. Those 3 approaches allowed the identification of 146 putative VFs. Surprisingly, only 38 were predicted both by Biomérieux and the 2 databases. These 38 proteins included protein A, fibronectin binding proteins, fibrinogen binding proteins, multiple enterotoxins, serine proteases as well as leukotoxins LukD and LukE. Contrarily to Biomérieux, both VFDB and VirulenceFinder had a hit for eta, the exfoliative toxin A, which was of particular interest for this genome due to the clinical picture with exfoliation. In depth analyses suggested that it was a false positive. Biomérieux classified the Clp protease and the HtrA serine protease as VFs, which was not the case of the two databases. Altogether, those results highlight the lack of consistency of VF databases that reflects the difficulty of database curation. The various definitions of VFs add another level of complexity in identifying which gene to include in a virulence database. Moreover, VFs present in all S. aureus strains (e.g. protein A) are generally not the focus of virulence analyses, which rather search for VFs part of the mobilome. These variable factors might explain very unusual severe clinical presentations such as the lethal case reported here.

O-64

Environmental Microbiology, Session 1 / A-3

PLASMID-BORNE MCR-1 COLISTIN RESISTANCE GENE IN ESBL-PRODUCING ENTEROBACTERIACAE IN RIVER WATER AND IMPORTED VEGETABLES IN SWITZERLAND

K ZURFLUH[1], L POIREL[1], P NORDMANN[2], M NÜESCH-INDERBINEN[1], H HÄCHLER[1], R STEPHAN[1]

[1]Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich Winterthurerstrasse 272, 8057 Zurich, Switzerland , [2]Emerging Antibiotic Resistance Unit, Department of Medicine, Faculty of Science, University of Fribourg, 1701 Fribourg, Switzerland

The recent identification of Enterobacteriaceae harbouring the plasmid-mediated transferable colistin resistance mcr-1 gene is of great concern to public health. Here, we report on the occurrence of mcr-1 harbouring extended-spectrum -lactamase (ESBL)-producing Enterobacteriaceae from river water in Switzerland and from ready-to-eat imported vegetables.

Seventyfour ESBL-producing Enterobacteriaceae isolated from 21 rivers and lakes sampled in 2012 in Switzerland and 60 ESBL-producing Enterobacteriaceae isolated from 42 imported vegetable samples from the Dominican Republic, India, Thailand, and Vietnam were screened by PCR for the presence of the mcr-1 gene.

The mcr-1 gene was detected in one out of 74 water strains, and 2 out of 60 vegetable strains (products from Thailand and Vietnam). All 3 genes showed 100% homology with the published mcr-1 sequence. Colistin resistance was transferable by transformation experiments into Escherichia. coli DH5-alpha. All 3 original isolates were identified as E. coli and belonged to different multi locus sequence types. They harboured different blaESBL genes and showed a multiresistance phenotype.

The results prove that mcr-1 has arrived in Switzerland as in other countries. This is worrisome, because polymyxins are currently being chemically modified to become less toxic in order to replace currently used antimicrobials that are increasingly corrupted by multiple resistance.

O-65

Prokaryotic Biology, Session 1: The envelope – from walls to molecules / A-1

THE STAPHYLOCOCCUS AUREUS CHAPERONE PRSA IS A NEW AUXILIARY FACTOR OF OXACILLIN RESISTANCE AFFECTING PENICILLIN-BINDING PROTEIN 2A

A JOUSSELIN[1], C MANZANO[1], A BIETTE[1], P REED[2], M PINHO[2], A ROSATO[3], W.L. KELLEY[1], A RENZONI[1]

[1]Service of Infectious Diseases, University Hospital and Medical School of Geneva, 4 rue Gabrielle-Perret Gentil CH-1205 Geneva, Switzerland, [2]Laboratory of Bacterial Cell Biology, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal., [3]Houston Methodist Research Institute, 6670 Bertner Ave., Houston, TX 77030, USA

Expression of the methicillin-resistant S. aureus (MRSA) phenotype results from the expression of the extra penicillin binding protein PBP2A, which is encoded by mecA and acquired horizontally on part of the SCCmec cassette. PBP2A can catalyze DD-transpeptidation of peptidoglycan (PG) because of its low affinity for ?-lactam antibiotics and can functionally cooperate with the PBP2 transglycosylase in the biosynthesis of PG. Herein, we focus upon the role of the membrane-bound PrsA foldase protein as a regulator of ?-lactam resistance expression. Deletion of prsA altered oxacillin resistance in three different SCCmec backgrounds and, more importantly, causes a decrease of PBP2A membrane amounts without affecting mecA mRNA levels. The N- and C-terminal chaperone domains of PrsA were found to be critical features for PBP2A protein membrane levels and oxacillin resistance. We propose that PrsA has a role in post-transcriptional maturation of PBP2A, possibly in export and/or folding of newly synthetized PBP2A. This additional level of control in the expression of the mecA dependent MRSA phenotype constitutes an opportunity to expand the strategies to design anti-infective agents.

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Prokaryotic Biology, Session 1: The envelope – from walls to molecules / A-3

RPIRC IS A REGULATOR OF STAPHYLOCOCCUS AUREUS BIOFILM SUSCEPTIBILITY TO ANTIBIOTICS AND VIRULENCE

A FISCHER[1], M GIRARD[1], P FRANÇOIS[1], J SCHRENZEL[1]

[1]Genomic Research Laboratory, Department of Medical Specialties, Geneva University Hospitals, 1211 Geneva 14, Switzerland.

Biofilm is the most common mode of bacterial growth on medical devices and has also been reported on human tissues, e.g. during lung infection by Pseudomonas aeruginosa. Some antibiotics are inefficient against susceptible bacteria growing in biofilm, even in the presence of very high drug concentration, either because molecules are too large to penetrate the biofilm matrix or the antibiotic has higher affinity for matrix components (e.g. vancomycin and extracellular DNA, eDNA), but other mechanisms likely remain to be deciphered. Matrix is composed of proteins, glucids (PIA for Staphylococcus aureus) and eDNA. eDNA provides structuration and stability in mature biofilms and is degraded by DNase. In many bacterial biofilms, eDNA originates from cell lysis although eDNA can also be actively secreted or exported by bacterial membrane vesicles. By screening the Nebraska transposon library, we identified rpiRc as a biofilm regulator involved in eDNA regulation. We tested wt and mutant biofilm susceptibility to antibiotics using a similar protocol as the Calgary biofilm device. Involvement of rpiRc in Staphylococus aureus virulence was assessed ex vivo by internalization experiments in HEK293 cells and in vivo in a mouse model of subcutaneous catheter infection. RpiRc is a transcription factor from the pentose phosphate pathway whose product is a PIA precursor. However, rpiRc mutant strain showed neither susceptibility to DispersinB® (a commercially available enzyme disrupting PIA biofilms) nor alteration of ica transcription (the operon regulating PIA production). While MICs of planktonic cells were not affected in the mutant strain, we observed increased biofilm susceptibility to almost all tested antibiotics, regardless of their mode of action. More importantly, the rpiRc mutant showed reduced virulence in both ex vivo and in vivo experiments related to decreased fnbpA-B transcription and eDNA production. RpiRc is an important regulator involved in eDNA degradation inside the matrix of mature PIA independent biofilms. Our results illustrate that RpiRc contributes to increased antibiotic tolerance in mature bacterial biofilm and also to S. aureus cell adhesion and virulence during subcutaneous infection.

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Clinical Microbiology, Session 3: Molecular Diagnostics and Serology / A-4

A UNIVERSAL SCREENING CULTURE MEDIUM FOR COLISTIN-RESISTANT GRAM NEGATIVE BACTERIA

P. NORDMANN[1], A. JAYOL[1], L. POIREL[1]

[1]Medical and Molecular Microbiology Unit, Department of Medicine, University of Fribourg

Emergence of colistin resistance among multidrug resistant Gram negative rods are emerging rapidly worldwide. To avoid the development of nosocomial outbreaks due to colistin-resistant gram negatives, an early detection of carriers is mandatory. Colistin-based selective culture media have been developed for screening intrinsic colistin-resistant bacterial species but they are not adapted for screening Gram negatives with acquired resistance to colistin that display low- to high-level resistance to colistin. Therefore, we have developed a selective culture medium, the SuperPolymyxin medium. This medium was prepared using an Eosine Methylene Blue agar (EMB)-based culture medium wherein colistin, daptomycin and amphotericin B were added. Performance of the SuperPolymyxin medium was evaluated with a total of 82 Gram-negative strains from various enterobacterial and non-fermenters species. Seven strains were from intrinsically colistin-resistant species, 44 strains exhibited acquired resistance to colistin (chromosomal mutations, plasmid-mediated mcr-1 gene acquisition or mechanisms not yet known) and 31 strains were colistin-susceptible. MICs of colistin were determined using the broth microdilution method and results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing breakpoints. The basis of the SuperPolymyxin medium is the EMB medium that allows to distinguish between lactose fermenters and lactose non-fermenters. Colistin-resistant strains grew on the SuperPolymyxin medium in 24 to 48 h. The lowest limit of detection was below the cut-off value of 103 CFU/ml for all colistin-resistant strains, whereas the limit of detection of the colistin-susceptible strains was ≥ 106 CFU/ml. The sensitivity and specificity of the SuperPolymyxin medium for selecting colistin-resistant gram negatives were 100% in both cases regardless of the nature of the colistin resistance mechanism (intrinsic, chromosomally or plasmid-encoded) and of its level (high or moderate). The SuperPolymyxin medium is the first screening medium that is aimed to detect intrinsic and acquired colistin resistant Gram negative rods. It will contribute to an early identification of carriers of colistin-resistant strains, therefore preventing and containing outbreaks due to colistin-resistant isolates.

O-70

Prokaryotic Biology, Session 1: The envelope – from walls to molecules / A-3

INCREASED RESISTANCE TO CARBAPENEMS IN PROTEUS MIRABILIS CAUSED BY AMPLIFICATION OF THE BLAVIM-1 GENE

S. BONTRON[1], L. POIREL[1], G. KUEFFER[1], E. SAVOV[2], P. NORDMANN[1]

[1]Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg, Switzerland, [2]Laboratory of Microbiology, Military Medical Academy, Sofia, Bulgaria

A series of 26 Proteus mirabilis clonally-related clinical isolates recovered during an outbreak in a Bulgarian hospital showed heterogeneous resistance levels to carbapenems. All isolates produced the VIM-1 enzyme known to hydrolyze efficiently carbapenems. MICs of the carbapenem meropenem ranged from 0.25- to >32-mg/L. Resistance levels to carbapenems correlated with the blaVIM-1 copy number, present at 1, 2, or 4 copies per bacterial cell. In all cases, the blaVIM-1 gene was chromosomal, and laid within a class 1 integron, embedded in a transposon delimited by two IS26 elements. In isolates with 2 or 4 blaVIM-1 copies, the transposon was duplicated in tandem, the genetic structure being consistent with non-equal homologous recombination between directly orientated repeats. In-vitro, clones with higher resistance levels to β-lactams, displaying amplification of the blavim-1 gene from 1 copy to 10 to 70 copies, were selected with high concentrations of ceftazidime, a substrate of the VIM-1 carbapenemase. The blaVIM-1 bearing transposon was found in tandem in some but not all clones, suggesting at least two distinct mechanisms of gene amplification. Conversely, exposure to meropenem selected clones with a marked increased resistance to this antibiotic, never in association with amplification of the blavim-1 gene, but rather with permeability defects. This work showed that within a given epidemic, bacterial isolates may evolve towards an increased resistance to carbapenems by amplification of the carbapenemase gene. In addition, it showed that gene amplification can be induced in vitro, preferentially upon exposure to specific antibiotics.

O-75

SSM Annual Meeting 2016, Session 8: Microbe replication / A-2

ACTIVE VERSUS DORMANT: ADAPTATIONS OF FIRMICUTES TO THRIVE IN SALINE ENVIRONMENTS

S FILIPPIDOU[1], T JUNIER[2], T WUNDERLIN[1], I PALMIERI[1], N JEANNERET[1], P JUNIER[1]

[1]Laboratory of Microbiology, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland, [2]Vital-IT group, Swiss Institute of Bioinformatics, Lausanne, Switzerland

How do endospore-forming Firmicutes (EFF) survive in saline and hypersaline environments? The order of Halanaerobiales are halophilic Clostridia found in saline environments according to culture-based and metagenomic diversity studies. They mainly accumulate KCl, instead of an organic molecule, as compatible solute for osmotic balance. This metabolic adaptation, interesting as it may be, is only one part of the story. EFF are also known for the production of endospores, specialized survival structures that could allow tolerance to high salt concentrations, under a dormant metabolic state. In this study we analyzed the interplay of these two strategies: halotolerance and dormancy, to understand the distribution and diversity of EFF in halophilic environments. In the case of halotolerance, extensive enrichment and isolation were conducted from halophilic sites. Eighty aerobic and anaerobic EFF were isolated from 9 saline habitats worldwide including marine environments, brines, salt crusts, and saline geothermal springs. These isolates cover a large taxomomic range of Bacilli and Clostridia. We verified if these isolates are halotolerant or halophilic, and their ability to accumulate KCl. The results were correlated to their phylogeny. In addition, the genetic mechanisms of tolerance were investigated in a selection of these strains, whose genome was fully sequenced. In halophilic Clostridia, the typical acidic signatures of the 'halophilic' proteins are absent. The genomic imprints in halophilic and halotolerant Bacilli are described herein for the first time. To evaluate the role of sporulation, we analyzed the in situ diversity of EFF using a molecular marker specific for endospore formation. Twenty-nine samples collected along salinity gradients in different sites at the Atakama dessert, Chile, were investigated. Finally, the diversity in situ was compared to the knowledge gained with the cultures. Our findings suggest that in saline habitats, both survival strategies are deployed, resulting in an impressive diversity of EFF. Biochemical, genomic, ecological and environmental data are pieces to fill in the puzzle of halophilic adaptations in saline habitats.

O-79

Environmental Microbiology, Session 2 / A-1

ENDOSPORE-FORMING FIRMICUTES DOMINATE BACTERIAL COMMUNITIES ACROSS THERMAL GRADIENTS IN NISYROS VOLCANO

F. PALMIERI[1], S. FILIPPIDOU[1], P. JUNIER[1]

[1]Laboratory of Microbiology, University of Neuchâtel, Neuchâtel, Switzerland

Volcanos are extreme environments with elevated temperatures, low pH, high pressure and high concentration of heavy metals. In order to thrive in these environments and to withstand unfavourable conditions, microorganisms have developed adaptations and survival strategies. One of these strategies is endospore formation. Thanks to their structure, endospores are highly resistant to heat, UV radiation, chemicals and other stressors. The aim of this study was to assess the role of endospore formation as a survival strategy along a temperature gradient in volcanic fumaroles. We hypothesize that endospore-forming Firmicutes (EFF) are predominant in the higher temperature sites as they withstand harsher environmental conditions. In order to address this hypothesis, soil was sampled across two thermal gradients from two different fumaroles in Nisyros volcano. Soil across a macrogradient from the crater to the vegetation was also sampled. The total bacterial community was assessed by qPCR of the 16S rRNA gene, while the endospore-forming community was assessed by qPCR of the spo0A (sporulation transcriptional factor) gene. Dipicolinic acid (DPA) will be also quantified as a proxy to endospores. The results showed that endospore-formers dominate the community at high temperature sites. The relative proportion of EFF compared to the total community decreases further away from the fumaroles. Similarly, endospore-formers are supposed to be predominant in the crater sample compared to the other soil samples in the macrogradient towards vegetation. DPA quantification should show the same tendency. These results suggest that bacterial communities stratify across a thermal gradient, with a dominance of EFF at higher temperatures. This study is the first to give an insight into the role of endospore-formation as a survival strategy in volcanic habitats.

O-80

Environmental Microbiology, Session 4 / A-3

COOPERATION IN CARBON SOURCE DEGRADATION SHAPES SPATIAL SELF-ORGANIZATION OF SURFACE-ATTACHED MICROBIAL CONSORTIA

R TECON[1], D OR[1]

[1]Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland

In natural environments, microorganisms often live in rich multispecies assemblages (e.g., biofilms), where resource utilization is constantly optimized and could yield stable spatial patterns. In unsaturated soils where aqueous habitats are highly fragmented, the complex nutrient diffusion fields promote microbial self-organization when motion is possible. The principles and conditions that control microbial self-organization remain understudied. Here, we examine how carbon source utilization, surface attachment and water film configuration shape the spatial organization of a two-member bacterial consortium. In our experimental system, two fluorescently-tagged mutant strains of the soil bacterium Pseudomonas putida were forced to cooperate to degrade and utilize toluene, resulting in mutualistic trophic interactions. Replacing toluene by benzoate, a carbon source degraded by both strains, changed the interaction from cooperation to exploitation competition. On agar surfaces, we showed that mutualism was expressed by distinct intermixing patterns, whereas competition promoted genotypic segregation. Characteristics of these patterns were quantified using image analyses. Results show that the ratio of cooperators in the mutualistic consortium converged to nearly 1:1 on solid surfaces and in liquid cultures regardless of the initial ratio. The consortium productivity, as measured by cell growth, was affected by the initial ratio on surfaces but not in liquid cultures. The consortium was grown on a porous surface model that mimics unsaturated soils to evaluate the role of hydration conditions on consortium spatial self-assembly. The study suggests that trophic dependencies among multispecies surface-attached microbial communities result in specific spatial patterns for optimal ecological functioning.

O-81

Environmental Microbiology, Session 2 / A-3

FOLDING OF COMPLEX REDOX PROTEINS BY DEDICATED MOLECULAR CHAPERONES

C GROB[1], Z JULIER[1], J MAILLARD[1]

[1]Laboratory for Environmental Biotechnology, ENAC-IIE-LBE, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Chlorinated compounds (so-called organohalides) are widespread soil and groundwater pollutants. Only few bacteria have the ability to degrade these compounds via organohalide respiration (OHR). Reductive dehalogenases (RDases) are complex redox enzymes involved in the reduction of organohalides, and contribute to the biodegradation of these pollutants. RDases need to be folded and loaded with iron-sulfur centers and a corrinoid cofactor prior to their transport across the cytoplasmic membrane via the Twin-arginine translocation (Tat) pathway. A new family of Tat molecular chaperones, named RdhT, was recently shown to participate in the maturation of RDases (1-2), and successfully applied for heterologous production of these complex redox enzymes (3).
The present study focuses on the interaction of RdhT molecular chaperones with their cognate RDases. PceT (the paradigmatic member of the RdhT family) interacts as a dimer with the Tat signal peptide of PceA, its cognate RDase, as shown by isothermal titration calorimetry. When recombinant pceT and pceA-His genes are heterologously expressed in E. coli, both proteins co-purify on Ni-NTA chromatography which indicates that PceT binds to PceA also in vivo. Although recombinant PceA is not functional in E. coli, it is produced in a soluble form when pceT is co-expressed and represent the basis for reconstitution experiments.
Currently, in vivo strategies are developed in E. coli to allow a rapid screening of interacting RdhT chaperones with the Tat signal peptides of RDases. This will further help evaluating the cross-reactivity of RdhT chaperones towards Tat signal peptides, and help identifying specific amino acids of the chaperones that are involved in the interaction event.

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O-82

Environmental Microbiology, Session 3 / A-2

COMPETITION OF TWO SULFUROSPIRILLUM POPULATIONS FOR TETRACHLOROETHENE

G BUTTET[1], C HOLLIGER[1], J MAILLARD[1]

[1]Laboratory for Environmental Biotechnology, ENAC-IIE-LBE, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Tetrachloroethene (PCE) represents a major groundwater pollutant. Some bacteria are able to use PCE as electron acceptor in an anaerobic respiration process called organohalide respiration (OHR). An anaerobic enrichment culture (named SL2-PCEb) consisting of two different Sulfurospirillum populations was obtained from a fixed-bed bioreactor sludge treating PCE-contaminated groundwater (1). The peculiarity of this bacterial consortium resides in stepwise dechlorination of PCE to trichloroethene (TCE) and cis-dichloroethene (cis-DCE), which is catalyzed by the two populations successively (2). Two subcultures were derived from SL2-PCEb, each one harboring one Sulfurospirillum population and showing distinct dechlorination potential: SL2-PCEc dechlorinates PCE to TCE only, while SL2-TCE (selected on TCE) kept the potential to dechlorinate both PCE and TCE. A molecular fingerprinting method targeting small differences in their rdhA genes was developed to follow the dynamics of both populations. The dechlorination activity of both populations suggested that the RdhA enzyme produced by SL2-PCEc has a higher turnover rate than the one produced by SL2-TCE (3). The present work proposes to study the competition of both SL2-PCEc and SL2-TCE populations for PCE by mixing them with different proportions and following their growth and activity. To this purpose, a new experimental set-up was developed by combining quantitative PCR and fragment analysis. This should highlight the physiological and molecular basis of the two RdhA enzymes for substrate affinity and enzymatic activity.

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O-84

Environmental Microbiology, Session 4 / A-1

A MICROFLUIDIC CHIP TO MEASURE BACTERIAL CHEMOTAXIS IN A BIOSENSORY PERSPECTIVE

C. ROGGO[1], J. R. VAN DER MEER[1]

[1]Department of Fundamental Microbiology, University of Lausanne

Chemotaxis is a behavior by motile bacteria to sense the environment and swim in the direction of or away from chemical compounds. In a uniform environment bacteria swim randomly to explore the maximum space but when they are in presence of a gradient of attractant, they bias their swimming direction toward the highest concentration of attractant. Chemotaxis is rapid, and could thus be exploitable for developing biosensors with quick response. In addition it is conserved among motile bacteria and some species show chemotaxis toward toxic compounds.
Here we pursue the design of microfluidic chips in which a gradient of attractant can be generated which enables measurement of bacterial chemotaxis. We demonstrate two different design principles. In the first, three parallel flow channels are produced, separated by 700 nm-shallow filters, which allow the diffusion of small chemical molecules but prevent the passage of the bacterial cells. The chemical gradient forms perpendicular to the flow and cells are flown in the inner channel. The accumulation of cells on the side with the highest attractant concentration is observed by microscopy. We show the working principle both on Escherichia coli chemotaxis to serine and aspartate, as well as on Cupriavidus necator and the herbicide 2,4–dichlorophenoxyacetic acid (2,4-D).
To observe the chemotactic response of cells to a gradient even more directly, we fabricated a second device with valves permitting rapid opening and closing of channels. This allows to create a stable gradient and to bring in a package of cells, upon which individual trajectories can be followed. We show the functioning of the chip and how individual E. coli cells react to serine within minutes after exposure to the gradient.

O-85

Environmental Microbiology, Session 2 / A-2

FLUORESCENT PSEUDOMONAS BACTERIA AS TOOLS FOR MONITORING AND IMPROVING THE HEALTH OF AGRICULTURAL SOILS IN SWITZERLAND

N IMPERIALI[1], F DENNERT[2], F MASCHER[3], M FESSELET[3], T TURLINGS[4], R CAMPOS HERRERA[4], M MAURHOFER[2], C KEEL[1]

[1]Department of Fundamental Microbiology, University of Lausanne, Switzerland, [2]Institute of Integrative Biology, ETH Zurich, Switzerland, [3]Institute for Plant Production Sciences, Agroscope Changins, Switzerland, [4]Laboratory for Fundamental and Applied Research in Chemical Ecology, University of Neuchâtel, Switzerland

In Switzerland there is a lack of knowledge about the impact of soil on the activity of bacteria expressing plant-beneficial traits. In the context of the National Research Programme 68 “Sustainable use of soil as a resource” , we evaluate the possibility of using plant-beneficial bacteria as bio-indicators of soil health. Of particular interest are root-colonizing Pseudomonas protegens and Pseudomonas chlororaphis, both displaying fungicidal and insecticidal activities and both useful to control fungal pathogens and pest insects. We constructed GFP-tagged reporter strains of these pseudomonads, which in addition express mCherry-based fusions reporting the activity of promoters relevant for the production of antifungal compounds (DAPG, HCN, pyrrolnitrin, phenazines) and the insecticidal toxin Fit. In ongoing studies, we test the activity of these bio-reporters in soil samples extracted from representative cereal-oriented fields and from long-term field trials subjected to different agricultural practices (crop rotation, tillage, fertilization). For monitoring, we developed a soil extraction protocol and a flow cytometry approach. Excitingly, our data reveal consistent differences in antifungal gene expression in the different Swiss soils. Another important aspect of our NRP 68 contribution is to develop novel environment-friendly strategies to control crop diseases and pests. To this end, we test combined applications of pseudomonads with other beneficials. In field experiments, seeds of spring wheat were inoculated with P. protegens and P. chlororaphis, alone and combined with entomopathogenic nematodes. The persistence of the inoculants was monitored and the performance and fitness of wheat plants assessed. One experimental field was invaded by Oscinella frit, an insect notorious for causing damage to cereal crops. We observed that plants treated with the two bacterial species were less damaged. In another field trial, seeds of maize were treated with the two Pseudomonas species, alone or in combination with entomopathogenic nematodes and mycorrhizal fungi and a positive effect of the bacteria and nematodes on the grain yield weight was observed.

O-88

Environmental Microbiology, Session 2 / A-4

IMMEDIATE GENOME-WIDE TRANSCRIPTIONAL RESPONSE OF PSEUDOMONAS VERONII STRAIN 1YDBTEX2 TO TOLUENE IN SOIL

M MORALES[1], V SENTCHILO[1], J VAN DER MEER[1]

[1]Department of Fundamental Microbiology. University of Lausanne. Biophore. UNIL-Sorge. Lausanne. 2015.

The natural recovery of the soils polluted by aromatic hydrocarbons such as BTEX (benzene, toluene, ethylbenzene and xylene) can be enhanced by introducing specific biodegrader bacteria in a process named bioaugmentation. Owing to their unique metabolic properties and to their adaptation potential these bacteria survive and propagate in the contaminated environment at the expense of the degraded pollutant. In this project we study the adaptive response of the BTEX biodegrader Pseudomonas veronii 1YdBTEX2 in laboratory scale microcosms. We performed an RNA-seq metatransciptomic analysis of the immediate genome-wide transcriptional response of P. veronii 1YdBTEX2 to toluene in the non-sterile soil environment and compared it to that in liquid medium. One hour exposure to toluene triggered massive transcription (up to 208-fold induction) of multiple gene clusters, among which those for toluene degradation pathway(s) and toluene efflux pumps, thus clearly underlining their key role in the adaptation. In addition we identified a variety of highly up-regulated genes coding for conserved hypothetical proteins, which would be interesting to follow up on. When comparing the transcriptome of the cells in liquid medium with that in sandy soil we found major changes in expression of genes involved with membrane functioning (e.g., lipid composition, lipid metabolism, cell fatty acid synthesis), with osmotic stress response (e.g., polyamine or trehalose synthesis, uptake of potassium) and putrescine metabolism. Stable growth and survival of P. veronii 1YdBTEX2 in the microcosms for extended periods of time (7-10 days) suggest that the immediate response mechanisms disclosed in this study are efficient for long-term establishment in the polluted soil.

O-90

Environmental Microbiology, Session 4 / A-4

EVOLUTION OF MICROBIAL COMMUNITIES AND NUTRIENT REMOVAL PERFORMANCES IN AEROBIC GRANULAR SLUDGE SEQUENCING BATCH REACTOR DURING CHANGE OF SUBSTRATE

A ADLER[1], V BERCLAZ[1], M HORISBERGER[1], J MAILLARD[1], C HOLLIGER[1]

[1]Laboratory for Environmental Biotechnology, ENAC-IIE-LBE, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland

Aerobic granular sludge (AGS) is a promising alternative wastewater treatment to the conventional activated sludge system. As AGS has enhanced settling abilities and provides different redox conditions across the granules at the same time, the processes based on AGS allow substantial space, energy and chemical products savings. A few wastewater treatment plants (mainly hybrid or pilot plants) are already using AGS. Nevertheless the performances of lab-scale reactors fed with simple synthetic wastewater are usually different from those of plants treating real wastewater where nutrient removal performances are more versatile and granules have a fluffy structure. Moreover, if the microbial community structures from AGS reactor fed with simple synthetic substrate have been extensively studied, it is less the case of the biomass from AGS reactors fed with more complex substrates or real wastewater. In order to make a step toward the comprehension of AGS used to treat municipal wastewater, this experiment was designed to study the impact of polymeric organic compounds on nutrient removal performances and microbial communities. These properties were monitored on a lab scale AGS sequencing batch reactor during a progressive substrate transition from volatile fatty acids (VFAs), to a more complex substrate mixture containing VFAs, glucose and amino acids, and finally to a synthetic wastewater containing VFAs, starch and oligo-peptides. With the lowered VFA concentrations, phosphorus (P)-removal performances were impaired. At the same time, amino acids fermentation significantly increased the ammonium concentration, thus extending the time required for total nitrification. The composition of the artificial wastewater was further adapted in order to recover efficient P- and nitrogen (N)-removal. Molecular analyses will show how the changes in substrate composition have influenced the AGS bacterial community structure that has kept its high settling ability and the granule size distribution throughout the first substrate transition.

O-91

Environmental Microbiology, Session 1 / A-2

LINKING HIGH-RESOLUTION METHANE-OXIDATION RATES TO BACTERIAL COMMUNITY STRUCTURE IN FOUR STRATIFIED SWISS LAKES

M MAYR[1], M ZIMMERMANN[1], A BRAND[1], H BÜRGMANN[1]

[1]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Surface Waters – Research and Management, 6047 Kastanienbaum, Switzerland , [2]ETH Zurich, Institute of Biogeochemistry and Pollutant Dynamics, 8092 Zurich, Switzerland

Worldwide, lakes have been estimated to contribute 6-16% to the natural methane emissions, even though methane-oxidizing bacteria (MOB) remove a major fraction of the methane produced in lakes before it even reaches the atmosphere. Understanding the ecology of MOB is thus central to our understanding of this source of an important greenhouse gas. Aerobic methane-oxidizing bacteria (MOB), the most important methane sink in lakes, are diverse, consisting of distinct phylogenetic groups mainly within the Alpha- and Gammaproteobacteria. There is a significant gap in our understanding of the importance of the structure of MOB communities for the rates and dynamic response of methane oxidation. We hypothesize that several ecologically distinct types of MOB occur throughout the vertical water column of stratified lakes, forming discrete assemblies, driven by environmental conditions acting on traits inherent to the specific MOB types. In a study on four lakes (Rotsee, Greifensee, Luganersee & Zugersee), potential methane oxidation rates were obtained from incubation experiments with 13C-CH4. Illumina sequencing of the MOB community (DNA and RNA) is under way in order to link methane-oxidation to MOB community structure at a high spatial resolution throughout the oxycline of the four lakes. The four lakes were found to be extremely variable in terms of methane-oxidation patterns. We expect to find spatially distinct population maxima of MOB OTUs across the four lake oxyclines. Future experiments with MOB species and consortia from the lakes will reveal the importance of various traits, which will be used to mechanistically explain the observed MOB community patterns.

O-93

Environmental Microbiology, Session 3 / A-1

TWO NEW INTEGRATIVE CONJUGATIVE ELEMENTS IN PSEUDOMONAS AZELAICA HBP-1 ARE RESPONSIBLE FOR THE ADAPTATION TO THE POLLUTED ENVIRONMENT

V SENTCHILO[1], L CHALUPOVA[1], J VAN DER MEER[1]

[1]Department of Fundamental Microbiology, University of Lausanne, Biophore, UNIL-Sorge, 1015 Lausanne

Bacterial genomes are dynamic structures prone to constant reshuffling of their genetic material via horizontal gene transfer and recombination. A particular class of mobile genetic elements (MGEs) coined Integrative Conjugative Elements (ICEs) is currently becoming more and more appreciated as an important mean for large scale gene mobilization and integration in the whole domain Bacteria. We have completely sequenced a genome of a sewage sludge isolate P. azelaica HBP-1 and discovered two new ICEs named ICEPaz1 (96 kbp)and ICEPaz2 (171 kbp). First of them confers resistance to mercury (II) and to arsenate whereas the second encodes catabolism of a range of aromatic substrates (2-hydroxybiphenyl, 4-hydroxybenzoate, vanillate, salicylate, catechol and protocatechuate) -- thus both ICEs being involved in the host adaptation to the particular environment. The two ICEs in P. azelaica were found integrated into two non-identical copies of the glycine-t-RNA gene; they encoded their own tyrosine-type site-specific recombinase (integrase) and were flanked by 18 base pairs direct repeats. Pairwise comparison between the two ICEs from P. azelaica revealed that the region of structural similarity between them was limited to about 33 kbp with average DNA identity of 75% and was comprised of genes involved in conjugation and life-cycle control—the so called “ICE core-region”. When compared to a model ICEclc from P. knackmussii B13, ICE1 displayed higher degree of similarity over the whole “core” region (53 kb aligned at 86% nucleotide identity), whereas ICE2 - much lower (ca. 40 kb at 75% identity). Subsequent mating experiments with P. putida UWC1 as recipient revealed that both ICEs are currently active that is capable of self-transfer and self-integration and, interestingly, their integrases displayed both conservatism (integration site preferences) and flexibility (integration into less than 100% conserved DNA targets). The distinct and unrelated “cargo” genes carried on the two ICEs from P. azelaica and their mosaicism imply ICEs' divergent evolution trajectories on one hand and their access to the communal gene pool of several classes of Proteobacteria, on the other.

O-95

Environmental Microbiology, Session 3 / A-4

TRACKING BIOTRANSFORMATION OF HEXACHLOROCYCLOHEXANE ISOMERS BY COMPOUND-SPECIFIC ISOTOPE ANALYSIS

I. E. SCHILLING[1 2], T. B. HOFSTETTER[1], T. B. HOFSTETTER[2], H.-P. E. KOHLER[12]

[1]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Duebendorf, Switzerland, [2]IBP, Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Swiss Federal Institute of Technology Zurich, Zurich, Switzerland

Hexachlorocyclohexanes (HCHs) were excessively used as pesticides all over the world during the second half of the last century in agriculture and medicine. The persistence of HCHs led to their frequent detection as diffuse pollutants and point sources. In this work, we explore the use of compound-specific isotope analysis for assessing the extent and pathway of HCH biodegradation.
Degradation experiments are done with Lin enzymes isolated from HCH degrading soil bacteria of the Sphingomonadaceae family. First experiments with the enzyme LinA2, a dehydrochlorinase that catalyzes the first and second step in the metabolism of HCH, and γ-HCH as a substrate show that at high and low turnovers, C-isotope fractionation can be detected in substrate, intermediate and product. The apparent kinetic isotope effect (AKIE) for this elimination reaction is 1.019. Further experiments will be conducted with other enzymes from the Lin family and other isomers to not only determine AKIEs for the overall reaction but also model the AKIES for the single steps in the degradation. Current work also focuses on adapting and improving the experimental setup for model systems, in order to quantify hydrogen and chloride leaving group isotope effects.

O-96

Environmental Microbiology, Session 4 / A-5

BACTERIAL IRON REDUCTION PROPERTIES FOR HEALING CORRODED IRON ARTEFACTS

WAFA KOOLI[1], PILAR JUNIER[1], EDITH JOSEPH[1]

[1]Laboratory of Microbiology, University of Neuchâtel, Neuchâtel, Switzerland

In cultural heritage and especially metal conservation, conservators are particularly concerned with the phenomenon of corrosion of iron and its alloys, which are the most represented substrates for metal artworks. In fact, the preservation of archaeological iron artefacts encounters severe obstacles after excavation when salts containing chloride ions contaminate the corrosion crust surrounding the objects. As a result a loose of shape is observed on the objects. Research on iron conservation has focused on interventions aiming at controlling alterations on corroded/archeological iron objects. However, conventional methods of iron conservation present some disadvantages (i.e. toxicity, cost, durability). Therefore, exploiting microbial metabolisms that are environmentally friendly processes is an alternative for the stabilization of iron artefacts. To this purpose, this study focuses on the capacities of the bacterium Shewanella loihica, to stabilize iron corrosion and prevent further damage on the artefacts. This protection can be achieved by iron reduction and thus by forming stable iron biominerals with a low molar volume. These biominerals will be embedded in the corrosion layers increasing their porosity and allowing the release of chloride ions, which will be extracted from the object for example by osmotic diffusion.
The experiments performed aimed at evaluating the iron reduction abilities of S. loihica and investigating iron biominerals production from soluble and solid iron(III) phases. SEM-EDX analyses demonstrated that different compounds precipitated depending on the iron(III) phase and culture conditions used. In particular with soluble iron(III) citrate, the presence of a crystal phase, probably vivianite, was identified with FTIR analysis. While treatment of iron plates corroded under marine environment, a crystalline phase was also observed with SEM-EDX and identified as siderite and iron phosphate biominerals with XRD analysis.
The next step is to assess a stratification analysis of these iron plates and eventually the term stability of the resulting biomineral layer.

O-97

Environmental Microbiology, Session 4 / A-2

INFLUENCE OF ENVIRONMENTAL FACTORS ON SOIL MICROBIAL DIVERSITY AT ARABLE- AND GRASSLAND SITES ACROSS SWITZERLAND

F GSCHWEND[1], F WIDMER[1]

[1]Molecular Ecology, Institute for Sustainability Sciences, Agroscope, 8046 Zürich, Switzerland

Soil samples (N=152) originating from 9 different Swiss cantonal soil monitoring surveys were analysed using an amplicon-based metabarcoding approach, i.e. PCR-based amplification and high-throughput sequencing of genomic markers. We assessed alpha and beta diversity of soil bacterial and fungal communities and statistically tested the contribution of several environmental factors to their distinction. DNA extraction yielded concentrations from 7 to 198 µg/g dry soil. Bacterial and fungal diversities were detected by amplicon sequencing of the ribosomal SSU (V3-V4) and ITS2 regions, respectively. After thorough sequence filtering, we obtained at least 4078 bacterial and 9725 fungal sequences per sample. Clustering operational taxonomic units (OTU) at 97% sequence identity resulted in a total of 6944 fungal and 11258 bacterial OTU, corresponding to 317-472 and 881-1349 OTU per sample. Arable land harboured less fungal, but greater bacterial richness in comparison to grassland sites. Carbon content, pH, and other environmental variables revealed only weak correlations to fungal and bacterial alpha diversity. However, beta diversities, i.e. diversity of microbial community structures among sites, revealed strong responses to different factors. DISTLM analysis indicated that pH, land-use, clay content, and altitude were the most important forces shaping both bacterial and fungal community structures in our dataset. With 22.7% of total variance, pH was the strongest factor for bacterial communities, while fungal communities lacked such a strongly influencing factor. For them, land-use was the most important factor explaining 5.2% of the total variance. A canonical analysis of principal coordinates (CAP) showed that arable land and grassland harboured distinctly structured microbial communities. Reclassification of single samples to the two land-use types yielded 96.7% and 96.1% successful classifications for bacterial and fungal communities, respectively. These results build a step towards the establishment of a baseline for classifying soil microbial diversity in Swiss land-use types and the development of a soil biomonitoring system based on amplicon sequencing.

O-99

Environmental Microbiology, Session 1 / A-1

SNOW IS MORE THAN FROZEN WATER – THE BACTERIAL BIOGEOGRAPHY OF HIGH ALTITUDE SNOW

T WUNDERLIN[1], B FERRARI[2], M POWER[3]

[1]Molecular Ecology, Agroscope Reckenholz, 8046 Zurich, Switzerland, [2]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney 2052, Australia, [3]Department of Biological Sciences, Macquarie University, Sydney 2109, Australia

Approximately 12% of the Earth's surface is seasonally snow-covered, yet little is known about microbial life in snow. The long-standing hypothesis of snow being near sterile has recently been challenged by demonstrations of actual microbial activity in snow. These findings on the one hand are surprising, because the extreme environmental conditions make life in snow very challenging. On the other hand, the existence of active snow microbes is coherent with the fact that every living organism today has ancestors who survived during Snowball earth.
In this study, we explored abundance and diversity of bacteria in alpine snow and revealed biogeographic distribution patterns. For this, we collected surface snow from two sites in Switzerland (Jungfraujoch (n=2) and Rosstock (5)) and two sites in Australia (Mt. Kosciuszko and Thredbo(10;5)). Bacterial abundance was 6.9 ± 0.7 x 10^5 cells/ml, which corresponds to previously determined cell numbers in alpine snow. Biomass in melted snow was filtered, subjected to DNA extraction and Illumina sequencing (V1-V3 amplicon of 16S rRNA gene). Short of 1.8 Mio. sequences were obtained, resulting in 703 to 64'699 seqs/sample. Substantially diverse bacterial communities (up to 2286 taxonomic units) were detected, which spanned across 25 bacterial phyla, with Alpha- and Betaproteobacteria, Acido-, Cyano-, Actinobacteria, Bacteroidetes and Firmicutes, accounting for 72-98% of the abundance. Isolations of strains directly from snow were also performed and their distribution corresponded to the one obtained by amplicon sequencing, with exception of Gammaproteobacteria, which were most abundant among the isolates, but rarely detected in sequence data. Detailed analysis of community patterns show that there is a significant variation in alpine snow communities across hemispheres, regional and local positioning. Surrounding vegetation and exposition to wind seem to influence the bacterial community. In addition to athmospherically deposited bacteria our results suggest the existence of cosmopolitan taxa that have previously been associated with snow or cold environments, ie. there may be cold-adapted bacterial ecotypes in snow.

O-100

Prokaryotic Biology, Session 3: Cell biology – seeing is believing / A-1

CHARACTERIZING INDIVIDUAL DONOR CELL FATES DURING HORIZONTAL GENE TRANSFER OF AN INTEGRATIVE AND CONJUGATIVE ELEMENT

F DELAVAT[1], S MITRI[1], S PELET[1], JR VAN DER MEER[1]

[1]Department of Fundamental Microbiology - University of Lausanne - Switzerland

Integrative and Conjugative Elements (ICEs) are widespread mobile DNA. ICE are integrated in the host genome and are maintained through vertical descent. Occasionally, they excise and form a circular intermediate, which can conjugate to recipient cells and re-integrate into the genome at one or more specific sites. It is poorly understood what the consequences of such occasional excision and conjugation are for "donor" cells that harbor the ICE.
We have previously shown how a subpopulation of 3 to 5% of cells of Pseudomonas knackmussii B13 in stationary phase conditions becomes "transfer competent" (tc) for conjugation of an ICE called ICEclc. In this study, we developed a specific single-cell tracking tool to study the fate of individual donor cells during ICEclc transfer. The tool is based on differential activation of fluorescent proteins coupled to ICEclc promoters and can differentiate initial activation of tc development in cells, ICEclc excision, transfer and integration. Results of time-lapse microscopy from both donors alone or donors mixed with recipients showed that while ICEclc is activated when cells enter in stationary phase, excision and transfer predominantly occur when cells obtain new carbon substrate. Although tc donor cells can still divide a few times, division becomes very irregular and half of the individual tc donors arrest growth altogether or lyse. ICEclc also shows a variety of fates in donor cells: in some but not all cells ICEclc excises, in some it replicates during or after transfer, whereas in others it is lost. A probabilistic model was developed to explain the different cell fates. Interestingly, the model predicts lower ICE-fitness if the proportion of cells activating tc development increases from 5% to 20%, or if activation occurs already during growth and not in stationary phase. Our results highlight a new facet of ICE life-style and shed a new light on bacterial cellular differentiation during horizontal gene transfer.

O-103

Environmental Microbiology, Session 3 / A-3

BIOGEOGRAPHY IN THE WESTERN SWISS ALPS: UNDERSTANDING THE INTERPLAYS AMONG BACTERIA, PLANTS, AND THE ENVIRONMENT

E. YASHIRO[1], E. PINTO[2], A. BURI[3], H. NICULITA-HIRZEL[4], A. ANTOINE GUISAN[2], J. R. VAN DER MEER[1]

[1]University of Lausanne, Department of Fundamental Microbiology, 1015 Lausanne, [2]University of Lausanne, Department of Ecology and Evolution, 1015 Lausanne, [3]University of Lausanne, Institute of Earth Surface Dynamics, 1015 Lausanne, [4]Institut Universitaire Romand du Santé au Travail, 1066 Epalinges-Lausanne

The possible effects of climate change on biodiversity is of major concern for scientists, policy-makers, and laypeople. We are studying potential microbial biodiversity changes and co-occurrence patterns with plants and insects within a 700 km2 area of the Western Swiss Alps. Alpine top-soils were sampled across an elevation gradient of 500-3000 m at a hundred sites, and bacterial communities were characterized by sequencing of amplified 16S rRNA gene fragments. Extensive correlations were determined between a wide set of physico-chemical and soil parameters, and bacterial communities, in order to understand the major abiotic drivers shaping community structure. Correlations were further drawn between the occurrence of plant species and bacterial communities at the same sites, in order to understand possible interplays between bacteria, plants, and the environment. Our study helps to understand alpine biodiversity across wide elevational and spatial gradients, and can be used to support diversity models predicting future trends.

O-104

Mycology, Session 3 / A-4

HOW GENOMIC PLASTICITY CONTRIBUTES TO VIRULENCE EVOLUTION IN A FUNGAL PATHOGEN OF WHEAT

D CROLL[1], F HARTMANN[1]

[1]ETH Zurich, Institute for Integrative Biology, 8092 Zurich

Crop plants are colonized by a multitude of highly adapted fungal pathogens that show extraordinary adaptive potential to overcome host resistance and evolve tolerance to fungicides. Zymoseptoria tritici is the major fungal pathogen on wheat in Europe. Frequent sexual reproduction, the capacity to disperse and large population sizes are thought to be the main drivers of rapid evolution. However, how adaptive genetic variation is generated in the genome and how this variation contributes to virulence evolution in populations is poorly understood. A major challenge in understanding mechanisms generating genomic plasticity is the lack of complete genome assemblies. Hence, we assembled the genome of an isolate from a Swiss wheat field complementing the previously available reference genome. We used long-read technology and high-density genetic maps to produce accurate and complete chromosomal sequences. Comparative genomics analyses showed that core chromosomes of the newly assembled genome harbored extensive orphan regions not found in the reference genome. A subset of genes in orphan regions carried signatures indicative of a role in virulence. To understand the role genetic variation plays in virulence evolution at the population level, we performed a genome-wide association study (GWAS) of 106 whole-genome sequenced isolates. Localization of GWAS hits showed that differences in virulence were most likely caused by non-synonymous substitutions in genes encoding cell wall-degrading enzymes and deletion polymorphisms affecting a candidate avirulence gene. In conclusion, chromosomal plasticity and frequent recombination generated highly divers pathogen populations capable to rapidly gain or lose virulence loci in response to selection pressure imposed by the host.

O-105

Mycology, Session 4: Antifungal development / A-3

SELECTION, SEX AND SWEEPS; RESISTANCE EVOLUTION OF THE DMI TARGET GENE CYP51

PC BRUNNER[1], BA MCDONALD[1]

[1]ETH Zurich, Universitaetstrasse 2, 8092 Zurich

The rapid development of fungicide resistance in crop and human pathogens is an increasing global threat but surprisingly little is known about the underlying evolutionary processes. We evaluated early, mid and late stages in the evolution of DMI resistance using the wheat pathogen Zymoseptoria tritici, taking advantage of a global isolate collection spanning +30 years. We analyzed sequences of the nuclear CYP51 gene implicated in multiple-mutation resistance to DMI fungicides. Most surprisingly, we found an increasing genetic diversity – mainly attributable to the accumulation of adaptive mutations at DMI resistance sites. Results are discussed with particular regard to the roles of selection, soft/ hard-sweeps, and intragenic recombination as driving evolutionary forces.

O-107

Mycology, Session 2: Fungal Interactions / A-4

DIFFERENTIAL SUPPRESSION OF PATHOGENIC AND SAPROPHYTIC FUNGI BY NATURALLY OCCURRING YEASTS

F. M. FREIMOSER[1]

[1]Federal Department of Economic Affairs, Education and Research EAER, Agroscope, Institute for Plant Production Sciences IPS, Schloss 1, P.B., 8820 Wädenswil, Switzerland.

Fungi are the most important plant pathogens in soil and cause rot or wilt diseases by colonizing roots. Soilborne fungal pathogens are also notoriously difficult to control, because they can grow in soil and persist even in the absence of a suitable host. Infestations by aggressive, soilborne, fungal pathogens can constrain crop production and often the only resort is crop rotation, fallow, or an abandonment of the cropland. Due to the complexity of the soil microbiome, the detection of low pathogen numbers is often difficult and their management by fungicides is rarely effective and in many countries (including Switzerland) not permitted. Naturally occurring yeasts are an unused resource for the management of soilborne, plant pathogenic fungi. Our research therefore focuses on antagonistic and beneficial yeasts and their application for the control of plant pathogenic fungi in soil. We have quantified the antagonistic activity of 40 yeast isolates (a representative selection of a larger collection obtained from soil, leaves, and flowers) against a diverse group of 16 filamentous fungi (antagonists, pathogens, and saprophytes belonging to the Zygomycota, Basidiomycota and Ascomycota). This initial screen of over 600 interactions identified weakly and strongly antagonistic yeast isolates, as well as species with differential competitiveness against different filamentous fungi (e.g., plant pathogens and known antagonists). Further characterization of the most promising species (growth characteristics, metabolic activities, rhizosphere competence, and antagonistic activity in soil) links the in vitro results on the competitiveness of the 40 yeast species to specific phenotypic characteristics and the behavior in a complex environment. These studies are the foundation for elucidating modes of action and for identifying the most promising species for the management of soilborne fungal pathogens.

O-108

Mycology, Session 1: Fundamental mycology, Yeast Molecular Biology / A-3

CHARACTERIZATION OF A NEW VIRULENCE GENE IN CANDIDA ALBICANS

S AMORIM-VAZ[1], V TRAN[2], S PRADERVAND[2], M PAGNI[2], AT COSTE[1], D SANGLARD[1]

[1]Institute of Microbiology, University Hospital Lausanne and University Hospital Center, Lausanne, Switzerland, [2]Vital-IT Group, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland

Candida albicans systemic infections in immunocompromised patients result in over 40% fatalities, despite the available treatments. Novel therapeutic agents are needed, and specific mediators of pathogenesis could be used as targets. To better understand C. albicans pathogenesis mechanisms, a genetic screen was recently carried out to identify factors necessary to establish an infection in animal models (mice and larvae of Galleria mellonella). Among the genes identified as playing a role in virulence, we identified MBF1, a putative transcriptional regulator in C. albicans, and which identified was required for a successful systemic infection in both infection models. To identify potential MBF1 targets, we compared the gene expression profiles of a null mutant (mbf1Δ/Δ) and wild type strain SC5314, both in in vitro conditions and during systemic infection of mice and G. mellonella larvae. In vivo genome-wide transcriptional profiling was made possible by an RNA enrichment approach that allowed deep sequencing of C. albicans transcripts directly from infected animals. Our results suggested, among other observations, a connection between MBF1 and GCN4, since some known GCN4 targets were significantly regulated in the mbf1Δ/Δ mutant, such as GLN3, TTR1 or RHR2. It is known that GCN4 and MBF1 interact in Saccharomyces cerevisiae and this association is involved in regulation of nitrogen metabolism. Consistent with this hypothesis, the mbf1Δ/Δ mutant was sensitive to 3-amino-1,2,4-triazole (3-AT, a competitive inhibitor of the HIS3 product) as did a gcn4Δ/Δ mutant. Undergoing work includes co-immunoprecipitation (Co-IP) of Mbf1 and Gcn4, in order to verify whether a direct interaction exists between the two proteins. In addition, since Mbf1 is a putative transcriptional coactivatior of other regulators, we are currently determining other client proteins of Mbf1 by tandem affinity purification. In conclusion, we achieved the in vivo transcriptome of a mutant with reduced virulence, thus highlighting the utility of the fungal RNA enrichment from infected host tissues. MBF1 is being further characterized in order to clarify its involvement in C. albicans pathogenesis.

O-109

Mycology , Session 3 / A-1

TH17 CELLS AND THEIR ROLE IN PROTECTIVE IMMUNITY TO VULVOVAGINAL CANDIDIASIS

R MARZI[1], G GYUELVESZI[1], S BECATTINI[1], B BECHER[2], S LEIBUNDGUT-LANDMANN[3], F SALLUSTO[1]

[1]Institute for Research in Biomedicine, Università della Svizzera Italiana, Bellinzona, Switzerland, [2]Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland, [3]Section of Immunology, Institute of Virology, University of Zurich, Zurich, Switzerland

Vulvovaginal candidiasis (VVC) is the most common manifestation of C. albicans infection, affecting ~75% of healthy women worldwide during their childbearing age. Chronic mucocutaneous candidiasis (CMC) patients with mutations in genes associated with the IL-17 pathway display symptoms of VVC (in addition to oral candidiasis), but whether the disruption of the IL-17 pathway is a direct cause for VVC or rather an indirect effect due to the general increase in fungal colonization in these patients is unclear. Mice lacking IL-23, either IL-17R subunit or the adaptor Act1, are susceptible to oral and dermal candidiasis. However, IL-17RA–/– and IL-23–/– mice are not susceptible to vaginal infection. These previous studies analyzed only the early phases of the host-pathogens interplay, which is generally life-long in humans. In this study, we set out kinetic experiments to assess the relative contribution of Th17 cells in persistent VVC. We found that wild type (WT) mice, CD3ε–/– mice, lacking T cells, and μMT mice, lacking B cells, rapidly recovered from infection. However, while WT and μMT mice survived even at later time points (>90 days), completely recovering from the infection, CD3ε–/– mice succumbed to the challenge. CFU assessment from collected organs revealed massive C. albicans presence in vagina, ovary/uterus, gut and stomach in CD3e–/– mice, implicating failure of containment strategies. In order to define the mechanism of T cell-mediated protections, we adoptively transferred CD3ε–/– mice with WT, IL-22–/– or IL-17–/– CD4+ T cells before vaginal infection. CD3ε–/– mice transferred with WT or IL-22–/– CD4+ T cells, that produce normal amount of IL-17, survived upon challenge, while mice transferred with IL-17–/– CD4+ T cells succumbed at late time points as CD3ε–/– mice. We are currently performing experiments of adoptive transfer with Candida-specific TCR-transgenic T cells to follow T cell differentiation and assess T cell plasticity. Our results suggest that, although not required in the initial phase of the challenge, IL-17 production by Th cells is required for long-term containment of C. albicans vaginal infection.

O-111

Mycology, Session 1: Fundamental mycology, Yeast Molecular Biology / A-1

DYNAMIC MEASUREMENT OF KINASE ACTIVITY IN LIVE SINGLE CELL

ED DURANDAU[1], MM MA[1], DA AYMOZ[1], SP PELET[1]

[1]Département de Microbiologie Fondamentale

The phosphorylation of signaling cascade components is a key post-translational modification used in signal transduction. Although all kinases catalyze this bio-chemical reaction, each individual kinase possesses its specific pool of substrates. Moreover, the temporal activity of these enzymes varies from cell to cell depending on intra- or extra-cellular cues. Knowing that kinase mis-regulation is implicated in many diseases, such as cancer, it becomes crucial to quantify the heterogeneity of the dynamic kinase activity at the single cell level. Micros- copy is an ideal technique for such investigation. However, fluorescent assays have to be established to quantify this enzymatic activity in living cells.
Using a synthetic biology approach, we designed a fluorescent biosensor that undergoes nuclear-to-cytoplasmic relocation upon phosphorylation by the kinase of interest. Combination of time-lapse microscopy measurements and automated image analysis allows the quantification of the dynamics of kinase activity in hundreds of single cells. As proof of concept, we generated a Synthetic Kinase Activity Relocation Sensor (SKARS) for Mitogen Activated Protein Kinases (MAPK) of the mating pathway in S. cerevisiae. The kinetics of the MAPK acti- vation exhibits a large heterogeneity between single cells due to an inhibition of signal transduction at specific stages of the cell-cycle. The modularity of the architecture of the SKARS allows us to expand the range of kinases that we can monitor in yeast, providing biosensors for all MAPKs as well as cyclins. In addition, based on the same sensor structure, we also developped reporters for the mammalian MAPKs ERK, JNK and p38.

O-114

Mycology, Session 3 / A-2

IMPACT OF NATURAL DIVERSITY OF C.ALBICANS ON THE BALANCE BETWEEN FUNGAL COMMENSALISM AND PATHOGENICITY IN VIVO

F SCHÖNHERR [1], C FRAGOSO-CORTI [2], O PETRINI [3], S LEIBUNDGUT-LANDMANN [1]

[1]Section of Immunology, Vetsuisse Faculty, University of Zürich, Winterthurerstrasse 266a, 8057 Zürich, Switzerland , [2]Laboratory of Applied Microbiology, DACD, SUPSI, Via Mirasole 22A, 6500 Bellinzona, Switzerland , [3]POLE Pharma consulting, Breganzona, Switzerland

Candida albicans is a member of the normal human microbiota, but as an opportunistic pathogen it can also cause severe infections in immunocompromised individuals. It is generally believed that the host immune status alone determines the outcome of the interaction between the commensal fungus and the host, resulting in either health or disease. Interleukin 17 (IL-17)-mediated immunity has emerged as a critical mechanism of the host to regulate the antimicrobial response, thereby limiting fungal overgrowth at the epithelial barriers. Complementarily, neutrophils contribute to host defense by preventing systemic dissemination of the fungus. Whether in addition to host factors, differences in C. albicans that exist between individuals may also contribute to disease development remains unclear. We used the well-established mouse model of oropharyngeal candidiasis (OPC) to probe the host response to diverse natural isolates of C. albicans in a uniform and Candida-naïve host environment. The isolates displayed no gross differences in growth, hyphenation, drug resistance or cell wall composition in vitro, but they triggered highly variable degrees of inflammation in vivo. The weak and delayed induction of IL-17 and antimicrobial peptides by some isolates correlated with their persistence in the mucosal epithelium. Importantly, however, the requirement of IL-17 for preventing fungal outgrowth was conserved with all isolates tested, highlighting the key role of this cytokine in host protection from C. albicans. Differences in the host response induced by the diverse isolates in vivo was reflected by their capacity to induce the release of ‘alarmins’ such as IL-1α from keratinocytes. This supports the notion that the epithelium can sense variations in the fungus and translate them into host signals that mediate fungal clearance or persistence. This study demonstrates the relevance of the natural diversity of C. albicans for determining the fine balance between commensalism and pathogenicity in vivo.

O-118

Mycology, Session 3 / A-3

THE ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS DURING SYSTEMIC C. ALBICANS INFECTION

E GUIDUCCI [1], S LEIBUNDGUT-LANDMANN[1]

[1]Section of Immunology, Vetsuisse Faculty, University of Zürich, Switzerland

NETs are web-like structures composed of decondensed chromatin and antimicrobial substances, which restrain and subsequently kill pathogens, including bacteria and fungi. NET formation is associated with citrullination of histone H3 by peptidyl arginine deiminase, type IV (PAD4), histone degradation by neutrophil elastase (NE) and myeloid peroxidases (MPO)-driven chromatin decondensation. PAD4 is expressed prominently in mature neutrophils and it has been shown to be central for NET formation, as PAD4 knockout mice show abolished induction of histone citrullination and fail to form NETs.
C. albicans hyphae (but not C. albicans yeast) can efficiently induce NET formation, as in vitro studies have shown. The observation that Candida hyphae are the predominant morphotype found in infected organs and that these structures are too large to be phagocytosed efficiently, suggests that NETs may also be relevant during infection in vivo. However, the contribution of NETs to fungal control and host protection has not been clarified. Using a mouse model of systemic candidiasis, we analysed citrullination of histone H3 in neutrophils in the infected kidney, the main target organ of C. albicans during infection, and found NETs to be strongly induced. Their abundance increased over time, paralleling the increase in neutrophil numbers and the presence of fungal hyphae. The relevance of NET induction during systemic candidiasis is also addressed in this project.

O-119

Mycology, Session 4: Antifungal development / A-1

IDENTIFICATION OF A PLANT NATURAL PRODUCT TARGETING FUNGAL HUMAN PATHOGENS

S DORSAZ[1], Q FAVRE-GODAL[2], E FERREIRA QUEIROZ[2], K GINDRO[3], H RIEZMAN[4], J-L WOLFENDER[2], D SANGLARD[1]

[1]Institute of Microbiology, University Hospital of Lausanne, Lausanne, Switzerland, [2]School of pharmaceutical sciences, University of Geneva, University of Lausanne, Switzerland, [3]Swiss Federal Research Station, Agroscope Changins Wädenswil ACW, Switzerland, [4]Department of Biochemistry, University of Geneva, Switzerland

Candida infections are a major cause of fungal diseases in humans and their increasing resistance against available drugs is of major concern. There is an urgent need for the development of new antifungals and plant natural products may represent a potential reservoir for new discoveries.
We initiated a small scale screening of a library of natural compounds (NPs) extracted from plants(1). We submitted the selected compounds to diverse in vitro profiling experiments including antimicrobial susceptibility test (MIC), activity spectrum against different Candida and bacterial species as well as toxicity assays with mammalian HeLa cells. Over 240 compounds that were screened so far, 41 exhibited in vitro activities against C. albicans (MIC50 ≤ 32 µg/ml). Among these NPs, we identified one promising substance, the sterol alkaloid tomatidine from Solanum lycopersicum (tomato). It showed high activity against Candida spp. such as Candida albicans (MIC50 = 1 µg/ml) and the azole-resistant Candida krusei (MIC50 = 0.5 µg/ml), but no activity on E. coli species (MIC50 > 100 µg/ml) and low toxicity against mammalian cell culture (lethal dose 50 (LD50) > 100 µg/ml).
It has been earlier reported than tomatidine has an antifungal activity against Saccharomyces cerevisiae in vitro and it may interfere with the ergosterol biosynthetic pathway (2). Sterol profile analysis of tomatidine-treated Candida cells showed that two enzymes involved in ergosterol synthesis, ERG6 (24-C methyl transferase) and ERG4, were inhibited.
RNA-seq analysis of cell treated with tomatidine revealed that the drug induced several ERG genes, ERG6 being the most upregulated gene (9.5 times). Several other genes were upregulated, especially those belonging to the TAC1 regulon.
In vivo efficacy of the drug is under investigation in two different Candida infection models, the mini host wax moth Galleria mellonella model and the murine systemic infection model.

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(2) Simmons, V, et al (2006). AAC. 50 :2732.

O-120

Mycology, Session 4: Antifungal development / A-2

IDENTIFICATION OF MEDIATORS OF ANTIFUNGAL TOLERANCE IN CANDIDA ALBICANS

E DELARZE[1], S ZNAIDI[2], M LEGRAND[2], M ARENDRUP[3], C MUNRO[4], C D'ENFERT[2], D SANGLARD[1]

[1]University of Lausanne and University Hospital Center, Lausanne, SWITZERLAND, [2]Pasteur Institute, Paris, FRANCE, [3]Statens Serum Institut, Copenhagen, DENMARK, [4]University of Aberdeen, Aberdeen, UNITED KINGDOM

Antifungal tolerance can be defined as the ability of C. albicans cells to survive high drug concentrations without acquiring mutations associated with resistance. The study of drug tolerant strains may reveal factors contributing to treatment failures and facilitate the design of improved therapies. In this study we aimed to identify mediators of tolerance to fluconazole (FLC), using two different approaches. The first was to assess the tolerance profile of 26 clinical C. albicans isolates against FLC using the EUCAST drug susceptibility assay. Tolerant strains were identified by their ability to sustain residual growth after 24 h at a drug concentration above the FLC resistance breakpoint. This screening revealed different profiles grouped from low to high tolerance. These FLC tolerant strains are candidates for further characterization by genetic and transcriptomic approaches and will be used to test the importance of drug tolerance in animal models. In a second approach, in order to identify genetic mediators of tolerance, we used a collection of 582 tetracycline-inducible overexpression barcoded strains, which were pooled and maintained under FLC pressure for five days of repeated subculture. This strategy was used to enrich the pool in FLC resistant and/or tolerant strains. After amplification and sequencing of all barcodes from the cultures, the fractional index of each strain was calculated. This analysis yielded 53 strains that were at least 2-times more enriched under FLC pressure than in conditions without FLC or ORF overexpression. The FLC tolerance profile of these strains will be tested individually.

O-122

Mycology, Session 4: Antifungal development / A-4

BIOCONTROL OF DIABROTICA VIRGIFERA VIRGIFERA IN MAIZE – ASSESSING EFFECTS OF BIOCONTROL AGENTS ON SOIL MICROBIAL COMMUNITIES

J MAYERHOFER[1], H RAUCH[2], CO PABST[3], A LEUCHTMANN[4], M HARTMANN[5], H STRASSER[3], F WIDMER[1], J ENKERLI[1]

[1]Molecular Ecology, Institute for Sustainability Sciences, Agroscope, 8046 Zurich, Switzerland, [2]Research Centre for Agriculture and Forestry Laimburg, 39040 Auer, Italy, [3]Institute of Microbiology, Leopold-Franzens University Innsbruck, 6020 Innsbruck, Austria, [4]Plant Ecological Genetics, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland, [5]Forest Soils and Biogeochemistry Rhizosphere Processes, Swiss Federal Research Institute WSL, 8903 Birmensdorf, Switzerland

A major part of the terrestrial biomass is formed by microbial communities. Soil microbial communities have a great impact on nutrient cycling, soil fertility and plant health, representing important ecosystem services for productive and sustainable agriculture. Biological and chemical plant protection treatments are well accepted measures for pest control, however, the impact particularly of biological control agents on soil microbial communities has been poorly investigated. In this study potential effects of two biological control treatments and an insecticide were assessed in a pot experiment with maize and Diabrotica virgifera virgifera as pest insect. One biological control agent was the entomopathogenic fungus Metarhizium brunneum, which was formulated as fungal colonized barley kernels or as fungal alginate capsules and the other one was a suspension of the entomopathogenic nematode Heterorhabditis bacteriophora. The pot experiment was performed in a greenhouse and consisted of eight treatments with six replicates per treatment.
Treatments including FCBKs led to a significant increase of Metarhizium spp. (3-8 x 104 CFU g-1 soil dry weight compared to about 103 CFU in untreated pots), which provided an ideal system to study effects of the applied Metarhizium brunneum strain on soil microbial communities. In order to assess microbial community composition, soil samples were collected from each pot before application, and 9 and 18 weeks after application. Soil genomic DNA was extracted and the internal transcribed spacer region 2 (fungi) and the variable regions V3-V4 of the small ribosomal subunit (prokaryota including bacteria and archaea) were amplified and sequenced using the Illumina MiSeq platform. The community structure of fungi changed over time, but no effects of the treatments were observed. Prokaryotic communities also changed over time, however, they were also affected by the application of FCBKs, nematodes as well as their combination 9 weeks after application. Eighteen weeks after application the effects had decreased suggesting resilience of the communities.

O-123

Mycology, Session 2: Fungal Interactions / A-2

ROOT MICROBIOME DYNAMICS IN RESPONSE TO PHOSPHATE

N BODENHAUSEN[1], M VAN DER HEIJDEN[1], K SCHLAEPPI [1]

[1]Institute for Sustainability Science, Agroscope, Switzerland, Reckenholzstr. 191, 8046 Zurich

Plants require phosphorus (P) for growth. A small fraction of soil P is directly available for plant growth while the larger fraction is complexed to minerals and inaccessible for plants. Farmers optimize their yields by fertilizing their fields with plant-available phosphate. This P pool, however, is a limited resource and the world reserves will be depleted within the next 50 to 100 years. Therefore, understanding how plants tap the complex P fraction is crucial. Microbes - mainly bacteria - can support plants to access this P source in soil through their capacity to solubilize complex soil P. In addition, symbiotic arbuscular mycorrhizal fungi (AMF) connect to plant roots and through their large hyphal network they allow plants to access a larger soil volume and distant sources of P. The P availability in soils determines the extent of the symbiosis between AMF and their host: in soils with low P, plants are heavily colonized by the fungus and little in soils with high P. If the P availability in soil is low, plants secrete a hormone called strigolactone, which attracts and promotes the symbiotic relationship with AMF. In this study, we investigated the interplay between bacteria and fungi that are associated with the roots of Petunia plants as a function of the P availability and strigolactone exudation using 16S rRNA gene profiling. We found differentially abundant OTUs both under the high P and low P conditions; interestingly, we found little overlap of P-dependent OTUs between Petunia, which is colonised by AMF, and Arabidopsis, a plant that does not form symbiosis with AMF.

O-124

Mycology, Session 2: Fungal Interactions / A-3

ENHANCED ACCESS TO ORGANIC NITROGEN AS A DIRECT BENEFIT IN THE INTERACTION OF THE FUNGUS MORCHELLA CRASSIPES WITH SOIL BACTERIA

A. LOHBERGER (1,3 - AFFILATION NUMBERS)[1], JE SPANGENBERG[2], Y VENTURA[1], N JEANNERET[1], K JEANNERET[1], S BINDSCHEDLER[1], R BSHARY[4], E VERRECCHIA[3], P JUNIER[1]

[1]Laboratory of Microbiology, Institute of Biology, University of Neuchâtel. Rue Emile-Argand 11, 2000, Neuchâtel, Switzerland, [2]Institute of Earth Surface Dynamics, University of Lausanne, Géopolis, 1015 Lausanne, Switzerland, [3]Laboratory of Biogeosciences, Institute of Earth Sciences, University of Lausanne. Géopolis, 1015 Lausanne, Switzerland, [4]Laboratory of Eco-ethology, Institute of Biology, University of Neuchâtel. Rue Emile-Argand 11, 2000, Neuchâtel, Switzerland

Fungi and bacteria have a long co-evolutionary history in terrestrial ecosystems. In soils, they are the basis for nutrient recycling. Therefore, not only do they co-exist, but they are also potential competitors for resources. In our laboratory, it was shown that the bacterium Pseudomonas putida benefits from the interaction with fungi by using the so-called “Fungal Highways” to disperse in porous unsaturated soil-like media. Dispersal allows this bacterium to reach inaccessible nutrient sources, as its mobility is limited in soil-like conditions (1, 2). However, the direct fitness benefit for the fungi remains unknown. Until now, a long-term benefit was shown for the fungus Morchella crassipes, which acted as a farmer of the dispersing bacteria and stored bacterial carbon into resting structures (2). However the role of other trophic exchange mechanisms favouring fungal highways remains unknown. In terms of organic nitrogen exploitation we have determined that fungal proteolysis can be enhanced by dispersal of P. putida and this is not the result of direct competition as it occurs even when fungal cells are removed, but living bacterial cells are required. We tested if a mutual benefit was dependent on a given nitrogen/carbon (N/C) ratio. When organic nitrogen and carbon, or just the former, were highly available, bacteria were not farmed by the fungi and thus benefited from dispersal and nutrient acquisition. The fungus also gained from the interaction when nitrogen and carbon were highly available, but when the C/N ratio was higher, fungal biomass decreased. Our results show that the sharing of the benefits of fungal-driven bacterial dispersal is strongly context-dependent and that nutrient acquisition might be the driving factor of this type of bacteria-fungi interaction.

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2. Pion M, Spangenberg JE, Simon A, Bindschedler S, Flury C, et al. Bacterial farming by the fungus Morchella crassipes2013 2013-12-22 00:00:00.

O-125

Mycology, Session 2: Fungal Interactions / A-1

HIGH-RESOLUTION COMMUNITY PROFILING OF ARBUSCULAR MYCORRHIZA

K SCHLAEPPI[1], F BENDER[1], M VAN DER HEIJDEN[1]

[1]Plant-Soil-Interactions, Institute for Sustainability Sciences, Agroscope, Zürich, Switzerland

Community analyses of arbuscular mycorrhizal fungal (AMF) using ribosomal small subunit (SSU) or internal transcribed spacer (ITS) DNA sequences often suffer from low resolution or coverage. We developed a novel sequencing based approach for a highly resolving and specific profiling of AMF communities. We took advantage of AMF-specific PCR primers that amplify an ~1.5 kb long fragment covering parts of SSU, ITS and parts of the large ribosomal subunit (LSU) and we sequenced the resulting amplicons with single molecule real-time (SMRT) sequencing. The method was applicable to soil and root samples, detected all major AMF families and discriminated successfully closely related AMF species, which would not be discernible using SSU sequences. In inoculation tests we could separate the strain of the introduced AMF species (Rhizoglomus irregulare) from the strains of the native community, indicating resolution at strain level. Further, the introduced strain almost replaced the local strain(s) revealing that AMF inoculation can have a profound impact on the native community. The presented methodology offers researchers a powerful new tool for AMF community analysis because it unifies high specificity and enhanced resolution while the drawback of medium sequencing throughput appears of lesser importance for low-diversity groups such as AMF.

O-127

Mycology, Session 1: Fundamental mycology, Yeast Molecular Biology / A-2

NGS FOR "NO-PAIN GENETIC SCREENS": USING TRANSPOSONS AND NEXT-GEN SEQUENCING TO UNVEIL ALL IMPORTANT YEAST LOCI IN ONE GO.

A.H MICHEL[1], P. KIMMIG[1], B. KORNMANN[1]

[1]ETHZ, Institute for Biochemistry/ Otto-Stern-Weg 3, 8093 Zürich

Yeast genetic screens have been absolutely instrumental in our understanding of cell biology. Yet they remain tedious and oftentimes incomplete. Next generation sequencing on the other hand is fast and exhaustive. I have implemented a transposon-based approach combined with deep sequencing to define the complete set of genes that are essential for growth in a particular condition, in one go. The idea is to saturate the yeast genome with independent transposon insertions. Transposons cannot insert in genes that are essential in a given condition. Deep-sequencing of the transposon-genome junctions of the whole library identifies the locations that tolerate the presence of the transposon and allows to deduce those that cannot, revealing the corresponding set of essential genes. The method is readily applicable to multiple growth conditions for comparison. Genes that are essential in one condition and not in another are expected to play a condition-specific physiological role. In addition to identifying essential genes, the method reveals essential protein domains at an unprecedented throughput and resolution.

O-128

Mycology, Session 4: Antifungal development / A-5

DE NOVO ASSEMBLY OF THE PNEUMOCYSTIS JIROVECII SUBTELOMERIC GENE FAMILIES ENCODING VARYING SURFACE ANTIGENS

E SCHMID-SIEGERT [1], S RICHARD [2], M PAGNI[1], PM HAUSER[2]

[1]Vital-IT Group, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland , [2]Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

Pneumocystis jirovecii is an obligate parasite that causes severe pneumonia in immuno-compromised individuals. The lack of culture in vitro for this fungal pathogen complicates its study. Using high throughput sequencing and a dedicated bioinformatics strategy, we recently succeeded in assembling the P. jirovecii genome from a single bronchoalveolar lavage fluid specimen from a patient. However, telomeres could not be assembled because of the repetitive nature of both their sequences and the subtelomeric gene families encoding varying surface antigens. In the present work, we used single molecule real-time sequencing and dedicated bioinformatics to assemble the P. jirovecii telomeres from a patient infected with a single strain. Three different families of genes encoding isoforms of the major surface glycoprotein were identified: (i) classic, (ii) without the 5' conserved sequence, and (iii) pseudogenic. A single classic gene would be expressed in each cell thanks to its localization downstream of a subtelomeric expression site which is present at a single copy in the genome. The localization of the classic genes within the proximal regions of the subtelomeres suggests that exchange of telomeres' ends through recombination at the 5' conserved sequence might be responsible for changes of the expressed isoform. The absence of the 5' conserved sequence in the second family, and the localization of this family in distal regions of the subtelomeres suggest that these genes are not expressed but might be involved in recombination events creating mosaic classic genes. The studied population of P. jirovecii cells appeared to share a core of telomeres which provided the isoform present at the expression site that we identified by PCR in several subpopulations. Other families of genes encoding other surface proteins were also present in the subtelomeres. The expression of the latter genes remains to be characterized.

O-137

Prokaryotic, Session 1: The envelope – from walls to molecules (continuation) / A-5

IDENTIFICATION OF THE ENTIRE GENOMIC PROTEIN CODING POTENTIAL OF PROKARYOTES

U OMASITS[1], AR VARADARAJAN[1], DJ STEKHOVEN[2], M QUEBATTE[3], M ROBINSON[2], C DEHIO[3], CH AHRENS[2]

[1]Agroscope, Institute for Plant Production Sciences, 8820 Wädenswil, Switzerland, [2]SIB, Swiss Institute of Bioinformatics, 8057 Zurich, Switzerland, [3]Biozentrum, Infection Biology Unit, University of Basel, 4056 Basel, Switzerland

Large advances in functional genomics technologies have enabled the description of complete transcriptomes with RNA-Seq. In contrast, the study of complete proteomes has largely lagged behind. We have previously devised a generic strategy to describe complete, condition-specific expressed prokaryotic proteomes using Bartonella henselae as a model system [1]. Applying this strategy under two conditions that mimic interaction of B. henselae with its major hosts led to the description of a - to our knowledge for the first time - complete membrane proteome, including expression evidence for all members of the VirB/D4 type IV secretion system (T4SS).

This unique dataset was further exploited to determine the predominant subcellular localization of more than 90% of the expressed proteins [2]. Importantly, the high quality proteomics data from this large dataset provided evidence for the expression of a number of short, unannotated ORFs. As the annotations of different reference genome annotations can largely differ, we have developed a generic proteogenomics strategy, that allows researchers to discover the full coding potential of prokaryotic organisms. So far, we were able to uncover missed protein-coding ORFs in each prokaryote tested.

We discuss the manifold implications of this generic approach, up to the identification of coding differences from clinical genomes sequenced and assembled from long PacBio reads, a capability urgently needed for personalized medicine.

[1]. Omasits U et al., Genome Research 2013, 23:1916-1927.
[2]. Stekhoven DJ, Omasits U et al., J Proteomics 2014, 99:123-137.

O-139

SSM Annual Meeting 2016, Session 8: Microbe replication / A-1

CHARACTERIZATION OF NOVEL MEMBERS OF THE CHLAMYDIAL DIVISOME THAT LINK SEPTUM FORMATION AND PEPTIDOGLYCAN REMODELLING

N JACQUIER[1], PH VIOLLIER[2], G GREUB[1]

[1]Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland, [2]Department of Microbiology & Molecular Medicine, Institute of Genetics & Genomics in Geneva (iGE3), Faculty of Medicine / CMU, University of Geneva, Switzerland

Chlamydiales are obligate intracellular bacteria, such as Chlamydia trachomatis and Chlamydia pneumoniae, which are important human pathogens. All Chlamydiales share a common peculiar division mechanism, which is independent of the bacterial division organizer FtsZ, a bacterial homologue of tubulin, as an FtsZ-encoding sequence is absent from all the chlamydial genomes sequenced up to date. Nevertheless, Chlamydiales divide by binary fission in a process that highly resembles the peptidoglycan(cell wall)-driven constriction of other bacteria. Using the Chlamydia-related bacterium Waddlia chondrophila as a model, we recently provided pharmacological and cytological evidence for a role of the actin homologue MreB and its regulator RodZ in cell division of W. chondrophila (1). We could also show that the Pal-Tol complex involved in integrity maintenance of the link between the inner and the outer membrane is conserved in Chlamydiales and is highly enriched at the division septum (2). In this study, we further investigated the link between septum formation by RodZ and MreB and peptidoglycan synthesis and remodelling. For this purpose, we initiated several screens to find potential interactors of both RodZ and peptidoglycan. Using immunoprecipitation and mass spectrometry analysis, we could identify several candidates that are conserved in all Chlamydiales and that might play a role in the coordination of septum formation and peptidoglycan remodeling. We could confirm that some of these candidates (i) localize to the division septum, (ii) directly bind peptidoglycan and (iii) cause division defect when overexpressed in an E. coli system, highlighting their role in bacterial division. We need now to investigate if these proteins have a structural effect on peptidoglycan remodeling and how their recruitment to the division septum is regulated. These proteins can be potential targets for new antibiotics specifically targeting Chlamydiales, avoiding development of resistance in other bacteria.

1. Jacquier N, Frandi A, Pillonel T, Viollier PH, Greub G. Nat Commun. 2014 Apr 8; 5:3578.
2. Jacquier N, Frandi A, Viollier PH, Greub G. Chem Biol. 2015 Sep 17; 22(9):1217-27.

O-140

Prokaryotic Biology, Session 1: The envelope – from walls to molecules / A-2

CHARACTERIZATION OF ABERRANT BODIES SUBTYPES IN WADDLIA CHONDROPHILA

A. SCHERLER[1], N. JACQUIER[1], G. GREUB[1]

[1]Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland

Waddlia chondrophila is an obligate intracellular bacteria belonging to the Chlamydiales order which is associated with abortion in ruminants and miscarriage in humans. Members of the Chlamydiales order are characterized by a biphasic developmental cycle involving adhesion and internalization of elementary bodies, which differentiate into proliferative reticulate bodies. In presence of stress stimuli such as β-lactam antibiotics, interferon-gamma or nutrient starvation, reticulate bodies proliferation is inhibited, inducing the formation of enlarged and persistent aberrant bodies (ABs). The removal of these stress stimuli allows the bacteria to re-enter the normal developmental cycle. From these observations, ABs are thought to play an important role in chronic infections due to Chlamydiales. Nevertheless, the link between chronic infections and presence of ABs has yet to be demonstrated. The aim of this study was to describe W. chondrophila ABs formation, their morphology and potential differences in DNA, RNA and protein content following treatment with different stimuli. ABs morphology, DNA replication and transcription pattern were assessed by immunofluorescence, quantitative PCR and reverse transcription PCR. In this study, we observed two main subtypes of ABs that exhibited different morphologies and number per host cell. Furthermore, we showed that DNA replication of W. chondrophila was differentially affected by the different stimuli. Finally, mRNA expression patterns of important division proteins (MreB and RodZ) could be classified in distinct groups according to the different expression of their encoding genes. These results indicate that distinct subtypes of ABs are induced by the various stimuli, potentially reflecting different mechanisms of ABs formation. Further studies are now needed to increase our knowledge on this persistent stage, and consequently, to improve therapeutic options against chronic chlamydial infections.

O-142

SSM Annual Meeting 2016, Session 9: Symbionts / A-1

THE GROWTH CYCLE OF SIMKANIA NEGEVENSIS IN PNEUMOCYTES AND ENDOMETRIAL CELLS, EVIDENCE FOR ITS IMPLICATION IN HUMAN DISEASES?

M. VOUGA[1], D. BAUD[2], G. GREUB[1]

[1]Center for Research on Intracellular Bacteria, Institute of Microbiology, Faculty of Biology and Medicine, University of Lausanne and University Hospital, Lausanne, Switzerland, [2]Materno-fetal and Obstetrics Research Unit, Department "Femme-Mère-enfant", Maternity, University Hospital, Lausanne, Switzerland

Simkania negevensis (Sneg) is an emerging Chlamydia-related bacterium. As other Chlamydiales, it is an obligate intracellular bacterium characterized by a biphasic developmental cycle. A growing interest has developed towards Chlamydia-related bacteria as they provide new insights in the biology of the Chlamydiales and may represent potential emerging pathogens. Indeed, W. chondrophila and P. acanthamoebae have been associated with miscarriages and lower respiratory tract diseases, respectively.
Little is known about the biology and pathogenesis of Sneg. Evidence of human exposition has been reported worldwide and current data suggest an association with pneumonia and bronchiolitis. In addition, its similarities with adverse pregnancy outcomes-related pathogens (C. trachomatis or W. chondrophila) make it a putative candidate for genital infections and obstetrical complications.
Here, we characterized the growth cycle of Sneg in Vero cells, endometrial cells (Ishikawa cell line) and pneumocytes (A549 cell line) using a specific quantitative PCR, specifically developed, immunofluorescence and a mortality assay, based on propidium iodide incorporation.
Sneg efficiently replicated in these cell lines within 6 days and new infective particles were released in the cell culture supernatant starting from day 3. Infectivity was higher in pneumocytes. No cytopathic effects were observed as shown by similar mortality rates in both infected and uninfected cells. We confirmed that Sneg-containing vacuole is closely associated with the endoplasmic reticulum and mitochondria in these cell lines and showed that Sneg does not induce Golgi apparatus fragmentation. These findings emphasize the specificities of the replication cycle and host nutrients parasitism in each family level lineage of Chlamydiales members. From a clinical perspective, our work suggests that Sneg infection might persist for a prolonged time in pneumocytes or endometrial cells and induce chronic lung or endometrial infections. These data strongly support the role of Sneg as an agent of lower respiratory tract infections and should encourage clinicians to assess its implication in genital tract infections.

O-143

Prokaryotic, Session 1: The envelope – from walls to molecules (continuation) / A-6

GENETIC VARIABILITY AND LIMITED CLONALITY OF MYCOPLASMA HYORHINIS IN PIG HERDS

B TRÜEB[1], E CATELLI[2], A LÜHRS[3], H NATHUES[4], P KUHNERT[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland, [2]Department of Veterinary Science, University of Parma, Italy, [3]Field Station for Epidemiology, University of Veterinary Medicine Hannover, Foundation, Bakum, Germany, [4]Clinic for Swine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Switzerland

In order to gain more insight into the epidemiology and population structure of Mycoplasma hyorhinis we genetically characterized 60 isolates by multi locus sequence typing (MLST). The M. hyorhinis strains originated from Swiss and German pig herds with knowledge on the clinical background. The MLST scheme of Tocqueville et al. (1) using the six housekeeping genes dnaA, rpoB, gyrB, gltX, adk and gmk served as a basis to optimize the protocol. Primers with identical annealing temperatures were defined, allowing amplification of all targets with a single PCR amplification and sequencing protocol. A total of 27 ST were observed with the 60 strains, 26 of those were not previously known types. Generally identical genotypes were observed within a farm but they differed between farms. The identical genotype was also observed in three different Swiss farms. On the other hand different genotypes were found within three German farms. The Swiss isolates formed a distinct cluster but otherwise there was no geographical nor a clinical association with specific clusters observed. Data shows a high variability of M. hyorhinis comparable to what is observed for Mycoplasma hyopneumoniae. Similar to this pathogen the population structure of M. hyorhinis also shows some limited clonality with predominant genotypes within an animal and a single farm and different ones between farms. The comparable population structure of M. hyorhinis and M. hyopneumoniae could indicate a similar evolution of the two species in the common pig host.

1. Tocqueville V, Ferre S, Nguyen NH, Kempf I, Marois-Crehan C. Multilocus sequence typing of Mycoplasma hyorhinis strains identified by a real-time TaqMan PCR assay. J Clin Microbiol. 2014;52(5):1664-71.

O-144

Prokaryotic Biology, Session 1: The envelope – from walls to molecules (continuation) / A-2

COLISTIN RESISTANCE PLASMID PVT553 IN AVIAN PATHOGENIC ESCHERICHIA COLI

C STRAUSS[1], D GERBER[2], V PERRETEN[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]V-Tech, Midrand, South Africa

Since the discovery of the plasmid-mediated colistin resistance gene mcr-1 in November 2015, several studies have confirmed its dissemination in different human and animal environments across Asia, Europe, Africa and South America. We also detected this gene in avian pathogenic Escherichia coli (APEC) causing airsacculitis in South Africa and determined the complete nucleotide sequence of one mcr-1 carrying plasmid.
Whole genome sequencing of the plasmid carrying strain VT55363 performed on the MiSeq system using MiSeq Reagent Kit v2 and a read length configuration of 2 x 250bp (Illumina©, Labormedizinisches Zentrum Dr. Risch, Bern-Liebefeld, Switzerland) resulted in a single contig. The circular form of the plasmid was confirmed by PCR and subsequent Sanger sequencing (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems). The novel plasmid, pVT553, was related to the plasmid pHNSHP45 from China harboring the mcr-1 containing element ISApl1-mcr-1 and shared 96% identity with the plasmid pSH146_65 originally isolated from Salmonella enterica subsp. enterica serovar Heidelberg. These plasmids belong to the incompatibility group IncI2, like other mcr-1 containing plasmids from Asian and European origin. pVT553 had the ISApl1-mcr-1 integrated into a gene cassette determined for the formation of type IV pilus while the ISEc1-blaCMY-2 from the Salmonella plasmid is missing.
The complete sequence of pVT553 represents a new mcr-1 containing plasmid and showed that the colistin resistance gene can integrate in different plasmids. Plasmid pVT553 may also serve as basis for further epidemiological studies on genetic elements carrying mcr-1.

O-146

Prokaryotic Biology, Session 3: Cell biology – seeing is believing / A-2

REGULATORY ROLE OF CAMR IN CELL CYCLE CONTROLLED CAPSULATION AND MOTILITY IN CAULOBACTER CRESCENTUS

EZGI EROGLU[1], SILVIA ARDISSONE[1], PATRICK VIOLLIER[1]

[1]Department of Microbiology and Molecular Medicine, University of Geneva, 1211 Geneva 4, Switzerland

C. crescentus divides asymmetrically into two functionally specialized daughter cells: the motile non-capsulated swarmer cell and the non-motile and capsulated stalked cell. While the former resides in G1-like quiescent state, the latter is engaged in S-phase. In order to replicate, swarmer cells have to differentiate into stalked cells. Cell cycle studies in C. crescentus revealed that daughter cell-specific programs are implemented by conserved transcriptional activators and repressors that induce developmental and morphological transitions at key times, as in eukaryotic cells. For example, we found that expression of HvyA, a bacterial transglutaminase-like cysteine protease that prevents capsulation in swarmer cells, is activated by the G1-specific cell cycle transcriptional module.
In the effort to uncover new cell cycle regulated factors, I identified a previously uncharacterized gene (named camR for capsulation and motility Regulator) that regulates motility and capsulation. Although the camR transcript is abundant in G1-phase, I found that overexpression of CamR causes constitutive capsulation and reduced swarming motility. Moreover, my preliminary results showed that constitutive capsulation is due to a block in hvyA translation which is a bacterial transglutaminase-like cysteine protease (BTLCP) that prevents capsulation in swarmer cells. Interestingly, CamR also affects translation of other G1-specific genes, and therefore may represent a new cell cycle-dependent translational regulator in C. crescentus.
Bipolar localization of CamR-GFP points that in addition to temporal, CamR has spatial regulation. The polar localization pathway of CamR will be investigated further.

O-147

Prokaryotic Biology, Session 2: The 3 Cs – cytoplasm, chromatin and chaperones / A-2

TYPE VI SECRETION SYSTEM SUBSTRATES ARE TRANSFERRED AND REUSED AMONG SISTER CELLS

A VETTIGER[1], M BASLER[1]

[1]Focal Area Infection Biology, Biozentrum, University of Basel, Basel, Switzerland

Bacterial Type VI secretion system (T6SS) is a nanomachine that works similarly to a speargun and shares structural and functional homology to a membrane attached bacteriophage. T6SS physically punctures a target cell membrane by a tip composed of valine-glycine repeated protein G (VgrGs) and effector molecules located at the end of a long shaft composed of haemolysin co-regulated protein (Hcp). This is driven by a rapid contraction of a sling called sheath. The deletion of essential structural components (e.g. Hcp, VgrG2 or certain effectors) leads to a complete absence of T6SS activity. Using live-cell imaging of T6SS assembly in Vibrio cholerae, we show that the T6SS substrates are exchanged between neighboring sister cells within tens of seconds after initial contact. Upon T6SS-dependent translocation, both tip and Hcp shaft dissociate and are subsequently reused to form a fully functional T6SS assembly. Detailed analysis of T6SS dynamics in trans-complemented cells revealed that the availability of tip associated proteins (e.g. VgrGs) regulates the number of sheath assemblies per cell, whereas sheath length correlates with Hcp concentration.
We describe here a novel form of direct protein exchange and recycling among by-standing sister cells that could lower the overall cost of constitutively active secretion machinery for the bacterial community. Furthermore, we gained intriguing insights into the regulation and assembly of the T6SS.

O-148

SSM Annual Meeting 2016, SSM Award and lecture of Award Winner

PRIX SSM: GcrA/CcrM EPIGENETIC MODULE IN BACTERIAL CELL CYCLE CONTROL AND HOST-PATHOGEN INTERACTIONS.

G. PANIS[1], M. DELABY[1], C. FUMEAUX[1], L. THERAULAZ[1], P.H. VIOLLIER[1]

[1]Department of Microbiology & Molecular Medicine, Faculty of Medicine, University of Geneva, Switzerland.

Chemical modifications impart secondary regulatory information on primary DNA sequence at the post-replicative level to regulate fundamental processes in human health and disease. These so-called epigenetic modifications are also present in bacterial genomes and introduced by sequence-specific DNA methyltransferases and control many DNA transactions such as gene transcription, DNA replication and repair and virulence functions. Recently, we made an important leap forward towards understanding how an epigenetic regulatory pair, composed by the CcrM methyltransferase and the enigmatic transcriptional regulator GcrA, controls cell cycle transcription in the synchronizable Caulobacter crescentus model.

In Caulobacter, cell cycle progression is controlled by a cyclical genetic circuit comprising at least four transcriptional regulators. We found that deletion of the gene encoding in the GcrA regulator resulted in (ΔgcrA) cells that are viable, but slow-growing and elongated, with the latter mostly due to an insufficiency of the FtsN cell division protein. Furthermore, suppressor analysis showed that a second cell cycle regulator, the CcrM methyltransferase, also causes a strong (filamentation) cell division defect when inactivated in isolation, while the simultaneous gcrA/ccrM disruption ameliorated the cytokinetic and growth defect of ΔgcrA cells, rather than aggravating it. As no direct biochemical and regulatory relationship between GcrA and CcrM were known before, we discovered that GcrA preferentially targets promoters harboring CcrM-dependent N6-methyl-adenosine promoter marks. Within the alphaproteobacteria, gcrA and ccrM are consistently present or absent together, rather than either gene being present alone, suggesting that gcrA/ccrM constitutes an independent, dispensable genetic module.

In addition, we found that functional analogues of the GcrA/CcrM pair are carried by a cell cycle-specific bacteriophage ϕCbK infecting Caulobacter. We identified that ϕCbK infection prevents expression of the host GcrA/CcrM module. Interestingly, ϕCbK replace the host epigenetic pair by phage encoded variants to reprogram the host cell cycle transcriptional program.

O-149

SSM Annual Meeting 2016, Session 9: Symbionts / A-2

THE ESSENTIAL GENOME OF THE PLANT ENDOSYMBIONT SINORHIZOBIUM MELILOTI CULTURED IN RICH AND MINIMAL MEDIA.

C.E. FLORES-TINOCO[1], F. TSCHAN[1], M. BARNETT[2], M. CHRISTEN[1], S. LONG[2], B. CHRISTEN[1]

[1]IMSB, ETH Zürich, CH-8093 Zürich, Switzerland, [2]Stanford University, Stanford, CA 94305, USA

Sinorhizobium meliloti is an alpha-proteobacterium that can adopt a free-living lifestyle or engage in endo-symbiosis with legume plants. Associated with its distinct role as a nitrogen fixing endosymbiont of legumes, S. meliloti has evolved a remarkable adaptability to grow in nutritionally diverse environments. In the absence of its plant host, S. meliloti thrives as oligotrophic soil bacteria and hence must have evolved sophisticated strategies to adapt to diverse environmental conditions present within the rhizosphere. To identify the complete set of essential genes required for growth under variations in carbon source availability, we designed a systems-wide genetic screen using hyper-saturated Tn5 transposon mutagenesis in conjunction with next generation sequencing (TnSeq). We defined the essential genome of S. meliloti required for propagation under defined sets of nutrients. Across the 6.7Mb tripartite genome of S. meliloti, we mapped 750128 and 674886 unique Tn5 transposon insertion events under rich and minimal media growth. We identified 320 genes essential under both conditions. Surprisingly, we found more conditional genes for rich media (190 genes) than for minimal media (132 genes) growth. Under rich media conditions, a significant portion of 56 conditional essential genes (29.4%), participate in cell cycle regulation, while in minimal media 67 conditional essential genes (50.8%) participate in auxotrophic processes. In sum, our studies provide systems-level insights how the essential genome responds to variations in nutrient availability enabling persistence of S. meliloti in soil, and consequently improving its ability to colonize and survive within host plants.

O-151

Prokaryotic Biology, Session 2: The 3 Cs – cytoplasm, chromatin and chaperones / A-1

MULTIPLE APPROACHES TO DISCOVER NEW T3SS EFFECTORS OF CHLAMYDIALES BACTERIA

LP PILLOUX[1], CK KEBBI-BEGHDADI[1], AC CROXATTO[1], G GREUB[1]

[1]Center for Research on Intracellular Bacteria (CRIB), Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland

Like other obligate intracellular microorganisms, Chlamydia-related bacteria are able to modulate important functions of their host cells to allow their survival and replication. All bacteria of the Chlamydiales order exhibit a common "core genome" encoding bacterial proteins essential for their intracellular way of life, in particular those forming the Type III secretion system (T3SS). T3SS is a large proteic complex able to inject bacterial effector proteins within the eukaryotic host cells. In Chlamydiales bacteria, effectors are secreted in the inclusion lumen, inserted in the inclusion membrane or translocated into the host cell cytosol. Their functions are multiple, including cytoskeletal rearrangements, subversion of signalling pathways, activation or inhibition of host cell death and modulation of intracellular trafficking. In classical chlamydiae, several proteins have been identified as effectors secreted by the T3SS but nothing is known about those secreted by Chlamydia-related bacteria. Since these bacteria are emerging pathogens for humans and animals, identifying their secreted effector proteins and investigating their mode of action is essential.
We used multiple approaches to identify T3SS effectors of Chlamydia-related bacteria. The first one is based on bioinformatic algorithms that predict T3SS effectors even though no consensus sequence for type III secretion has been identified. We have also developed and optimized two alternative methods to recover bacterial proteins secreted in the host cell cytoplasm. These methods rely on selective permeabilization of the eukaryotic membrane or on fractionation of host cell components; the latter technique also allows the recovery of bacterial proteins localized into the inclusion membrane. After mass spectrometry analysis, we obtained a list of putative effectors, whose secretion by T3SS was confirmed using a heterologous system. These effectors were further expressed in eukaryotic cells to identify their interactants.
This large screening led to the identification of new chlamydial T3SS effectors, increasing our understanding of chlamydial pathogenesis and opening perspectives for new drug targets.

O-152

Prokaryotic Biology, Session 1: The envelope – from walls to molecules (continuation) / A-1

THE HETERODIMERIC ABC TRANSPORTER EFRCD MEDIATES MULTIDRUG EFFLUX IN ENTEROCOCCUS FAECALIS

L. M. HÜRLIMANN[1], M.A. SEEGER[1]

[1]Institute of Medical Microbiology, University of Zurich, 8006 Zürich, Switzerland

Increasing numbers of nosocomial infections caused by Enterococcus faecalis were reported and associated with serious diseases such as endocarditis, bacteremia, urinary tract infections and surgical wound infections. Interestingly, the contribution of drug efflux pumps to intrinsic resistance against antibiotics is poorly studied in E. faecalis. Recent studies on other Gram-positive bacteria have revealed heterodimeric ABC multidrug transporters to play an important role in multidrug efflux, including LmrCD of Lactococcus lactis and PatAB of Streptococcus pneumoniae. Here we investigated the complete set of seven heterodimeric ABC exporter homologs from E. faecalis, which are annotated as drug efflux pumps.
Unmarked gene deletion knockout of ef0789/ef0790 on the chromosome of E. faecalis resulted in increased susceptibility towards daunorubicin, doxorubicin, ethidium and Hoechst 33342 and the corresponding transporter was named EfrCD. Unexpectedly, the previously described heterodimeric multidrug ABC transporter EfrAB contributes marginally to drug efflux in the endogenous context of E. faecalis. In contrast, heterologous expression in L. lactis revealed EfrAB, EfrCD and the gene product of ef2226/ef2227 (EfrEF) to mediate efflux of fluorescent substrates and confer resistance towards multiple dyes and drugs including fluoroquinolones. Four out of seven transporters did not exhibit any measurable drug efflux activity even upon overexpression in L. lactis. Since all seven transporters were purified as heterodimers after overexpression in L. lactis, lacking drug efflux activity is unlikely attributed to poor expression or protein aggregation. Reconstitution of purified multidrug transporters EfrAB, EfrCD and EfrEF in proteoliposomes revealed a robust functional coupling between ATP hydrolysis and drug binding. Our analysis builds an experimental basis for the accurate prediction of drug efflux transporters and indicates that many annotated multidrug efflux pumps are in fact incapable of drug transport and thus fulfil other physiological functions in the cell.

O-153

Virology, Session 4: Basic Virology / A-6

LAUSANNEVIRUS AND ESTRELLA LAUSANNENSIS EVOLUTIONARY DYNAMICS

L MUELLER[1], T PILLONEL[1], C BERTELLI[2], N SALAMIN[3], G GREUB[1]

[1]Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland, [2]SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland , [3]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Amoeba-resisting microorganisms (ARM) such as Nucleocytoplasmic large DNA viruses (NCLDVs) and Chlamydia-related bacteria, evolved to survive and replicate inside amoebae. These ARM are under a strong selection pressure, being in a very adverse environment; the professional phagocyte. However, the evolution rate of their genomes is yet unknown. Therefore, we investigated the mutation rates of two recently discovered ARM: Lausannevirus (346 kb, GC content 42.9%), a large DNA virus and Estrella lausannensis (~ 2.8 Mb, GC content 48.2%), a bacterium belonging to Criblamydiaceae bacterial family. During 1 year (144 passages) both ARM were maintained in coculture within Acanthamoeba castellanii. Each 3 months culture were split in two lineages, for a total of 8 different subcultures at the end of the year. Quantitative real-time PCR allowed the evaluation of viral and bacterial abundance of each subculture. Interestingly, bacterial subcultures showed similar profiles, while major differences in population size among viral subcultures were reported. Sequencing of all subcultures was performed with MiSeq Illumina, viral and bacterial genomes were de novo assembled and Single Nucleotide Polymorphism (SNP) as well as insertion/deletion (INDEL) calling assessed. Analyses showed that both ARM have stable genomes; after one year they acquired from 2 to 7 and from 4 to 10 mutations per culture for Lausannevirus and E. lausannensis, respectively. Interestingly, in Lausannevirus no mutations become fixed, suggesting that mixed populations coexist in the same subculture. While, in E. lausannensis 15% of the mutations reached the fixation (cutoff >= 99%). The presences of different mutations in the endonuclease encoding genes of Lausannevirus were detected in different subcultures, suggesting the importance of this gene product in viral replication. Conversely, mutations in E. lausannensis were mainly located in a gene encoding for a phospoenolpyruvate-protein phospotransferase (PtsI), implicated in sugar metabolism. Noteworthy, we detected no stable horizontal gene transfer among Lausannevirus and E. lausannensis.

O-155

Virology, Session 2: Virological toolbox / A-1

PERSISTENT MAMMALIAN ORTHOREOVIRUS INFECTION IN A CHILD WITH PRIMARY IMMUNODEFICIENCY DETECTED BY METAGENOMIC SEQUENCING

DW LEWANDOWSKA[1], R CAPAUL[1], S PRADER[2], O ZAGORDI[1], FD GEISSBERGER[1], M KNORR[1], C BERGER[3], T GÜNGÖR [4], J REICHENBACH [2]

[1]Institute of Medical Virology, University of Zurich, [2]Division of Immunology, University Children's Hospital Zurich, [3]Division of Infectiology and Hospital Hygiene, University Children's Hospital Zurich, [4]Division of Stem Cell Transplantation, University Children's Hospital Zurich

Here we report on a unique combination of viral infections diagnosed by metagenomic sequencing in a child with combined immunodeficiency. Following a flu-like infection with cough, headache and fever, the child suffered from persistent diarrhea for 18 months. Stool samples tested regularly positive for Enterovirus in a PCR assay but a specific enterovirus could not be typed by cell culture and subsequent indirect immunofluorescence staining. To define the infecting virus, we used an unbiased metagenomic approach to sequence cell culture supernatants and stool suspensions from several time points.
For metagenomic sequencing, virus particles were enriched by filtration, total nucleic acids extracted and randomly amplified. Sequencing libraries were prepared with NexteraXT and sequenced on a MiSeq Illumina (1x150 bp). Quality filtered reads were aligned with BLAST against a database containing > 40,000 viral sequences.
Metagenomic sequencing identified Coxsackievirus A22 in stool suspensions and, very surprisingly, Mammalian Orthoreovirus (MRV) 3 in supernatants of stool cell culture, respectively, over at least a 12-month period. Phylogenetic analysis of MRV showed high similarity to isolates from bats in Germany and from a child with acute gastroenteritis in Slovenia; to our knowledge, the only other reported case of MRV originating from European bats with clinical manifestations in humans.
MRVs have a broad host range and can also infect humans often causing mild enteric and respiratory infections. More severe cases include hemorrhagic enteritis and encephalitis. Recently, novel MRVs have been isolated from various bat species in Asia, Australia and Europe. Zoonotic transmission is often implicated as the origin of human infection. As our patient is living in close contact to pets and in the vicinity of an animal farm, this is a likely possibility.
In summary, cell culture combined with open metagenomic sequencing was crucial in identifying MRV, as no specific routine tests are available for these viruses. This highlights the potential of metagenomic sequencing in complex diagnostic settings for the identification of atypical virus infections.

O-156

SSM Annual Meeting 2016, Session 4: Clinical Virology / A-2

MULTI-RESISTANCE OF ENTEROVIRUS TO UV254 DISINFECTION AND MUTAGENIC NUCLEOTIDE ANALOGS IS LINKED TO MODIFICATIONS IN THE REPLICATION MACHINERY

A CARRATALÀ[1], V BACHMANN[1], Q ZHONG[1], T KOHN[1]

[1]Environmental Chemistry Laboratory, School of Architecture, Civil and Environmental Engineering. École Polytechnique Fédérale de Lausanne (EPFL).

Disinfection is an important strategy to control the environmental transmission of human viruses. Specifically, UV254 is increasingly used to disinfect from clinical items or surfaces to drinking water. RNA virus populations have high mutation rates and large population numbers that allow them to rapidly adapt to stressors. It is thus likely that variants with enhanced resistance to disinfection may emerge. The genetic basis and mechanisms that underpin the resistance to disinfection remain unknown. Our goal was to investigate the emergence of echoviruses resistant to UV254, and to characterize their genotypic and phenotypic changes.
Resistant virus populations emerged in mutation-accumulation experiments consisting in exposing echovirus suspensions to UV254 such that a 4.5 log10 reduction was achieved, and then regrowing the survivors in BGM cells at a very low multiplicity of infection. Unexposed control experiments were conducted in parallel by diluting the virus suspensions prior to regrowth.
Echovirus populations became increasingly resistant to UV254. Interestingly, a similar extent of resistance was observed in the unexposed control experiments. Fixed mutations were identified by next generation sequencing in the 2C and 3D protein region, both involved in the genome replication. The wild-type and resistant echovirus populations exhibited an equivalent replicative fitness under standard culturing conditions. However, the UV254-resistant mutants were more resistant to ribavirin and guanidine hydrochloride. Finally, the exposed populations were able to maintain the production of infective progeny despite the effect of the chemical mutagens. Combined, these findings suggest that multi-resistance of echovirus to UV254 disinfection and treatment with mutagenic nucleotide analogs may arise in virus populations that, subjected to repeated dramatic bottlenecks, evolve towards an increased replication fidelity. The outcomes of this work show that enteric human pathogens may exhibit multi-resistance to disinfection and clinical antiviral therapies. Ultimately, our results may impact future strategies to ensure adequate control of virus environmental transmission.

O-158

A SYSTEMS SURVEY OF PROGRESSIVE HOST CELL REORGANISATION DURING ROTAVIRUS INFECTION

V. A. GREEN[1], L. PELKMANS[1]

[1]Institute of Molecular Life Sciences, University of Zurich, Winterhurerstrasse 190, Zurich 8057, Switzerland

Pathogen invasion is often accompanied by widespread alterations in cellular physiology, which reflects the hijacking of host factors and processes for entry and replication. Although genetic perturbation screens have revealed the complexity of host factors involved for numerous pathogens, it has remained challenging to disentangle this complexity along the progression of host cell reorganisation during the infection process. We here address this by combining the first high confidence, image-based, genome-scale RNAi screening of rotavirus infection in human intestinal cells with an innovative approach to infer a continuous trajectory of virus infection progression from fixed cell populations. We uncover a complex, yet ordered host cell reorganisation program during rotavirus infection that provides a replication-permissive cellular environment for the virus. This includes an alternative mechanism for host protein synthesis shut-off during early stages of infection, consumption of lipid stores, rearrangement of mitochondria, and ER shape remodelling throughout infection, modulated by the ER shaping protein REEP2. Finally, by integrating the large-scale gene perturbation dataset with single-cell trajectories, we propose a model for host factor-mediated cellular reorganisation during rotavirus infection, many elements of which can be linked to calcium-activated AMPK signalling. Our work provides a powerful approach to order the complexity of host cellular requirements along a trajectory of cellular reorganisation during pathogen invasion, an approach that would enhance the analysis of perturbation screens from any field.

O-159

Virology, Session 5: Basic Virology / A-1

EARLY ENDORIBONUCLEASE-MEDIATED EVASION OF RNA SENSING ENSURES EFFICIENT CORONAVIRUS REPLICATION

E KINDLER[1], J SPANIER[2], C GIL-CRUZ[3], Y LI[4], MH HWANG[5], M KELLER[6], J ZIEBUHR[7], U KALINKE[2], V THIEL[8]

[1]Federal Department of Home Affairs, Institute of Virology and Immunology, Bern and Mittelhäusern, Switzerland, [2]Institute for Experimental Infection Research, Twincore, Hannover, Germany, [3]Institute of Immunobiology, Cantonal Hospital, St.Gallen, Switzerland, [4]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA, [5]Department of Neurosciences, Lerner Research Institute, Cleveland Ohio, USA, [6]Institute of Novel and Emerging Infectious Diseases , Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany, [7]Institute for Medical Virology, University of Giessen, Giessen, Germany, [8]Department of Infectious Diseases and Pathobiology, University of Bern, Bern, Switzerland

Coronaviruses are of veterinary and medical importance and include highly pathogenic zoonotic viruses, such as SARS-CoV and MERS-CoV. They are known to efficiently evade early innate immune responses manifesting in almost negligible induction of type-I interferon (IFN-I) responses. This evasion strategy suggests an evolutionary conserved viral function that has evolved to prevent RNA-based sensing of infection in a variety of vertebrate hosts. Here we show that the coronavirus endoribonuclease (EndoU) activity, an integral component of the viral replication complex, is key to prevent early induction of double-stranded RNA (dsRNA) host cell responses. Replication of EndoU-deficient coronaviruses is greatly attenuated in vivo and severely restricted in primary cells already during the early phase of the infection. In macrophages we found immediate Mda5-mediated induction of IFN-I expression and RNaseL-mediated breakdown of ribsosmal RNA. Accordingly, EndoU-deficient viruses can only retain replication in cells that are deficient in IFN-I expression or sensing, and in cells lacking both RNaseL and PKR. Collectively our results demonstrate that the coronavirus EndoU activity efficiently prevents simultaneous activation of host cell dsRNA sensors, such as Mda5, OAS and PKR. The localization of the EndoU activity at the site of viral RNA synthesis suggests that coronaviruses have evolved a viral RNA quality control pathway to evade early innate and intrinsic antiviral host cell responses.

O-163

HETEROGENEOUS ANTIGENIC PROPERTIES OF THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) NUCLEOCAPSID AMONG GENOTYPE I STRAINS

J RAPPE[1], N RUGGLI[1]

[1]Institute of Virology and Immunology IVI, Sensemattstrasse 293, 3147 Mittelhäusern, Switzerland

Porcine reproductive and respiratory syndrome virus (PRRSV) is an arterivirus responsible for a widespread contagious disease of domestic pigs with high economic impact. Switzerland is one of the rare PRRSV-free countries in Europe, although sporadic outbreaks have occurred in the past. PRRSV strain IVI1173 isolated during the latest short Swiss outbreak of 2012 was entirely sequenced, and a full-length functional cDNA clone was constructed. Genomic characterization of IVI1173 revealed the importance of amino acid 90 of the nulceocapsid (N) as part of a conformational epitope. Indeed, IVI1173 was not detected by SDOW17, a commercial monoclonal antibody against PRRSV N widely used to detect PRRSV-infected cells. Substitution of alanine at position 90 – N(A90) – with a threonine present in most isolates at this position restored reactivity of the recombinant IVI1173-N(T90) to SDOW17 completely. The relevance of this amino acid for PRRSV in general was further studied by mutagenesis of N at position 90 in the backbone of the genotype II clone RVB-581 and in a panel of nucleocapsid proteins of 12 genotype I strains. N(A90) totally disrupted or severely affected the discontinuous epitope in 9 out of 10 tested strains. Due to the loss of antigenicity implied by this mutation, its prevalence among 225 genotype I strains was screened: while N(A90) is rare in the other subgroups, alanine at this position is highly frequent in the Russian subtype 1 (70%) and the subtype 2 (45%) isolates. In conclusion, this study highlights the variable antigenic features of N among genotype I PRRSV strains.

O-164

Virology, Session 2: Virological toolbox / A-2

SINGLE-CELL ANALYSIS APPROACH TO IDENTIFY CELLULAR BIOMARKERS FOR HIV PERMISSIVENESS

S RATO[1], A RAUSELL[1], S QUENNEVILLE[1], M MUÑOZ[1], A TELENTI[2], A CIUFFI[1]

[1]Institute of Microbiology, University of Lausanne, Lausanne, Switzerland, [2]J. Craig Venter Institute, La Jolla, USA

Cellular permissiveness to HIV infection is highly heterogeneous across individuals. Heterogeneity is also found across CD4+ T cells from the same individual, where only a fraction of cells gets infected. We used a single-cell RNA-Seq approach to investigate cellular heterogeneity and identify biomarkers of HIV permissiveness. CD4+ T cells from healthy donors were activated by TCR-mediated stimulation for three days and tested for their permissiveness to HIV infection. Non-infected activated CD4+ T cells from a highly and a poorly susceptible individual were selected and used for single-cell RNA-seq analysis using fluidigm C1 technology. RNA-Seq profiles from 85 highly permissive and 81 poorly permissive single cells were successfully obtained, with ~25 million reads per single cell. Transcriptional heterogeneity translated in a continuum of intermediary cell states in both highly and poorly permissive donor cells, which was mainly driven by TCR-mediated cell activation. Genes whose expression was differential across cells, across both donors and that encoded proteins expressed at the cell surface were further investigated as candidate biomarkers of HIV permissiveness. Single biomarkers were tested for their ability to identify permissive cells, showing enrichment in HIV infection. The combination of multiple candidate biomarkers further selected for highly permissive cells, thus defining the “HIV-permissive cell”.
Our data identified activation as a major determinant driving cellular heterogeneity in HIV permissiveness and further revealed the role of single candidate biomarkers in defining the HIV highly permissive cell.

O-165

Virology, Session 4: Basic Virology / A-2

MEMBRANE REMODELING PROPERTIES OF CORONAVIRUS NON-STRUCTURAL PROTEIN 6 AND ITS ROLE IN REPLICATION COMPARTMENT FORMATION.

P VKOVSKI[1], V GASCHEN[2], M STOFFEL[2], V THIEL[3]

[1]Federal Institute of Virology and Immunology, Mittelhäusern and Bern, Switzerland; Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland, [2]Vetsuisse Faculty, Division Veterinary Anatomy, University of Bern, Bern, Switzerland., [3]Federal Institute of Virology and Immunology, Mittelhäusern and Bern, Switzerland; Vetsuisse Faculty, Department of Infectious Diseases and Pathobiology, University of Bern, Bern, Switzerland.

Coronaviruses are positive-sense RNA viruses known to extensively remodel intracellular membranes and induce double-membrane vesicles (DMVs) that support viral RNA synthesis. Transmembrane non-structural proteins 3, nsp4 and nsp6 play an essential role in diverting ER-derived membranes and anchoring the viral replication complex to modified membranes. The potent human CoV inhibitor K22 specifically prevents the formation of DMVs and viral RNA synthesis. Remarkably, K22-resistant hCoV-229E viruses containing substitutions in nsp6 are able to form DMVs, although the latter are reduced in number and structurally impaired.

Our results describe the effects of hCoV-229E nsp6 and nsp6 K22-resistant mutant (nsp6*) on membrane modifications using both immunofluorescence microscopy and electron microscopy (EM). Monitoring of nsp6 expression in MRC5 cells results in a punctate perinuclear accumulation comparable to viral replication complexes. In contrast, K22 treatment prevents accumulation and nsp6 distributes homogenously in the cytosol. Interestingly, K22-resistant nsp6* evenly distributes in the cytosol both in presence or absence of K22. At the ultrastructural level, nsp6 induces massive membrane rearrangements resulting in vesicular structures accumulated in the nucleus vicinity. However, nsp6* is unable to induce such structures. Rather, ER compartments appear highly dilated as a consequence of nsp6* expression. Both phenotypes are disrupted or modulated by the addition of K22. We are currently processing our samples for immuno-EM in order to assess the location of nsp6 and nsp6* constructs to modified membranes.

In addition to hCoV-229E, K22 was also shown to efficiently inhibit the replication of SARS-CoV, MERS-CoV and IBV. Nsp6 constructs of these additional viruses, which display high conservation of their amino-acid sequences, were subjected to IF and EM analyses to determine the membrane remodeling properties of homologous nsp6 proteins. Moreover, the assessment of homologous nsp6 sensitivities towards K22 will establish whether K22 supposedly inhibits CoV replication by interfering with functions of nsp6.

O-168

Virology, Session 1: Antiviral defense / A-3

INVESTIGATION OF IFITM INCORPORATION INTO INFLUENZA A VIRUS PARTICLES AND ITS CONSEQUENCES ON VIRUS INFECTIVITY

C. LANZ[1], E.E. MÜLLER[1], S. STERTZ[1]

[1]Institute of Medical Virology, University of Zurich, Zurich, Switzerland

Interferon-inducible transmembrane proteins (IFITMs) have been identified as potent antiviral factors inhibiting entry of a whole variety of viruses, including influenza A virus (IAV). Recently is has been described that in addition to blockage of fusion of viral and host cell membranes in target cells IFITMs are also incorporated into budding HIV virions. IFITM incorporation has been associated with a decreased fusion capacity of virions. Furthermore, IFITMs have been shown to interact with HIV envelope glycoproteins in virus producer cells, resulting in decreased envelope processing and incorporation into virions.
Here, we describe studies on IFITM incorporation into IAV particles and its consequences on virus infectivity. We assessed the effect of IFITM incorporation on the envelope-mediated fusion process by comparing the entry efficiency of HIV virus-like particles (VLPs) pseudotyped with envelope proteins of IAV, HIV or VSV in the presence or absence of IFITM3. We observed that all virions tested incorporated IFITM3, which led to a reduction in entry efficiency. To detect potential effects specific to IAV, IFITM incorporation and its consequences were also studied in an IAV-based VLP system. Our results suggest that also in the IAV-based system the presence of IFITM3 in the producer cells leads to reduced entry efficiency at or before the fusion step. Ongoing studies aim to test whether the results obtained in the VLP system can be extrapolated to wildtype virus.

O-170

Virology, Session 1: Antiviral defense / A-1

RESPIRATORY VIRAL INFECTIONS IN RECONSTITUTED HUMAN AIRWAY EPITHELIA

M ESSAIDI-LAZIOSI[1], S BENAOUDIA[1], M FERNANDEZ-ROCHA [1], S CONSTANT[1], L KAISER [1], C TAPPAREL [1]

[1]Laboratory of Caroline Tapparel, Faculty of medicine of Geneva

The human airway epithelium is the first line of defense against respiratory infections. Mucociliary clearance serves as a mechanical barrier against microorganisms while epithelial cells recognize and fight the infection via cytokine secretion. Viral respiratory infections are the most frequent etiologies of acute illnesses worldwide and cause mild to severe diseases such as common cold, bronchiolitis and pneumonia. This study aimed to compare infections by the most frequent human respiratory viruses using standardized in vitro reconstituted human airway epithelia. Differentiated tissues were infected in parallel with clinically relevant strains of rhinovirus, respiratory enterovirus, influenza virus, respiratory syncytial virus and coronavirus and different parameters were compared. For each virus, the replication kinetics, the cell tropism and the impact of the virus on tissue integrity and cytokine secretion was assessed. We observed that all viruses affected negatively the mucociliary clearance except rhinovirus B and coronavirus OC43, while enterovirus D68 and influenza virus H3N2 were the only viruses affecting tissue integrity. Altogether this work provides new insights on the differential in vitro pathogenesis of the most frequent agents of respiratory infections

O-171

Virology, Session 4: Basic Virology / A-3

INFECTIOUS BAT-DERIVED INFLUENZA A VIRUS (H18N11) REVEALS A CELL TYPE-SPECIFIC BUT NOT SPECIES-SPECIFIC TROPISM

G. ZIMMER[1], E. MOREIRA[2], H. BOLTE[2], S. LOCHER[1], M. JUOZAPAITIS[2], T. AYDILLLO[3], A. GARCÍA-SASTRE[3], M. SCHWEMMLE[2]

[1]Institute of Virology and Immunology (IVI), Mittelhäusern, Switzerland, [2]Institute of Virology, Medical Center, University of Freiburg, Freiburg, Germany, [3]Icahn School of Medicine at Mount Sinai, New York, New York, USA

Two novel influenza A-like viral genome sequences have been recently identified in South American fruit bats and provisionally designated “H17N10” and “H18N11”. All efforts to isolate infectious virus from bats or to generate these viruses by reverse genetics have failed to date. Vesicular stomatitis virus (VSV) pseudotyped with bat influenza virus glycoproteins were used to identify cell lines that are susceptible to bat influenza virus infection. Madin-Darby canine kidney type II (MDCK II) cells were found to support replication of H18 but not H17 pseudotype virus. Entry in these cells was dependent on a low-pH triggered membrane fusion activity and sialidase pre-treatment did not abrogate infection. In contrast to conventional influenza A viruses infection occurred preferentially at the basolateral site. Cells of varies species revealed limited susceptibility to HA pseudotype virus infection with the notable exception of human U87-MG, SK-Mel 28 and Calu3 cells. Although to very low levels, infectious H18N11 but not H17N10 virus was recovered from transfected cells and passaged on MDCK II cells. Infection with H18N11 virus was efficiently neutralized by anti-H18 but not by anti-H17 immune sera. H18N11 preferentially infected polarized MDCK II cells from the basolateral site and sialidase pre-treatment did not abrogate HA18N11 infection, highlighting marked differences to conventional influenza A viruses. The ability of H18N10 to infect human cells might indicate increased zoonotic potential of these bat influenza A-like viruses.

O-172

Virology, Session 4: Basic Virology / A-4

NOVEL BAT INFLUENZA VIRUS NS1 PROTEINS: USUAL AND UNUSUAL FEATURES

H.L. TURKINGTON[1], B.G. HALE[1]

[1]Institute of Medical Virology, University of Zürich, Zürich, Switzerland

The influenza A virus (IAV) NS1 protein is a multifunctional virulence factor which primarily acts as an interferon (IFN) antagonist, but also promotes cell survival signalling through activation of the host phosphoinositide-3-kinase pathway (PI3K). Identification in 2012/13 of two novel influenza virus genomes (H17N10 and H18N11) in Central and South American bat species presented two unique NS1 proteins with high sequence divergence from other known NS1 proteins. How these divergent NS1s have evolved to function in the bat host-cell environment and whether they possess strain-specific functions, is unknown. We show that both the H17 and H18 NS1 proteins are capable of antagonising the human IFN response in transfected cells, a property strictly dependent upon their capacity to bind double-stranded (ds)RNA. High-resolution crystal structures of their RNA-binding domains (RBDs) revealed a dimeric fold similar to other classical NS1 RBDs, with conservation of important RNA-binding residues. Strikingly, recombinant chimeric bat IAVs expressing NS1 proteins defective in dsRNA-binding were highly attenuated in IFN-competent cells, but replicated similar to wild-type viruses in IFN-deficient cells. This confirms the highly-conserved functional importance of dsRNA-binding by IAV NS1 proteins for antagonising host innate immunity. Interestingly, we found certain well-described IAV NS1 functions associated with the C-terminal effector domain to be lacking in the H17 and H18 NS1 proteins such as the ability to inhibit general host gene expression. Both bat IAV NS1 proteins also lack the ability to interact with the human p85β subunit of host PI3K, with a subsequent inability to activate downstream signalling molecules in this pathway. We show the NS1:p85β interaction can be restored with a small number of amino acid changes in the H17 and H18 NS1 proteins. Our ongoing work seeks to elucidate whether this interaction may be species-specific and additionally whether the bat IAV NS1 proteins preferentially interact with other intracellular factors in order to modulate the bat host-cell environment.

O-173

Virology, Session 4: Basic Virology / A-5

GENERATION AND CHARACTERIZATION OF NOVEL INFLUENZA A REPORTER VIRUSES ENCODING A VARIETY OF FLUORESCENT AND LUMINESCENT REPORTER AS WELL AS A CRE-RECOMBINASE

P. REUTHER[1], K. GÖPFERT[2], A. DUDEK[2], D. DORNFELD[2], M. SCHWEMMLE[2]

[1]Department of Biomedicine, Experimental Virology, University of Basel, 4009 Basel, Switzerland, [2]Department of Virology, University of Freiburg, 79104 Freiburg, Germany

Reporter viruses represent valuable tools to study dynamics of a viral infection in cell culture and animal models. Furthermore, they facilitate screens for host factors or antiviral agents. We generated A/SC35M (H7N7) viruses encoding a Renilla luciferase, a secreted Gaussia luciferase, GFP-derivates (GFP, dsRed, Azurite) as well as a virus encoding a Cre-recombinase. These viruses grow to high titers and, importantly, do not loose the inserted gene while passaging.
To evaluate the suitability of the luciferase-encoding viruses for drug screenings, we treated infected MDCKII cells with different concentrations of the nucleosid inhibitor ribavirin. We could show that antiviral effects can be visualised by a luciferase assay already 3-6 hours post infection. We also tested these viruses for their suitability for screens for host factors. Knock down of the antiviral host protein MxA resulted in a significant increase of luciferase activity in human A549 cells upon infection with the reporter viruses.
The Cre/loxP system is a widely used method to specifically control the expression of target genes. To evaluate the applicability of the Cre-encoding virus as a tool for in vivo studies, we employed rosamT/mG reporter mice. Upon infection of these mice with a sublethal viral dose, we made use of the Cre-mediated switch from red to green fluorescence in order to localize and characterize cells that survived acute viral infection by fluorescent microscopy as well as flow cytometry. Surviving cells were present in different lung tissues and belonged to diverse lineages. Strikingly, we identified fort he first time a population of stem/progenitor cells, which survived viral infection.

O-175

Virology, Session 3: Basic Virology (supported by CUSO) / A-4

RESTRICTED SIRNA SCREEN REVEALS DIFFERENTIAL EFFECTS OF CELLULAR PROTEINS ON GENE EXPRESSION FROM SINGLE-STRANDED AND DOUBLE-STRANDED AAV2 VECTORS

F.D. FRANZOSO[1], A. YAKIMOVICH[2], K. TOBLER[1], A. MAN[1], R. VOGEL[1], U. GREBER[2], M. ACKERMANN[1], C. FRAEFEL [1]

[1]Institute of Virology, University of Zürich, Zürich, Switzerland, [2]Institute of Molecular Life Sciences, University of Zürich, Zürich, Switzerland

Adeno-associated virus type 2 (AAV2) is a non-pathogenic, helpervirus-dependent parvovirus. AAV2 DNA replicates in nuclear compartments which contain both viral and cellular proteins. We performed a siRNA screen to assess the effect of these cellular proteins on reporter gene expression from single-stranded (rAAVGFP) and self-complementary (scAAVGFP) AAV2 vectors. Specifically, 65 cellular proteins were selected based on their previously reported recruitment to replicating AAV2 DNA in presence of either herpes simplex virus type 1 (HSV-1) or adenovirus type 5 as the helper virus. Reporter gene expression (GFP fluorescence) was recorded by using an ImageXpress Micro High Content Screening System (Molecular Devices) and further analyzed with CellProfiler (BROAD Institute, Cambridge, MA, USA) and KNIME (KNIME.COM AG, Zurich Switzerland) software. The post-transcriptional silencing of the target genes was confirmed and the data on gene expression validated by RT-qPCR and Western blotting. We found that knockdown of several cellular genes, such as Mre11, Nbs1 and Rad50, enhanced the transcription from both single-stranded and sc AAV2 vectors, while the knockdown of other genes, such as RPA1, RPA2, MSH2, MSH3, and MSH6, differentially affected gene expression from the two different vectors.
Interesting observations were made also when comparing the effect of post-transcriptional silencing on transcription from rAAVGFP or scAAVGFP in presence versus absence of HSV-1 as the helper virus. For example, the negative effect of some cellular proteins on AAV2 gene expression was neutralized by the helper virus, or helper virus infection compensated for proteins that appeared to enhance AAV2 gene expression.
By using a knockdown and replacement strategy we are currently investigating the role of different domains and activation statuses of selected cellular proteins on AAV2 gene expression and replication.

O-177

SSM Annual Meeting 2016, Session 2: Microbe entry / A-1

PHOSPHORYLATION EVENTS IN EARLY STEPS OF INFLUENZA A VIRUS INFECTION

E. YÁNGÜEZ[1], M.P. DOBAY[2], S. STERTZ[1]

[1]Institute of Medical Virology, University of Zurich, Zurich, Switzerland. , [2]Swiss Institute of Bioinformatics, Lausanne, Switzerland.

Influenza A viruses (IAVs) harness the cellular endocytic machinery to enter the cell and traffic through the cytoplasm to reach the replication sites in the nucleus. Coordinated early activation of particular signalling pathways, such as EGFR-mediated signalling, has been shown to be crucial for the entry process and the outcome of viral infection. However, the complexity of these signalling cascades, tightly connected with each other, requires a broader analysis to identify the main routes, the key mediators and the direct effectors involved in the early steps of the viral replication cycle. The aim of the presented work is the identification of signalling and functional pathways that are selectively activated during IAV entry and could play a crucial role in the early steps of the viral infection. We have initially performed an ELISA-based multiplex analysis for the identification of Receptor Tyrosine Kinases (RTKs) that are activated upon IAV entry and observed that the ErbB family, a group of RTKs closely related with EGFR, is consistently activated in response to the virus infection. Moreover, and in order to obtain a more comprehensive view of the signalling events induced during IAV entry, we have conducted a proteome-wide SILAC-based quantitative phosphoproteomic screen of A549 cells within minutes post-infection with IAV. We find that both ErbB and MAPK signalling pathways are activated as early as 5 min after the initial contact of the virus with the cells. This early activation is accompanied by significant changes in the phosphorylation of key proteins of the cytoskeleton, the vesicle-mediated transport and the nuclear import machinery. Currently, we are focusing our efforts on the characterization of the influence of these early events on viral entry and the outcome of viral infection. The key proteins of the identified activated pathways could be used as promising clinical targets for future antiviral interventions.

O-178

Virology, Session 1: Antiviral defense / A-2

SUMOYLATION IN THE REGULATION OF ANTIVIRAL HOST INNATE IMMUNITY

C. PATZINA[1], F. GOLEBIOWSKI[1], B.G. HALE[1]

[1]Institute of Medical Virology, University of Zurich, Zurich, Switzerland

Type-I interferon (IFN) signaling is an essential part of the body`s innate defense against viruses. Upon detection of foreign pathogen-associated patterns, every cell is capable of mounting this rapid response to put itself and its neighbors into an antiviral state. Since exacerbated IFN signaling is detrimental to the host, while insufficient activation can result in severe infection, the pathway is tightly regulated by mechanisms including posttranslational protein modifications like SUMOylation. SUMO has just recently been tied to innate immunity, and can have a profound impact on the antiviral action of IFN, as well as negatively regulating IFN signaling. Since many aspects of IFN regulatory functions of protein SUMOylation are still unknown, we aim to elucidate the IFN regulating activity of SUMO by characterizing the specific cellular targets of (de-)SUMOylation upon induction of IFN signaling, as well as by defining the SUMO enzymes involved in this response. Therefore, we established a screening system using reporter cell lines that allowed us to identify SUMO enzymes, i.e. putative and known SUMO E3 ligases and SUMO-specific proteases (SENPs), that mediate SUMOylation of specific target proteins involved in the IFN induction and signaling pathways. In addition, we defined the subset of target proteins that change in their SUMOylation status upon induction of IFN signaling by performing a SILAC-based comparative mass spectrometry screen of SUMO targets in the presence or absence of a strong IFN-inducing agent. Functional validation of these factors will give insights into their impact on the IFN pathway, as well as on their influence on virus replication. These findings will ultimately broaden our knowledge on how innate immunity is fine-tuned by SUMO to prevent excessive as well as insufficient responses to pathogens, and might eventually reveal novel treatment options for innate immune disorders.

O-179

Virology, Session 3: Basic Virology (supported by CUSO) / A-2

VIRAL BUDDING REGULATED BY A CRITICAL RESIDUE LOCATED AT THE PUTATIVE DIMERIC INTERFACE OF THE CANINE DISTEMPER VIRUS MATRIX PROTEIN

F. BRINGOLF[1], M. HERREN[1], M. WYSS[1], A. ZURBRIGGEN[1], P. PLATTET[1]

[1]Division of Neurological Sciences, Department of Clinical Research and Veterinary Public Health (DCRVPH),Vetsuisse Faculty, University of Bern, Bern, Switzerland

Paramyxoviruses rely on the matrix (M) protein to orchestrate viral assembly and budding at the plasma membrane. Recent structural data from different paramyxovirus M-proteins revealed a monomeric unit composed of N- and C-terminal modules (NTD and CTD, respectively) that are connected via a flexible linker and which can self-assemble into inverted “head-to-tail” dimers. Because growing evidences support the notion that M-dimerization and/or higher-order oligomerisation play a critical role in virus particle formation, we investigated the role of the asparagine 138 (N138) of CDV M, which putatively locates at the core of the dimeric interface. While intracellular expression of M-mutants carrying N138 substitutions into alanine (N138A) or arginine (N138R) remained unaltered, the overall steady state levels of M-N138 mutated into phenylalanine (N138F), methionine (N138M) or glutamate (N138E) amino acids were impaired. Surprisingly, while cross-linking analyses indicated that all M-mutants displayed proper dimeric-like assembly, M-M co-immunoprecipitation experiments conversely revealed that, except for M-N138A, all mutants displayed a modulated affinity of interaction with another M-unit. Remarkably, all M-mutants remained well transported to the cell periphery and could properly relocate N proteins from the cytosol to the cell periphery, thereby indicating some preservation of the overall M-monomeric/dimeric structure. Furthermore, the expression-deficient M-mutants N138F, N138M and N138E were additionally impaired in triggering the formation of cell protrusions and virus-like particles (VLP). Taken together, while our data suggest that residue N138 of CDV M contributes to the regulation of dimer stabilization, expression and VLP formation, M may self-oligomerize and interact with N through additional discrete microdomains.

O-181

Virology, Session 3: Basic Virology (supported by CUSO) / A-1

WHAT'S IN THE COWS' BRAIN? –TACKLING THE ETIOLOGY OF BOVINE ENCEPHALITIS BY VIRAL METAGENOMICS

D. WÜTHRICH[1], C.L. BOUJON[3], L. TRUCHET[3], S. SELIMOVIC-HAMZA[2], I.G. BOUZALAS[3], R. BRUGGMANN[1], T. SEUBERLICH[3]

[1]Interfaculty Bioinformatics Unit, University of Bern and Swiss Institute of Bioinformatics, 3001 Bern, Switzerland; , [2]Graduate School for Cellular and Biomedical Sciences, University of Bern, 3001 Bern, Switzerland, [3]NeuroCenter, Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Non-suppurative encephalitis is one of the most frequent pathological diagnosis in cattle with neurological disease, but there is a gap in the knowledge on disease-associated pathogens. In order to identify viruses that are associated with non-suppurative encephalitis in cattle, we established a viral metagenomics based analysis pipeline for virus detection and discovery. RNA and DNA libraries were prepared from brain tissue samples of diseased cattle and subjected to Illumina next-generation-sequencing (NGS). Collected sequence reads were (i) mapped to a reference virus genome database for the detection of known viruses and (ii) assembled to contigs and searched for similarities to viral proteins in order to identify novel viruses. We found parainfluenza virus 5, bovine polyomavirus 2 (BoPV-2 SF), ovine herpesvirus 2 and bovine herpesvirus 6 (BHV-6) in a proportion of the affected cattle. In addition we discovered two novel bovine astroviruses (BoAstV CH13 and BoAstV CH15) as well as a putative new bovine betaretrovirus (1, 2). In case-control studies using PCR protocols and in-situ RNA hybridization BoAstV-CH13, BoPV-2 SF and BoHV-6 were statistically associated with the disease. Retrospective studies indicate that BoAstV CH13 is present in brain tissues of around one quarter of cattle with virus encephalitis of unknown etiology and that astroviruses have been involved in the pathogenesis of this disease entity since the mid-20th century. These data expand our knowledge on encephalitis-associated pathogens in cattle and point to the value of NGS in resolving complex infection scenarios in a clinical disease setting.

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O-182

SSM Annual Meeting 2016, Session 3: Host Responses to Microbes / A-2

HEPATITIS C VIRUS STIMULATES MURINE CD8A-LIKE DENDRITIC CELLS TO PRODUCE TYPE I INTERFERON IN A TRIF-DEPENDENT MANNER

S. PFAENDER[1], E. GRABSKI[2], C. DETJE[2], N. RIEBESEHL[1], S. LIENENKLAUS[3], E. STEINMANN[1], U. KALINKE[2], T. PIETSCHMANN[1]

[1]Institute of Experimental Virology, TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany, [2]Institute for Experimental Infection Research, TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany, [3]Department of Molecular Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany

Hepatitis C virus (HCV) strongly induces interferon (IFN) stimulated genes in the infected liver despite of distinct innate immune evasion mechanisms. Recent studies indicate that a rare subset of myeloid dendritic cells (CD141+ DC) produces type III IFN upon stimulation with cell-culture derived HCV whereas plasmacytoid dendritic cells (pDC) are able to produce large amounts of type I IFN. To improve our understanding of the interplay between HCV and dendritic cells, we aimed at dissecting the requirements for DC stimulation by using mouse-derived DC and mouse genetics. Using murine bone marrow-derived in vitro differentiated DC cultures we observed an IFN response upon coculture with HCV RNA transfected human or murine hepatoma cells. Both full length and subgenomic HCV replicons triggered IFN-release by murine DC. Injection of human HCV subgenomic replicon cells into type I interferon reporter mice confirmed the interferon induction upon HCV replication in vivo. Moreover, transfer of conditioned culture fluid from HCV-replicating cells activated DC and elicited IFN release indicating a cell-cell contact independent stimulation of the DC. Using differentiated DC from mice with distinct genetic lesions in innate immune signalling we observed that IFN secretion by HCV-stimulated murine DC was independent of MyD88 and CARDIF, but dependent on TRIF and IFNAR signalling. Separating mouse DC cultures into pDC and conventional CD11b-like cDC and CD8α-like cDC revealed that the CD8α-like cDC, which are homologous to the human CD141+ DC, release interferon upon stimulation by HCV replicating cells. In contrast, the other cell types did not. Collectively, these results indicate that HCV-replicating cells stimulate IFN secretion from murine CD8α-like cDC. This model should be useful to explore the interaction between dendritic cells during HCV replication and to define how viral signatures are delivered to immune cells to trigger IFN release.

O-184

Prokaryotic Biology, Session 2: The 3 Cs – cytoplasm, chromatin and chaperones / A-4

µFACS - A Multi-parametric High-Content Image Analysis Approach to Study Co-Infections across Pathogens

A. YAKIMOVICH[1], C. RACHMÜHL[2], A. RUDNICKA[1], D. SEILER[1], R. WITTE[1], V. ANDRIASYAN[1], Y. YAMAUCHI[1], A. S. ZINKERNAGEL[2], U. F. GREBER[1]

[1]Institute of Molecular Life Sciences, University of Zurich, Switzerland, [2]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Switzerland

Dozens of years of research in the area of microbiota have brought little understanding of how bacteria and viruses influence each other in the way they infect mammalian cells. This is in part due of the considerable complexity of viral and bacterial infections alone. Nonetheless, bacterial and viral co-infections severely impact on clinical disease development and outcome, and can have challenging therapeutic issues, especially since both viral and bacterial infections can be persistent or acute. To deconvolve the complexity of viral-bacterial co-infections, we have developed high content single-cell infection readouts. This can in principle be achieved by microscopy or fluorescence associated cell sorting analyses. While the former approach lacks scale, the latter lacks subcellular resolution and time-resolved information from live cells. Our novel method termed Microscopy-based Fluorescence pAthogen Co-infection Setting (µFACS) scores bacterial and viral infections of eukaryotic host cells at multi-parametric single- cell readouts in high-content. The µFACS assay employs bleeding edge machine learning (deep learning) approaches for the identification of specific infection phenotypes. It is augmented by open source image analysis and data mining software, which potentially allows other researchers to build upon our efforts. Using three major human respiratory viruses, adenovirus, influenza virus, rhinovirus, and Staphylococcus aureus bacteria we show how virus infection is influenced by persistent bacterial infection. This project will help enhance molecular understanding in the emerging field of viral and bacterial co-infections and immunity with medical relevance.

O-186

Virology, Session 3: Basic Virology (supported by CUSO) / A-3

HAX-1 INHIBITS VIRAL REPLICATION OF AVIAN INFLUENZA A VIRUSES AND IS COUNTERACTED BY PB1-F2

B. MAZEL-SANCHEZ[1], I. CARVALHO[1], M. SCHMOLKE[1]

[1]Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland

Influenza A viruses (IAV) are the etiological agent causing a common respiratory infection, influenza. PB1-F2 was identified an important pathogenicity factor. However, this small protein does not contain any intrinsic enzymatic activity and thus likely requires host proteins to mediate its phenotype. We identified a number of host interactors of PB1-F2 under infection conditions using highly sensitive LC-MS/MS. Here we decided to focus on the mitochondrial factor HCLS1-associated protein-X (HAX-1) in more detail. HAX-1 is ubiquitously expressed and was initially described as an anti-apoptotic factor, albeit this largely depends on the investigated cell type. We hypothesized that HAX-1 might play a role in the pathogenicity induced by PB1-F2. First, we confirmed the interaction of HAX-1 and PB1-F2 by IP-WB and confocal IF. Next, we generated cell lines overexpressing or knock-out for HAX-1 and analysed virus replication. HAX-1 overexpression inhibits viral replication of a virus lacking PB1-F2. This dependence on PB1-F2 to overcome HAX-1 restriction appears to be specific for avian viruses but is alternatively compensated in human isolates. Thus, human HAX-1 might be a specific inhibitor of avian influenza A viruses in humans. We are now investigating the functional mechanism by which HAX-1 inhibits avian isolates but not human ones on a molecular level.

O-194

Prokaryotic Biology, Session 1: The envelope – from walls to molecules (continuation) / A-4

PSEUDOMONAS AERUGINOSA IN VIVO OUTER MEMBRANE PROTEOME: ROLE OF SIMPLE PORINS

J.M. BUYCK, [1], P. SAINT AUGUSTE[1], C. SCHLEBERGER[1], D. BUMANN[1]

[1]Focal area infection biology, Biozentrum, University of Basel, Basel, Switzerland.

Pseudomonas aeruginosa is a major opportunistic pathogen. Because of its high intrinsic resistance to antibiotics and the emergence of additional resistance mechanisms, it has become one of the most dangerous infectious agents in hospital-acquired infections. It is often assumed that the P. aeruginosa outer membrane simple porins crucially determine antibiotic translocation and efficacy. Indeed, inactivation of one of its 35 different simple porins, OprD, decreases P. aeruginosa susceptibility specifically to carbapenems. However, if similar links also exist between other antibiotics and simple porins remains largely unclear. To determine which porins P. aeruginosa expresses, we have developed a sensitive targeted mass spectrometry-based proteomic approach. The results showed that diverse P. aeruginosa strains including human clinical isolates, express only a rather small subset of simple porins under various in vitro conditions. As expected, OprF was the dominant simple porin. In addition, five other simple porins (OprD, FadL, OprG, OprQ, OprE) were commonly detected. Six other porins were detected at low and variable levels (Tsx, OpdQ, OpdC, OpdH, OpdP, OprB). All other porins were below our detection threshold of ca. 30 molecules per cell. To assess the functional relevance of these porins, we generated a series of single or multiple gene deletions on PA14 strain. Antimicrobial susceptibility testing revealed that none of the simple porins other than OprD affected minimal inhibitory concentrations (MIC) of diverse antibiotics. Surprisingly, even a delta34 strain lacking all simple porins had identical MIC values compared to parental PA14 (except for carbapenems where the delta34 strain phenocopied the oprD mutant). This strain grew normally on rich media but had severe growth defects on minimal media containing single carbon sources. Interestingly, we did not detect any compensatory expression of cryptic porins in various multiple knock-outs. These results suggest a role of simple porins in nutrient uptake, but do not support current models of antibiotics membrane translocation. In addition of simple porins, alternative translocation routes likely exist. These findings have major implications for the mechanism of antibiotic action against P. aeruginosa and developments of urgently needed novel drugs.

O-202

SSM Annual Meeting 2016, Mini-symposium: “Making the bridge: talking about epidemic outbreak to the broad public” / A-1

MICROBIOLOGY DAY: A TRUE 10-YEARS EXPERIENCE FROM GENEVA

P. LINDER

Professor of Microbiology, Director of the Department of Microbiology and Molecular Medicine University of Geneva Medical School, President of the Biology Platform, Swiss Academy of Science.

On the news, journals, magazines and in the streets, laypersons are continuously overwhelmed with worrisome information and apocalyptic perspectives about microbes. As a consequence, the hidden world of microbiology is very often only associated with dangerous diseases or nasty contaminants. On the contrary, although some microbiological topics must be addressed with the proper concern (e.g. antimicrobial resistance), microbiology can be a marvelous land to be discovered with amazing landscapes and new dimensions to be explored. Launched for the first time in 2007 by the communication platform for life sciences, called BiOutils (University of Geneva, UNIGE), the Microbiology Days want to reduce this gap between concerns and reality. Planned as an annual event, the Microbiology Days take place in the city of Geneva and aim to bring microbiology closer to laypeople. Each year, every event is designed to focus on a social-relevant topic of microbiology (e.g. Microbes: a threat or a new hope?; Microbes and humans: intimate relationship; Viruses without borders), so that seminars, workshops, laboratory visits and discussions may be proposed to the broad public. Thanks to the support of the Faculty of Medicine and the Faculty of Science (UNIGE), 200-350 citizens (on average) attend to these organized events. In 2017 the 10th Microbiology Days will take place in conjunction with the 10 years of BiOutils. While preparing special events that will be held during such an exciting celebration, this presentation will focus on the foundation, challenges, satisfactions and perspectives of the Microbiology Days, described by one of its original founders, Prof. Patrick Linder.

O-203

SSM Annual Meeting 2016, Mini-symposium: “Making the bridge: talking about epidemic outbreak to the broad public” / A-2

CLEAN CARE IS SAFER CARE: ACHIEVING PATIENT AND PUBLIC SAFETY

D. PIRES

Dr. Pittet Group - 1. Infection Control Programme and WHO Collaborating Centre on Patient Safety - Infection Control & Improving Practices, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland - 2. Department of Infectious Diseases, Centro Hospitalar Lisboa Norte and Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal.

Outbreaks are inherently unpredictable. There is no possible way to be ready for an unpredictable event. In this context, having a preparedness plan is the only way to be able to mount an adequate response when needed. Correct perception of an outbreak by the public, stakeholders and policy makers is essential for its management. Indeed, misunderstanding the magnitude or the scope of outbreaks can lead to lack of directed action by leaders and stakeholders. Moreover, misconceptions by the public can lead to dramatic consequences, such as noncompliance with recommendations, panic reactions, harassment of healthcare teams and patients or even complete social disruption. Effective communication is thus essential. The World Health Organization (WHO) developed a guidance document on best practices for communicating with the public during an outbreak. It established five essential practices: building trust, announcing early, being transparent, respecting public concerns, and planning in advance. The recent West Africa Ebola epidemic is a paradigmatic case in various ways. First, it demonstrated the fragility of world leadership to deal with large scale, fatal outbreaks. This was in part due to leadership’s poor perception of the real magnitude of the problem in its early stages. Second, this outbreak took place in an interconnected world. Communication takes place through channels not easily managed by authorities and at very high speed. Social media tools as Facebook®, Tweeter® and Instagram® are instantaneous, constantly updated, lively and open sources of information. This creates endless opportunities but also enormous challenges regarding the quality and flux of information. Our Infection Control Programme and WHO Collaborating Centre on Patient safety team has been working for a decade with WHO to spread the word on Infection Control and Hand Hygiene Promotion. Healthcare ministers of more 139 countries have pledge to take action to reduce healthcare-associated infections through hand hygiene promotion, transforming this programme on the most successful ever in WHO. Most importantly, implementation of the Geneva model of hand hygiene promotion is a reality in the most well equipped healthcare institutions in developed countries as well as in the deprived health facilities in sub-Saharan countries. Communication has been and still is critical to face this challenge. Indeed, “Clean Care is Safer Care” was only made possible through the effective communication of scientific evidence that not only addressed common problems but also common solutions. Building trust in partnership and empowering people is the best way to achieve a common goal: patient and public safety.

O-204

SSM Annual Meeting 2016, Mini-symposium: “From lab with love: how to promote scientific education using microbiology” / A-1

PLAYING WITH MICROBES: DEVELOPING A GAME TO ENHANCE THE POPULATION KNOWLEDGE ON THE THREATS AND PREVENTION OPPORTUNITIES RELATED TO EMERGING PATHOGENS

C KEBBI-BEGHDADI[1], G GREUB[1]

[1]Center for Research on Intracellular Bacteria (CRIB), Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland

In the past decades, important changes occurred in habits and lifestyle of the Swiss population as well as in climatic conditions worldwide. These changes provided new opportunities for microorganisms to emerge as pathogens and enhanced the risks for the population to get in contact with them. In addition, recent advances in microbiology techniques, such as metagenomics or improved culture conditions, allowed the discovery of several new bacterial species, some of them with significant impact on human health.
To inform the general population about emerging pathogens, we intend to create and develop, in collaboration with a game designer and communication professionals, a completely new and innovative board game. Currently available games on emerging pathogens mainly stimulate anxiety and exaggerated fears, our newly developed game will conversely convey a balanced, unbiased and scientifically strong message. We will focus on different aspects related to emerging pathogens such as their reservoirs and vectors, their mode of transmission, real risks to get infected, prevention opportunities, symptoms of related diseases and existing treatments. This communication will also deliver the key messages that many microbes remain to be discovered, that most new pathogenic microbes have been identified during large outbreaks and that research may help identifying these microbes before they cause large suffering in humans. And so, this project will also increase awareness of the general population on the importance of performing research in microbiology.
This new game will be the support of extended interactions with the public since it will be launched together with a cycle of general audience conferences, given by proficient microbiologists, that will particularly emphasize three modes of transmission of these new pathogens i.e. by arthropod bites, by exposure to mammals, birds or reptiles and by exposure to water aerosols (humidifiers, air conditioning systems). Interaction with the public will also takes place thank to an active collaboration with game libraries and to the organisation of “game evenings” followed by open discussions with scientists.

O-205

SSM Annual Meeting 2016, Mini-symposium: “From lab with love: how to promote scientific education using microbiology” / A-2

NEW BUSINESS OPPORTUNITY (NBO) IN MICROBIAL BIOTECHNOLOGY: AN INNOVATIVE EDUCATIONAL PROGRAMME

K. KOVAR

Head of the Bioprocess Technology Centre, Institute of Chemistry and Biotechnology, School of Life Sciences and Facility Management, Zurich University of Applied Sciences ZHAW

The potential for using microbial biotechnology is not fully exploited in many markets for products such as chemicals, dietary supplements, cosmetics, foods, or animal feed. This knowledge gap is challenged by the NBO, which was launched in 2010 and conferred Best Teaching Award 2015 by the ZHAW. This teaching/learning concept was developed for students in the Master’s programme in Pharmaceutical Biotechnology and aims to impart crossdisciplinary knowledge and develop competencies necessary to initiate, evaluate and implement a viable new product or process idea in the field of microbial biotechnology. The didactic concept facilitates collaboration between academia and industry and promotes the introduction of “green technologies” into the Swiss economy. The close interaction with industry practitioners as topic owners and/or coaches of the student teams is highly appreciated. Industry partners may become inspired by opportunities to substitute their traditional production technologies with more sustainable biotechnological processes and are offered a multi-perspective analysis involving technological, financial, regulatory and ecological issues.

POSTER PRESENTATIONS

P-1

Wednesday, June 15th

ANTIBIOTIC PRESCRIBED AND THE RESULTS OF ANTIBIOTIC SUSCEPTIBILITY TESTING IN BURN PATIENTS

LEILA AZIMI[1], SOMAYEH SOLEYMANZADEH-MOGHADAM[2], LALEH AMANI [3], FARANAK ALINEJAD[4], AIDA RASTEGAR LARI [5], ABDOLAZIZ RASTEGAR LARI[6], FERYAL POURMOMEN BOLURCHI[7]

[1]Pediatric Infectious Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2]Department of Biology, University of Qom, Qom, Iran, [3]Pediatric Infectious Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [4]Pediatric Infectious Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [5]Soins Infirmiers - CEGEP Limoilou, 1300 8e Av, Québec, QC G1J 5L5 Canada, [6]Pediatric Infectious Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [7]Pediatric Infectious Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Aim: The aim of this study was to determine relationship between antibiotics prescribed and the results of antibiotic susceptibility testing in microbial laboratory in teaching burn hospital in Tehran, Iran.

Introduction: Burns and subsequent consequences are a global problem. Burn wound infection is one of the most common causes of death in burn injuries. Further, burn patients are at high risk for nosocomial infection. Thus, infection control in burn patients especially in the first 5 days after hospitalization is important. Infection control during this time period can prevent morbidity and mortality in these patients. Appropriate and accurate antibiotic prescription can be considered an important factor in increasing the awareness of patients about proper antibiotic use.

Methods: In this cross sectional study, we consider Pseudomonas aeruginosa, Acinetobacter baumannii and Staphylococcus aureus as most important cause of nosocomial infection especially in burn patients. 525 strains of Pseudomonas aeruginosa, Acinetobacter baumannii and Staphylococcus aureus were isolated from 335 hospitalized burn patients. Identification of the strains was performed by biochemistry and microbiological tests. Antibiotic susceptibility tests were performed according to CLSI guide line for each bacteria genus. The records were audited to find the antibiotic used.

Results: The results indicated that P. aeruginosa is the most prevalent Gram negative bacteria. The results of this study showed some antibiotics prescribed like, imipenem, amikacin, ciprofoloxacin and cefepime regardless of the antibiotics susceptibility responds in the lab.

Conclusion: Infection control is very important in burn care units, because burn wound infection is one of the main causes of morbidity and mortality among burn patients. Thus, the appropriate prescription of antibiotics can be helpful, but unreasonable prescription can have detrimental consequences, including greater expenses to patients and community alike.

P-2

Tuesday, June 14th

SURVEY ON THE ROLE OF ADERS AND OXA23 GENES AMONG IMIPENEM RESISTANT ACINETOBACTER BAUMANNII ISOLATES FROM HOSPITALIZED PATIENTS OF TEHRAN

M HAKEMI-VALA[1], H OSTADASADI -MALAYERI[2], M GHOLAMI[3], H GOUDARZI[1]

[1]Department of microbiology, medical school, Shahid Beheshti university of medical sciences Tehran-Iran, [2]Department of Microiology, Faculty of Basic Sciences, Sanandaj branch, Islamic Azad University, Sanandaj-Iran , [3]PhD candidate, Iran university of medical sciences Tehran-Iran

Background: Acinetobacter baumannii (A. baumannii) has emerged as a highly problematic hospital-associated pathogen and it can be an opportunistic pathogen in humans and affecting people with compromised immune systems[1]. This study aims to evaluate the role of efflux pump regulators and OXA-23 genes in imipenem resistance A. baumannii strains isolated from hospitalized patients.
Material/methods: This study was conducted on 60 A. baumannii isolates collected from patients admitted to the Shahid Motahari and Taleghani Hospitals in Tehran during 2013-14. Antibiotic susceptibility tests (AST) and minimal inhibitory concentration (MIC) was determined by broth micro dilution methods according to CLSI 2014 guidelines [2]. The frequency of efflux pump adeRS and OXA-23 genes were detected by PCR and further sequencing.
Results: The resistance of A. baumannii isolates to tested antibiotics was (100%) to cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, cefepime, piperacillin, meropenem, co-trimoxazole and piperacillin/tazobactam, (97 %) to imipenem, (94%) to gentamicin, (83%) to amikacin, (76%) to tetracycline, and (0.0%) to colistin. The MIC of 58 (96.6%) strains to imipenem was highly decreased in the presence of efflux pump inhibitor (PaβN), by 4 to 64 folds. The adeR and adeS genes were detected in 36 (60%) and 59 (98.3%), respectively and the frequency of OXA-23 gene was 57 (95%) of isolates.
Conclusions: Existence of adeRS and OXA-23 genes in more than 50% of A. baumannii isolates in this study shows the presumptive role of efflux pump in simultaneous of carbapenemase production. So, using new strategies are required in order to stop the vertical or horizontal exchanges mentioned genes from the resistant A. baumannii isolates to sensitive strains. Also doing antimicrobial susceptibility test before any prescription can decrease the emergence of resistant bacteria. However, all tested A. baumannii isolates in this study were susceptible to colistin, hopefully.
Key words: Acinetobacter baumannii, oxa23 beta-lactamase, drug resistance, efflux pumps

P-3

Wednesday, June 15th

DETECTION (EMERGE) OF NDM-1-PRODUCING ESCHERICHIA COLI IN IRAN

M HAKEMI-VALA[1], S AYVAZI[2], A HASHEMI[1]

[1]Department of microbiology, medical school, Shahid Beheshti university of medical sciences Tehran-Iran, [2]Department of Biotechnology, School of Advanced Technologies in Medicine,Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: C The global emerging and spreading of carbapenemase, especially New Delhi metallo β-lactamase-1 (NDM-1) producing enterobacteriaceae, is a concern in treatment of multidrug resistant gram negative infections[1-2]. This is a frist report on emerging of NDM-1 producing Escherichia coli (E. coli), in Iran.
Material/methods: 70 E. coli isolates were obtained from clinical specimens (burn wound and urine) of Motahari burn and Sina hospitals‘ patients in Tehran during 2013. Antibiotic susceptibility test for carbapenems (imipenem, meropenem and ertapenem) and several common antibiotic (piperacillin, amoxiclav, gentamicin, amikacin, ciprofloxacin, cefazolin, ceftriaxone and aztreonam) was done by disk diffusion metod based on CLSI standards[3]. Minimum inhibitory comcentration (MIC) of imipenem and meropenem among resistance isolates was determined by microdilution method.The Modified Hodge Test (MHT) is done for detection of KPC carbapenemase. Double disk synergy test (DDST) by use of EDTA and boronic acid inhibitors was done for determination of MBL and AmpC production, respectively. Also, presence of VIM, IMP, KPC, DIM and NDM-1 genes were studied by PCR.
Results: Among 70 E. coli isolates, only 1isolate from burn wound was resistance to imipenem, meropenem and ertapenem. The MIC of imipenem and meropenem for carbapenem resistant isolate were 8 and 64 µg/mL, respectively. The resistant isolate was resistance to all mentioned antibiotics, except nitrofurantoin. The results of MHT and DDST tests showed the isolate produce MBL enzymes, but it could not produce KPC and AmpC enzymes. The presence of the VIM, DIM and NDM-1 genes were confirmed by PCR in carbapenem resistant isolate. But PCR results for IMP and KPC genes, are negative.
Conclusions: Despite the frequency of carbapenem resistance which is low in Tehran, but emerge of NDM-1 gene is an alarming threat in our country. So, early identification of NDM-1 harboring bacteria and prevention of their spreading must be perform in any region.
Key words: E. coli, ndm 1 beta lactamase, drug resistance

P-4

Tuesday, June 14th

COEXISTENCE OF PORIN OPRD AND MEX-AB-OPRM EFFLUX PUMP GENES AMONG IMIPENEM RESISTANT P.AERUGINOSA ISOLATES FROM BURN PATIENTS

M HAKEMI-VALA[1], F BAGHERY BEJESTANY[2], A HASHEMI[1], P KAVAKEB[3], R MOMTAHENI[2], S RAHIMIGOLLO[2]

[1]Department of microbiology, medical school, Shahid Beheshti university of medical sciences Tehran-Iran, [2]Pharmaceutical sciences branch, Islamic Azad University Tehran-Iran, [3]Sina hospital, Tehran university of medical sciences Tehran-Iran

• Background: Among burn wounds infection agents the role of P.aeruginosa is important because of their high resistant to common antibiotics[1]. So the aim of this study was evaluation the co-existence of oprD porin and MexAB-oprM efflux pump genes among P.aeruginosa isolates from burn patients.
• Materials and methods: During 2013-14, 80 P.aeruginosa isolates were identified and collected randomly from burnt patients who admitted to Burn unit of Motahari hospital. The antimicrobial sensitivity test (AST) by Kirby-Bauer method and Minimum inhibitory concentration (MIC) of imipenem by E-test was determined based on the CLSI 2014 protocol[2]. The co-existence of oprD and Mex-AB-oprM genes was evaluated by PCR and further sequencing.
• Results: Based on the results of AST, resistant to tested antibiotics was as follow; piperacillin 88.7%, ticarcillin 90%, piperacillin-tazobactam 81.3%, ceftazidime100%,cefepime 67.58%, azteronam 88.8%, imipenem 53.8%, meropenem 77%, gentamicin 68%, tobromycin 86.3%, ciprofloxacin 86.3%. By E-test, resistant isolates showed MIC≥ 8µg/mL for imipenem. Simultaneous existence of oprD and Mex-AB-oprM genes were detected in 90.2% and 67.5% of isolates, respectively. Gene submission was done for oprD genes with accession No; KJ154062-3 and is in process for Mex-AB-oprM gene. New mutations in OprD were detected after alignment and comparison with P.aeruginosa PAO1 as follow; T103S, K115T, F170L, P186G and V189T.Among detected mutations, changes of K to T (related to change of lysine to thereonine) and V to T (related to change of valine to thereonine) seems more important because of change in a nonpolar to polar acid amine.
• Conclusion: by the results of this study, sum of the changes of oprD porin and Mex-AB-oprM efflux pump, multidrug resistant (MDR) P.aeruginosa isolates was emerged. The importance of MDR isolates are related to their high mortality rate especially in immune deficient burn patients. Such changes in oprD gene not only can effect on the structural shape but also on the functional property of the OprD protein which is important in drug delivery.
• Key words: oprD, Pseudomonas aeruginosa, Mex-AB-oprM

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Wednesday, June 15th

PHENOTYPIC METHODS DETECTION OF CARBAPENEMASES IN GRAM-NEGATIVE BACTERIA

L. AZIMI[1], A. RASTEGAE LARI[1], P. OWLIA[2], M. POURSHAFIE [3], F. FALLAH[1]

[1]1. Pediatric Infectious Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2]2. Molecular Microbiology Research Center, Shahed University, Tehran, Iran, [3]3. Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran

Carbapenemases production can cause multi antibiotics resistance in Gram-negative bacteria. A simple phenotypic rapid and accurate test for detection of Gram-negative bacteria - carbapenemase-producer can be useful for treatment of infection that cause with them. The aim of this study was to detected carbapenemases and some specific type of that phenotypic and genotypic. In this study, 150 imipenem resistant Gram-negative bacteria were surveyed. Modified Hodge test, boronic acid, ethylenediaminetetraacetic acid and dipicolinic acid were used for detection of carbapenemase, KPC and Metallo-Beta-Lactamases as phenotypic methods, respectively. PCR was performed for detection of carbapenemases genes. Our results indicated that 52.7%, 31.6% and 69.5% Modified Hodge Test, boronic acid and dipicolinic acid positive tests respectively. Non synergism affect was observed between imipnem and ethylenediaminetetraacetic acid. Sixty-nine of strains was confirmed as carbapenemases-producer according to results of molecular tests. Comparison between results of phenotypic and genotypic methods, we can purpose that phenotypic methods just can use for primary screening of carbapenemases and PCR based methods remains as a gold standard for detection of them.

Table. Sensitivity and specificity of MHT for detection of carbapenemases
NPV PPV Specificity Sensitivity
51% 69% 60% 61% MHT for carbapenemases

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Tuesday, June 14th

HIGH LEVEL METHICILLIN RESISTANCE CORRELATES WITH REDUCED STAPHYLOCOCCUS AUREUS ENDOTHELIAL CELL DAMAGE

K. SEIDL[1], M. LEEMAN[1], M. MALHEIROS MARQUES[1], C. RACHMÜHL[1], N. LEIMER[1], F. ANDREONI[1], Y. ACHERMANN[1], AS ZINKERNAGEL[1]

[1]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich

Methicillin-resistant Staphylococcus aureus (MRSA) is a leading cause of nosocomial infections worldwide and has emerged as a community-associated pathogen. While numerous clinical studies suggest that MRSA are more virulent than methicillin-susceptible S. aureus (MSSA) several laboratory studies suggest the opposite. To address this discrepancy, we assessed the intrinsic virulence of 42 MRSA and 40 MSSA strains by testing endothelial cell (EC) damage, a surrogate marker for virulence in blood stream infections. More endothelial cell (EC) damage was induced by MSSA vs. MRSA isolates without reaching statistical significance (64.2 vs. 57.8%, $p = 0.12$). Invasive MSSA strains induced significantly more EC damage vs. invasive MRSA strains ($p = 0.0147$). MRSA isolates with high level oxacillin resistance (>32µg/ml) induced significantly less damage than isolates with relatively low level oxacillin resistance (MIC ≤ 32µg/ml, $p < 0.0001$). The level of oxacillin resistance negatively correlated with the ability to induce EC damage ($R2 = 0.4464$, $p < 0.001$). SCCmec excision in MRSA strains of different SCCmec types and different resistance levels had no significant effect on EC damage despite complete abolishment of oxacillin resistance suggesting that SCCmec itself has no impact on EC damage. Together, our findings suggest that worse clinical outcomes associated with MRSA are not due to a higher intrinsic virulence but due to other factors such as ineffective initial antimicrobial treatment or prognostic factors confounding with MRSA infections.

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Tuesday, June 14th

MOLECULAR BASIS FOR THE ADAPTATION OF STAPHYLOCOCCUS AUREUS ST398 TO THE HUMAN BLOODSTREAM

SDM DIENE[1], ARG CORVAGLIA[1], PF FRANÇOIS[1], NVDM MEE-MARQUET[2]

[1]Genomic Research Laboratory, Service of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland, [2]Département de Microbiologie, Centre Hospitalier Régional Universitaire, and UMR 1282, Infectiologie Santé Publique, Université François-Rabelais, Tours, France

The first bloodstream infection (BSI) due to Staphylococcus aureus sequence type 398 (ST398-BSI) was identified in 2007 during a survey of BSIs conducted since 2000 in France. The incidence of ST398-BSI increased 10-fold during the period 2007-2015, when 1520 S. aureus-BSIs were reported. Patients with ST398-BSI were younger than those suffering from non ST398-BSI. ST398-BSI isolates were mostly methicillin-susceptible (98.7%), erythromycin-resistant (84.2%), and of t571or t1451 spa-types (81.1%). Whole genome sequencing of 18 representative isolates identified: (1) 30 prophages carrying genes involved in S. aureus pathogenesis including virulence, resistance to foreign DNA uptake, cell division, biofilm formation and bacterial persistence; (2) most BSI isolates sharing a core genome and a variable accessory gene pool (from a sole φ3-prophage to multiple MGEs); (3) BSI isolates harboring animal-associated MGEs (SCCmec XI remnants and prophages); and (4) animal-infecting isolates harboring a φ3-prophage variant, a human niche signature. These results provide evidence of on-going and possibly multidirectional gene transfer within the ST398 lineage. Multiple, recent and independent acquisitions of genetic features by horizontal gene transfer appear responsible for the selection of isolates showing increased ability to cause infection in humans and/or to spread into environments dominated by humans.

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Wednesday, June 15th

THE MORE, THE BETTER? WHEN TO USE HIGHLY MULTIPLEXED ASSAYS FOR THE DETECTION OF GASTROINTESTINAL PATHOGENS.

L NAEF[1], L BERLINGER[1], E CLAAS[2], M ALTWEGG[1]

[1]Bioanalytica, Maihofstrasse 95a, Lucerne, Switzerland, [2]LUMC, Leiden, Netherlands

Background: Conventional methods reveal established pathogens only in a minority of patients with infectious diarrhea. Amplification methods are more sensitive and more rapid and thus have a higher clinical impact. However, the question remains whether highly multiplexed assays like the FilmArrayTM Gastrointestinal Panel (22 different pathogens detected) or more targeted assays like the BD MAXTM Enteric Bacterial Panel (detecting Salmonella, Shigella/EIEC, Campylobacter and EHEC) are more adequate for routine use also in view of the significantly different reagent costs. Methods: A total of 112 stool specimens (patients >12y; no suspicion for C. difficile-related infection) were selected based on EBP-result (10 positive for Salmonella, 8 for Shigella/EIEC, 26 for Campylobacter and 9 for EHEC; 59 negatives) and travel history. All specimens were analyzed by FilmArrayTM within 24 hours. Discrepancies were resolved by in-house PCR. Results for EPEC were excluded from the analysis due to little clinical significance in adults. Results: As compared to the EBP, the FilmArrayTM had a sensitivity/specificity of 100%/99.0% for Salmonella, 100%/100% for Shigella/EIEC, 95.8%/98.8% for Campylobacter and 100%/100% for EHEC. In patients with (N=51) and without (N=61) recent travel, 21 (41%) and 30 (49%) were found positive by the EBP whereas 35 (69%) and 34 (56%) were positive with the FilmArrayTM. EAEC (18/51; 35%) and ETEC (14/51; 27%) were the most prevalent organisms in travelers, Campylobacter (20/61; 33%) in non-travelers. More than one pathogen was found with the FilmArrayTM in 3/34 (9%) positive patients without but in 20/35 (57%) with recent travel. Of these, 10, 8, 1 and 1 harbored 2, 3, 4 and 5 different pathogens, respectively. Conclusions: We conclude that the highly multiplexed FilmArrayTM detects a putative pathogen in more patients and also reveals a higher number of double or multiple infections. However, this is valid only for patients with recent travel to a developing country and not for those with locally acquired infectious diarrhea. Our results may help to select the most appropriate and cost-effective diagnostic approach based on patient selection.

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Tuesday, June 14th

EVALUATION OF TWO NEW CHEMILUMINESCENCE IMMUNOASSAYS FOR THE DETECTION OF CLOSTRIDIUM DIFFICILE GLUTAMATE DEHYDROGENASE AND TOXIN A&B

A BLAICH[1], C CASTELLANO[1], C KIESSLING[1], A GESCHKE[2], K RENTSCH[2], A EGLI[1], R FREI[1]

[1]Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland, [2]Division of Clinical Chemistry, University Hospital Basel, Basel, Switzerland

This study evaluated the automated LIAISON C. difficile glutamate dehydrogenase (GDH) assay (DiaSorin) in comparison to the C. DIFF CHEK-60 assay (TECHLAB/Alere) and in GDH-positive stool samples the automated LIAISON C. difficile Toxins A&B assay (DiaSorin) in comparison to PCR and toxigenic culture. DiaSorin offers a stool extract preparation in a sealed tube prior to testing on the LIAISON. Stool samples submitted for the detection of C. difficile were routinely screened for C. difficile specific antigen GDH with the C. DIFF CHEK-60 (GDH-Alere) and the LIAISON GDH assay. GDH-positive samples with one or both of these assays were further tested with the Xpert C. difficile PCR assay (Cepheid) and toxigenic culture. GDH-LIAISON-positive samples were analyzed for toxin in a reflex-testing with the LIAISON C. difficile Toxins A&B assay. PCR and toxigenic culture were considered as gold standard. Of the 545 samples tested with the GDH-Liaison 454 samples were true-negative, 13 false-positive, 77 true-positive, and 1 false-negative; with the GDH-Alere 460 samples were true-negative, 7 false-positive, 73 true-positive, and 5 false-negative. Thus, the GDH-LIAISON and the GDH-Alere possess a sensitivity of 98.7% and 93.6% and a specificity of 97.2% and 98.5%, respectively. If PCR for toxin detection was negative, but culture positive, a PCR from culture (toxigenic culture) was additionally performed. Overall, we found 65 toxin-positive and 26 toxin-negative samples by PCR and toxigenic culture. With the Liaison toxin A&B assay 24 samples were true-negative, 2 false-positive, 39 true-positive, and 26 false-negative. Thus, the Toxin-LIAISON assay possesses a sensitivity of 60% and a specificity of 92.3%. The LIAISON GDH assay compared to the GDH-Alere assay is more sensitive (98.7% vs 93.6%) but slightly less specific (97.2% vs 98.5%). For a screening test like the GDH-assay high sensitivity is more important than specificity. For reflex-testing the sensitivity of 60% of the LIAISON Toxins A&B assay is in accordance to the sensitivity of other immunoassays described in literature. The stool sample preparation and automated analysis on the LIAISON are convenient and neat.

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Wednesday, June 15th

COMPARISON OF TWO RAPID BIOCHEMICAL TESTS AND TWO CHROMOGENIC SELECTIVE MEDIA FOR DETECTION OF CARBAPENEMASE-PRODUCING GRAM-NEGATIVE BACTERIA

V. HINIC[1], I. AMREIN[2], D. MEINEL[1], R. FREI[1], A. EGLI[1]

[1]Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland, [2]Bioanalytica AG, Lucerne, Switzerland

Objectives: We compared the performance of two commercially available biochemical screening tests and two chromogenic selective media for detection of carbapenemase-producing Gram-negative bacteria.

Materials and methods: RAPIDEC® CARBA NP (bioMérieux) was performed according to the recently published updated protocol (JCM 2015,53:12) and Neo-Rapid CARB test (Rosco Diagnostica) according to the manufacturer's protocol. The biplates chromID® CARBA SMART (CARB/OXA; bioMérieux) and BrillanceTM CRE/ESBL (Thermo Scientific) were streaked with 1 µl of 104 CFU/ml bacterial suspensions. Carbapenemase-positive strains not growing on chromID® CARBA SMART were additionally inoculated onto chromID® ESBL (bioMérieux). 54 well characterized carbapenemase-producing clinical isolates were tested: 35 Enterobacteriaceae (8 KPC, 1 IMI, 8 NDM, 7 VIM, 9 OXA-48-like and 2 OXA-48-like/NDM), 10 Pseudomonas aeruginosa (6 VIM, 3 IMP and 1 SPM) and 9 Acinetobacter baumannii (5 OXA-23, 3 OXA-40 and 1 OXA-23/NDM). Additionally, 46 carbapenemase-negative strains were analyzed including 12 ESBL, 17 AmpC and 1 K1. To ensure objective reading and interpretation, all strains were coded and tested blindly.

Results: The overall sensitivity of RAPIDEC® CARBA NP and Neo-Rapid CARB test was 87.0% (47/54) and 72.2% (39/54), respectively. Sensitivity calculated without A. baumannii strains was 95.5% (43/45) for RAPIDEC® and 86.7% (39/45) for Neo-Rapid CARB. Specificity for both tests was 100%. The sensitivity of chromID® CARBA SMART biplate was 90.7% (49/54), but when chromID® ESBL plate was added, the sensitivity increased to 100%. Sensitivity of the CRE part of BrillanceTM biplate was 75.9% (41/54), but together with the ESBL part 98.1% (53/54).

Conclusions: Although sensitivity of RAPIDEC® CARBA NP was significantly better than of Neo-Rapid CARB test, the performance with OXA-producing A. baumannii and OXA-48-like Enterobacteriaceae was not optimal in this blinded study. The highest sensitivity for carbapenemase screening could be achieved when an ESBL screening plate was added to chromID® CARBA SMART biplate and when BrillanceTM CRE was used in combination with BrillanceTM ESBL.

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Tuesday, June 14th

COMMON SKIN INFECTIONS DUE TO PANTON-VALENTINE LEUCOCIDIN-PRODUCING STAPHYLOCOCCUS AUREUS STRAINS IN ASYLUM SEEKERS FROM ERITREA

LJ JATON[1], TP PILLONEL[2], KJ JATON[2], ED DORY[1], GP PROD'HOM[2], DB BLANC[2], FT TISSOT[3], PB BODENMANN[1], GG GREUB[2]

[1]Policlinique Médicale Universitaire, Lausanne, [2]Institut de microbiologie, CHUV, Lausanne, [3]Service d'infectiologie, CHUV, Lausanne

Background: Since late 2014, a high number of abscesses and boils due to Staphylococcus aureus expressing the Panton-Valentine leukocidin (PVL) were observed in Eritrean asylum seekers. We used Whole Genome Sequencing (WGS) to identify if all strains were identical or not and we checked for the presence of different kind of toxins and adhesins encoding genes; in addition, a phylogenic tree was reconstructed based on core genome single nucleotide polymorphisms (SNPs).
Methods: A total of 15 S. aureus PVL-producing strains were analyzed. Clinical and epidemiological characteristics of patients were compared to presence of adhesins, toxins and the presence of clusters of similar strains was investigated.
Results: There was 14 MSSA and 1 MRSA infections. 14/15 PVL-positive patients exhibited a skin infection (abscess, boil, adenitis or cellulitis), and 1 presented a necrotising pneumonia. Genomic sequencing showed that 8 cases (all MSSA strains) were due to MLST Sequence Type (ST) 152, 3 from ST15 and 1 each from ST 121, 72, 80 and 5. The 8 cases infected by ST152 strains included 4 Eritreans men and one Ethiopian woman, as well as two Swiss and one Portuguese patients, all in contact with African persons.
Conclusions: This work suggests that these S. aureus subtypes 152 and 15 are widespread in East Africa and that our patients have been infected by different strains of the 152 and 15 subtypes, respectively. WGS analysis showed that we are not actually facing an ongoing outbreak of infection due to PVL-producing S. aureus strains. In front of an unusual dermatological infection in African refugees, and before searching for an exotic infection (leishmaniosis, rickettsiosis or mycobacteria), clinicians must be aware that it could be a S. aureus PVL-producer. Finally, this study showed that whole genomic sequencing is a good promising tool to rapidly assess the risk of facing an epidemic.

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Wednesday, June 15th

DEVELOPMENT OF RESISTANCE OF MUTANS STREPTOCOCCI AND PORPHYROMONAS GINGIVALIS TO CHLORHEXIDINE DIGLUCONATE AND FLUORIDE-CONTAINING MOUTHRINSES

E.M. KULIK[1], T. WALTIMO[1], R. WEIGER[1], I. SCHWEIZER[1], K. LENKEIT[1], E. FILIPUZZI-JENNY[1], C. WALTER[1]

[1]UZB - University Center for Dental Medicine, University of Basel, Hebelstrasse 3, 4056 Basel, Switzerland

Objective: The aim if this study was to determine the minimal inhibitory concentrations of chlorhexidine digluconate and an amine fluoride/stannous fluoride-containing mouthrinse against Porphyromonas gingivalis and mutans streptococci during an experimental long-term subinhibitory exposition.

Material and methods: Five P. gingivalis strains and four mutans streptococci were subcultivated for 20–30 passages in subinhibitory concentrations of chlorhexidine digluconate or an amine fluoride/stannous fluoride-containing mouthrinse.

Results: Pre-passaging minimal inhibitory concentrations for chlorhexidine ranged from 0.5 to 2 mg/l for mutans streptococci and from 2 to 4 mg/l for the P. gingivalis isolates. For the amine fluoride/stannous fluoride-containing mouthrinse minimal inhibitory values from 0.125 to 0.25 % for the mutans streptococci and from 0.063 to 0.125 % for the P. gingivalis isolates were determined. Two- to fourfold increased minimal inhibitory concentrations against chlorhexidine were detected for two of the five P. gingivalis isolates, whereas no increase in minimal inhibitory concentrations was found for the mutans streptococci after repeated passaging through subinhibitory concentrations. Repeated exposure to subinhibitory concentrations of the amine fluoride/stannous fluoride-containing mouthrinse did not alter the minimally inhibitory concentrations of the bacterial isolates tested.

Conclusion: Chlorhexidine and the amine fluoride/stannous fluoride-containing mouthrinse are effective inhibitory agents against the oral bacterial isolates tested. No general development of resistance against chlorhexidine or the amine fluoride/stannous fluoride-containing mouthrinse was detected. However, some strains showed potential to develop resistance against chlorhexidine after prolonged exposure.

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Tuesday, June 14th

THE ANTIBACTERIAL EFFECTS OF TEA

S HEDAYAT[1]

[1]Unit of clinical microbiology,Department of infectious diseases,Barts and the London school of Medicine and Dentistry, Blizard institute ,Queen Mary University of London, London, UK (United Kingdom)

Antibiotic properties of different types of tea against different microorganisms were detected whether the teas are able to inhibit growth of the microorganisms. At first, antibiotic properties of extract of different types of teas were investigated on different microorganisms staphylococcus aureus, staphylococcus epidermidis, streptococcus faecalis, E.coli, Pseudomonas aeruginosa, Listeria ivanovii, Acinetobacter, Klebsiella species by well method and BSAC method. In the second step, the disc diffusion sensitivity test was applied for MRSA and ESBL E.coli by BSAC method by measuring of zone sizes. The MRSA and ESBL E.coli were tested against all the tea hot extracts using two methods well cut in agar and paper disk dipped in the tea. Then synergy testing of MRSA and ESBL E.coli organisms was performed with the same antibiotics used for BSAC sensitivity test along with paper disc of hot extract of teas. Changes in zone size or shape were observed. Moreover, zone size of antibiotics and teas compared with second part. The results of testing of the all of the teas against the microorganisms showed zone of inhibition for Staph aureus and staph epidermidis with some tea types. There was no zone of inhibition for E.coli, Pseudomonas aeruginosa, Klebsiella species. These organisms are resistant to all of the teas. The results of testing the microorganisms MRSA and ESBL E.coli against all the hot tea extracts using well method and paper disc method showed that some teas were able to inhibit the growth of MRSA while ESBL E.coli was resistant to all of teas in both methods. There was no synergy between hot tea extracts and antibiotics used for BSAC sensitivity test for MRSA and ESBL E.coli. Tea extracts are not able to improve the effect of antibiotics on MRSA and ESBL E.coli. In this research, Gram-positive bacteria MRSA, Staph aureus and staph epidermidis are more sensitive than Gram-negative bacteria to antibiotic properties of tea extracts. The structure of the bacterial cell wall and the different affinities of the main catechin (EGCG) of tea with various cell wall components are responsible for the different susceptibilities of the observed antibacterial activity.

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Wednesday, June 15th

ANTIMICROBIAL RESISTANCE IN ENTEROPATHOGENIC ESCHERICHIA COLI FROM PIGS IN SWITZERLAND, 2014-2015

P BRAND[1], S GOBELI[1], V PERRETEN [1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Längassstrasse 122, CH-3001 Bern, Switzerland

Antibiotics like sulphonamides, tetracyclines and colistin are frequently used to treat diarrhoea in pigs caused by enteropathogenic E. coli posing the risk of selecting antibiotic resistant strains. The recent report of plasmid-mediated colistin resistance in E. coli raised the question whether this resistance is also emerging in enteropathogenic E. coli from pigs in Switzerland. We therefore screened a collection of 95 strains isolated in 2014 and 2015 by measurement of the minimal inhibitory concentration (MIC) of 14 different antibiotics and detection of resistance genes using a microarray. Forty-five percent of the isolates were susceptible to all of the antimicrobial agents tested with the MICs situated below the EUCAST resistance breakpoints. All isolates were susceptible to 3rd generation cephalosporins, carbapenems, colistin and tigecycline. Except for colistin, these antibiotics are not used in medicated feed in pigs. However, the most commonly found resistances were those to classes of antibiotics which are routinely used in pig husbandry. Indeed, resistance to sulfamethoxazole [44%, sul1, sul2, sul3], tetracycline [43%, tet(A), tet(B)] and trimethoprim [32%, dfr (A)] were the most frequent resistance traits, similar to those already observed in 2003 in Switzerland (1). This study showed that resistance to antibiotics of the latest generation as well as to colistin did not emerge yet in enteropathogenic E. coli from pigs in Switzerland. The most common resistances are those to antibiotics which are commonly used for the treatment of pigs via medicated feed like sulphonamides and tetracyclines. It is therefore of major importance to avoid selection of further resistance by inappropriate use of antibiotics, like e.g. colistin. Consequently, a correct identification of the causative agent and accurate antibiotic susceptibility testing prior to therapy of diarrhoeic pigs is strongly recommended for an adequate therapy of bacterial infections. Additionally, continuous efforts in reducing the use of antibiotics in animal husbandry have to be made to keep the level of resistance low.

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INTESTINAL COLONIZATION WITH EXTENDED-SPECTRUM CEPHALOSPORIN-RESISTANT ENTEROBACTERIACEAE IN HEALTHY SWISS PETS: PREVALENCE AND RISK FACTORS

J PIRES[1], O.J. BERNASCONI[1], R TINGUELY[1], S KASRAIAN[1], C HAUSER[2], M HILTY[1], V PERRETEN[3], A ENDIMIANI[1]

[1]Institute for Infectious Diseases, University of Bern, Bern, Switzerland, [2]Department of Infectious Diseases, Bern University Hospital, University of Bern, Bern, Switzerland, [3]Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland

Background: Pets represent a reservoir of extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESC-R-Ent) that may increase the risk for owners to be colonized. However, prevalence and risk factors for colonization with ESC-R-Ent in pets are poorly studied. We aimed to study this problem in pets living in Swiss households.Methods: 66 pets of 56 different households were analyzed. Stools were enriched in LB broth with cefuroxime and plated on BLSE, ChromID ESBL and Supercarba plates. Species ID was obtained using the MALDI-TOF MS. Trek panels were used to obtain MICs. Microarray CT103XL and PCR/sequencing were used to characterize bla genes. Clonality was determined by MLST. A questionnaire regarding pets was filled in by owners.Results: The prevalence of intestinal colonization was 9.1%. Past invasive procedures was found to increase the risk of being colonized (OR, 14.6; $P<0.05$). Other factors (age, previous antibiotic use or hospitalization, raw meat consumption) were not statistically significant. 7 E. coli were recovered from pets, of which 5 were ESBL (2 CTX-M-15 and CTX-M-1/-14, SHV-12) and 2 CMY-2 pAmpC producers. Interestingly, one dog was co-colonized with both CTX-M-15 and CMY-2 producers. Isolates were diverse, each belonging to an individual ST (ST73, ST949, ST963, ST162, ST127, ST1421, ST56). The ESBL diversity was similar to what is seen in colonized humans, whereas clonal diversity was fairly different (only ST73 and ST127 previously reported in humans), indicating that clones circulating among pets are different than those found in humans. However, presence of blaESBLs common in both E.coli from humans and pets suggests transfer of resistance between the different E. coli in both hosts.Conclusions: This is the first study assessing clinical and lifestyle data of pets colonized with ESC-R-Ent. The prevalence in pets is consistent with what is reported in healthy humans living in Switzerland. Since the ESBLs identified in pets are similar to those found in humans, but associated with different STs, a further characterization of the plasmids carrying these resistance genes is essential.

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Tuesday, June 14th

HIV-POSITIVE INDIVIDUALS IN SWITZERLAND AND INTESTINAL COLONIZATION WITH EXTENDED-SPECTRUM CEPHALOSPORIN-RESISTANT E. COLI STRAINS

J PIRES[1], O.J. BERNASCONI[1], S KASRAIAN[1], R TINGUELY[1], C HAUSER[2], A RAUCH[2], H FURRER[2], A ENDIMIANI[1]

[1]Institute for Infectious Diseases, University of Bern, Bern, Switzerland, [2]Department of Infectious Diseases, Bern University Hospital, University of Bern, Bern, Switzerland

Background. The intestinal microbiota of HIV-positive individuals has been shown to be in a state of dysbiosis. This might influence the susceptibility of this population to develop specific gastrointestinal diseases. However, nothing is known about the influence of HIV infection on the intestinal colonization with extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESC-R-Ent). In this study, we aimed to address this research question.
Methods. Sixty-seven HIV-positive volunteers followed by Bern University Hospital have been enrolled (March 2015 to January 2016). Stools of individuals and their pets (if any) were enriched overnight in LB broth with cefuroxime and plated on BLSE, ChromID ESBL, and Supercarba selective plates. At least five ESC-R-Ent colonies per sample were recovered. Species ID was obtained with the MALDI-TOF MS. Microarray CT103XL and PCR/DNA sequencing were used to characterize the bla genes. Clonality was assessed by MLST. An epidemiological questionnaire was obtained from the volunteers.
Results. The prevalence of intestinal colonization was 3% (n=2). All ESC-R-Ent recovered were E. coli. Interestingly, one of the volunteers was colonized with two different E. coli clones: a CTX-M-15-producing ST405 and a CTX-M-1-producing ST127. The other one was colonized with a CTX-M-1-producing ST410. All these STs are usually defined as hyperepidemic clones (HiRC). Remarkably, the individual colonized with two clones is the only case where the pets living within the same household were also colonized with ESC-R-Ent. In particular, one of the cats was colonized with the same CTX-M-1-producing ST127 clone identified in the human, while the other cat with unrelated clone (ST73) and ESBL (CTX-M-15).
Conclusions. The prevalence of intestinal colonization with ESC-R-Ent in the Swiss HIV-population is low. Nevertheless, ESBLs and clones identified resemble those frequently associated with HiRC causing serious infections. Moreover, the identification of the same clone within the same household highlights the potential silent spread of these pathogens between pets and humans in close contact.

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MRSA CLONE USA 300 IN PIGS FROM CUBA

M BAEZ [1], A COLLAUD[1], I ESPINOSA[2], V PERRETEN[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern Länggass-Strasse 122, CH-3012 Bern, Switzerland, [2]National Centre for Animal and Plant Health (CENSA), San José de las Lajas, Mayabeque, PC 32700, Cuba.

Methicillin-resistant Staphylococcus aureus (MRSA) represents a major cause of human infections in hospitals and community. Livestock, particularly pigs, are known to act as a reservoir for MRSA which may be transmitted to humans. It is therefore of public health importance to determine the prevalence and type of MRSA in pig husbandry. Up to date, the MRSA colonization status in pigs in Cuba was still unknown.
In 2015, 285 nasal swabs were collected at one slaughterhouse from pigs from 3 provinces of Cuba revealing a MRSA carriage prevalence of 7.7% (95% CI4.61% - 10.81%). MRSA were characterized using pulsed-field gel electrophoresis (PFGE), arginine catabolic mobile element (ACME), staphylococcal cassette chromosome mec (SCCmec), spa and multilocus sequence typing (MLST) methods, as well as virulence and antimicrobial resistance genotyping. Antimicrobial resistance phenotype was determined by the measurement of the minimal inhibitory concentration.
Eighteen of 22 MRSA belonged to a clonal lineage related to clone USA300 (ST8, SCCmec IV, PVL+). The pig MRSA were subdivided into two clonal clusters: i) MRSA clones ST8-t024-IV [PVL+/ACME I+] (PFGE A) (9/22) contained the Panton–Valentin leukotoxin (PVL) and the sek and seq enterotoxin genes, and were resistant to ß-lactams (mecA/blaZ), macrolides [msrA, mph(C)] aminoglycosides [aph(3')-III] and fluoroquinolones [grlA(S80-Y)/gyrA(S84-L)]; ii) MRSA clones ST8-t008-IV [PVL+/ACME-] (9/22) (PFGE B) were ACME negative variants and contained PVL but no enterotoxin, and were only resistant to ß-lactams (mecA/blaZ). This study revealed that pigs from Cuba predominantly carried MRSA clones associated with USA300, a clone which is spreading worldwide in human hospitals and community. The presence of such multi-resistant USA300 clones carrying leukotoxin in pigs is worrisome since they may spread into the community of people working with pigs in Cuba.

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PANTON-VALENTINE LEUCOCIDINE (PVL) IN STAPHYLOCOCCUS AUREUS ISOLATES: PREVALENCE IN HEALTHY CARRIERS AND CLINICAL SAMPLES IN SWITZERLAND

M. OBERLE[1], A. HASLER[1], C. OTTIGER[1], H. FANKHAUSER[1]

[1]Medizinische Mikrobiologie, Institut für Labormedizin, Kantonsspital Aarau, 5001 Aarau, Schweiz

Background: Panton-Valentine leucocidin (PVL) is a toxin responsible for increased severity in Staphylococcus aureus infections. PVL is encoded by the two cotranscribed genes lukS-PV and lukF-PV and is carried on a bacteriophage. Diagnosis for the presence of the PVL genes in clinical S. aureus isolates is rarely done. Therefore the prevalence of PVL in S. aureus from clinical isolates and from healthy carriers is largely unknown in Switzerland. The aim of this study was to measure the frequency of PVL positive S. aureus isolates in healthy Swiss carriers and in clinical specimens. Methods: S. aureus isolates from diverse clinical samples were selected retrospectively. Nasal swaps from healthy individuals were collected, and S. aureus isolates were analysed for PVL. Detection of the lukS-PV/ lukF-PV genes was done with real time PCR (RIDA PVL kit, rBiopharm on a LighCycler 2.0, Roche). Results: Sixty S. aureus carriers from 197 nasal swabs of healthy individuals were identified. No PVL-positive S. aureus was found among the healthy carries. Eighteen (30%) of the 60 clinical isolates were tested positive for PVL. Eleven isolates derived from wound swabs, six from biopsies and sterile fluids and one was isolated from a blood culture. Interestingly, one third (six) of the PVL-positive isolates were Methicillin susceptible S. aureus (MSSA) and the remaining (12) were MRSA. Conclusions: The prevalence of PVL-positive S. aureus among the healthy Swiss population (0 out of 60) seems to be very low in comparison to the clinical isolates ($p < 0.001$). This suggests that former healthy carriers of PVL have a high risk for severe S. aureus infections. Of interest is that not only MRSA but also MSSA do contain the PVL. Such isolates may worsen the clinical outcome and may require an adjusted treatment. Nevertheless, the detection of the lukS-PV/ lukF-PV genes is seldom asked in clinical microbiology laboratories suggesting underestimation of PVL-positive MSSA's.

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MOLECULAR MECHANISMS OF INTRINSIC STREPTOMYCIN RESISTANCE IN MYCOBACTERIUM ABSCESSUS

M. DAL MOLIN[1], M. GUT[1], K. HALDIMANN[1], P. SANDER[1]

[1]Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland

Mycobacterium abscessus is an emerging pathogen which exhibits a broad range of intrinsic drug resistance mechanisms. The high level of drug resistance not only limits treatment options for patients infected with M. abscessus but also hampers genetic experiments with M. abscessus. Amongst others, M. abscessus shows high levels of resistance towards first- and second-line TB drugs, including resistance towards Streptomycin (STR), the first effective TB drug. Clinically acquired STR resistance in Mycobacterium tuberculosis is either due to alterations in specific codons of rpsL (encoding for small ribosomal subunit protein S12) or due to mutations in specific regions of rrs (encoding for the small ribosomal RNA; 16S rRNA). Sequence alignments between M. tuberculosis and M. abscessus did not indicate any evidence for resistance associated polymorphism in rpsL or rrs of M. abscessus. Instead, an Open Reading Frame (MAB_2385) encoding a putative antibiotic modifying enzyme, a potential 3''-Streptomycin phosphotransferase, is present in the genome of M. abscessus, while a corresponding gene is missing in the genome of M. tuberculosis and Mycobacterium smegmatis, respectively. Heterologous expression of MAB_2385 in M. smegmatis significantly increased STR Minimal Inhibitory Concentration (MIC) of the recombinant strain as compared to the parental M. smegmatis thus indicating that MAB_2385 encodes a functional 3''-Streptomycin phosphotransferase. Accordingly, deletion of MAB_2385 from the genome of M. abscessus decreased the STR MIC, while MIC towards other aminoglycosides or unrelated drugs remained unaltered. Wild-type like STR resistance was restored upon transformation of the deletion mutant with a single copy complementation vector. Taken together, our experiments indicate that MAB_2385 is the major STR resistance determinant in M. abscessus.

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DECIPHERING AMINOGLYCOSIDE RESISTANCE MECHANISMS OF MYCOBACTERIUM ABSCESSUS

A. ROMINSKI[1], P. SELCHOW[1], J. K. BRÜLLE[1], K. BECKER[1], A. RODITSCHEFF[1], E. C. BÖTTGER[1], P. SANDER[1]

[1]Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland

Mycobacterium abscessus is one of the most pathogenic and chemotherapy-resistant organisms among rapidly growing mycobacteria. The M. abscessus genome encodes various enzymes that are able to modify crucial residues of aminoglycoside (AG) drugs leading to their inactivation. The presence of these drug resistance genes hampers treatment options. M. abscessus is predicted to possess a single AG 2´-N-acetyltransferase and two putative multi-acetylating AG acetyltransferases of the Eis (Enhanced intracellular survival) family. However, the exact function, their role in drug resistance and the interplay of these genes remain under cover. Elucidation of drug resistance heavily relies on the ability for genetic manipulation of M. abscessus, particularly on generation of mutants by targeted gene inactivation. By applying established and novel genetic tools, the aim of this study is to elucidate the detailed resistance mechanisms of M. abscessus towards AGs. Based on drug susceptibility patterns of clinical isolates, we exploited our newly developed genetic tools to generate targeted deletion mutants of M. abscessus aac(2´), eis1 and eis2 and characterized their drug resistance profile. Compared to the parental strain, the Δaac(2´) and Δeis2 mutants proved to be highly sensitive towards specific AGs. Our MIC data clearly demonstrate that antibacterial activity of AGs characterized by an NH2 group at the 2´-position against M. abscessus WT is low, as compared to AGs carrying a corresponding OH group. Genetic inactivation of aac(2´) in M. abscessus confers susceptibility to AGs carrying a 2´-NH2 group while not affecting susceptibility towards AGs with a 2´-OH group. Modification of AGs with an L-HABA side chain prevents Aac(2´) mediated drug inactivation. Genetic inactivation of eis2 increased susceptibility towards a distinct set of AGs, while targeted deletion of eis1 did not alter susceptibility against any of the AGs tested. Characterization of the M. abscessus Aac(2´) and Eis2 mediated AG resistance mechanisms at the molecular level may have significant implementation for the development of new intervention strategies against this highly resistant pathogen.

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MOLECULAR MECHANISM OF TOXIN-ANTITOXIN SYSTEMS IN M. TUBERCULOSIS

MM MANSOUR[1]

[1]laboratory of microbiology and molecular genetics

The aim of this project is to understand the molecular mechanism and the physiological role of toxinantitoxin systems (TAS) in mycobacteria. We propose to characterize the three HigBA systems of M.tuberculosis, focusing mainly on the toxins, which belong to the large RelE superfamily of ribosome-dependent RNases known to have very diverse mechanisms and substrate specificities. We will investigate possible crosstalk and cooperation among these systems, as well as their functional interplay with the SecB-like chaperone of the atypical tripartite HigB1-HigA1-Chaperone system (TAC) of Mtb, known to specifically control the HigB1 toxin inhibition cascade. A comprehensive analysis of the three Mtb-HigB cellular targets and the precise cleavage sites within these targets will be determined using the non-phosphorylated EMOTE methodology, which will allow the exact mapping of 5'-OH toxin cleaved sites in mRNA on a genome-wide scale (with PV and LF partners). Next, we will examine whether Mtb-HigB cleavage requires translating ribosome and possibly explore the nature of secondary structures of mRNA around cleavage sites (with LF). Finally, antibiotic profiling in the presence of each Mtb-HigB toxins will be performed and correlated with the identification of cellular toxin targets by nEMOTE (with PV). Such entirely novel approach will hopefully reveal interesting pathways for antibiotic tolerance/sensibility involving toxins, as well as compounds that block or stimulate toxins activity in vivo. If successful, the most interesting targets will be selected for further genetic studies.

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SUILYSIN STIMULATES HEPARIN BINDING PROTEIN RELEASE FROM NEUTROPHILS AND INCREASES VASCULAR PERMEABILITY IN MICE

S. CHEN[1], W. XIE[1], H. JIANG[1], Y. YUAN[1], Y. JIANG[1]

[1]State Key Laboratory of Pathogen and Biosecurity, Academy of Military Medical Sciences, Beijing 100071, China

Abstract
Most deaths occurred during the 2 large outbreaks of Streptococcus suis infection in 1998 and 2005 in China were caused by streptococcal toxic shock syndrome characterized by an increased vascular permeability. Heparin binding protein is thought to mediate vascular leakage. The purpose of this study is to investigate the molecular mechanism underlying the HBP release and vascular leakage induced by S. suis. Significantly higher serum HBP levels were detected in Chinese patients with STSS. Then suilysin, an exotoxin secreted by highly virulent strain 05ZYH33, was identified to stimulate HBP release from polymorphonuclear neutrophils and mediate vascular leakage in mice. SLY induced HBP release was caused by calcium influx-dependent degranulation. Analyses using pharmacological approach revealed that SLY interacted with TLR4 to activate p38 MAPK pathway and up-regulated the gene expression of 5-lipoxygenase to secret leukotriene B4, which then activated PI3K pathway through G protein-coupled seven-membrane spanning receptor and induced HBP release from PMNs. The 5-LO inhibitor Zileuton significantly reduced SLY-induced HBP release and vascular leakage in mice, further supporting that 5-LO is involved in SLY-regulated signaling pathways. These findings might lead to the discovery of potential therapeutic targets for S. suis-associated STSS.

Keywords: 5-lipoxygenase, Heparin binding protein, Streptococcus suis-associated streptococcal toxic shock syndrome, suilysin, vascular permeability.

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ELEVATION OF MATRIX METALLOPROTEINASE-9 LEVEL IN CEREBROSPINAL FLUID OF TICK BORNE ENCEPHALITIS PATIENTS IS ASSOCIATED WITH IGG EXTRAVASSATION

X. KANG[1], Y. LI[1], C. BIAN[2], K. WANG[2], X. WU[1], Y. YANG[1]

[1]State Key Laboratory of Pathogen and Biosecurity, Academy of Military Medical Sciences, Beijing 100071, China, [2]Mudanjiang Forest Hospital, Mudanjiang, China

Abstract

Backgrounds: Tick borne encephalitis (TBE), caused by tick-borne encephalitis virus (TBEV), is an infectious disease involving the central nervous system (CNS). The pathogenesis of CNS injury has not been clearly demonstrated. Matrix metalloproteinase-9 (MMP-9) and some cytokines like interleukin 6 (IL-6) may play important roles in the disruption of the blood–brain barrier (BBB) and the pathogenesis of TBE.

Methods: 72 Cerebrospinal fluid (CSF) samples were collected from TBE patients in north east China. IgG levels in CSF and serum were compared and MMP-9 and IL-6 levels were evaluated by ELISA. The correlation between the elevated MMP-9 levels and IgG extravassation, disease severity, and neuroinflammatory was analyzed.

Results: Increased concentration of MMP-9 could be detected in some CSF in TBE patients, and the elevation was closely related with the CSF TBEV IgG extravasation
and IL-6 expression enhancement. Moreover, the elevated level of MMP-9 was correlative with IL-6 enhancement. Most of all, among the 72 patients, the four dead cases presented with high CSF MMP-9 levels.

Conclusions: In TBE patients, elevated CSF MMP-9 levels were associated with blood brain barrier disruption, brain inflammatory reaction and disease severity.

Key words:

tick borne encephalitis; matrix metalloproteinase-9; cerebrospinal fluid; blood brain barrier; disease severity

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METHICILLIN RESISTANCE IN MACROCOCCUS FROM DOGS

K COTTING[1], S RODRIGUEZ[1], I BRODARD[1], V PERRETEN[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Macrococcus is a coccoid shaped, gram-positive bacterium which can be found on the skin of healthy dogs as well as in infection sites. However, little is known about the antibiotic resistance situation in this rarely reported bacterium in dogs. Twenty-six isolates were obtained from bacteriological culture of 122 skin samples of healthy dogs using swabs and 6 isolates were obtained from infection sites (rhinitis, dermatitis, pyoderma, otitis and mastitis). The isolates were identified using Matrix Assisted Laser Desorption/Ionization - Time of Flight Mass Spectrometry (MALDI TOF) as related to Macrococcus caseolyticus. Antibiotic susceptibility was determined by the measurement of the minimal inhibitory concentration (MIC) of 19 antibiotics. Antibiotic resistance genes were identified by PCR and using microarray capable of identifying up to 117 resistance genes from gram-positive bacteria. Eleven of the 32 strains were resistant to cefoxitin and contained the mecB-gene. Additional resistance genes including those conferring resistance to macrolides (erm(B)), fusidate (fus(C)), aminoglycosides (ant(6')-Ia, aph(2'')-Ia, aac(6')-Ie) and streptothricin (sat4) were detected. Macrococcus may represent a new challenge for veterinary medicine due to its association with infections and its ability to acquire resistance genes.

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DRAFT GENOME SEQUENCE OF CARDIOBACTERIUM HOMINIS, ISOLATED FROM BLOOD CULTURES OF A SWISS PATIENT WITH INFECTIVE ENDOCARDITIS

F TAGINI[1], T PILLONEL[1], S ASNER[2], G PROD'HOM[1], G GREUB[1]

[1]Institute of Microbiology, Department of Laboratory, University of Lausanne & University Hospital Center, Lausanne, Switzerland, [2]Unit of Pediatric Infectious Diseases and Vaccinology Unit, Department of Paediatrics, University Hospital Center, Lausanne, Switzerland

Cardiobacterium hominis, a member of the HACEK group (Haemophilus species, Aggregatibacter species, C. hominis, E. corrodens and Kingella species) is commonly encountered in the oral and nasal human floral. HACEK group bacteria are fastidious and Gram-negative organisms of infective endocarditis. This strain was isolated from a blood culture drawn from a 4-years old child hospitalized at the University Hospital Center, Lausanne, Switzerland. The patient presented with a complex congenital cardiopathy, which required extensive cardiac surgery with insertion of prosthetic material 3 years prior to this event. He was successfully treated with ceftriaxone for 6 weeks and gentamicin for 3 weeks concomitantly. Cardiac prosthesis material was replaced 1 month after discharge. De novo assembly was done using SPAdes Genome Assembler 3.6.2. Annotation was performed using the RAST version 2.0. We searched for antibiotic resistance genes with ResFinder 2.1 and for phage sequences with PHAST. We determined identity between translated coding sequences (CDSs) and corresponding proteins on NCBI database using CLUSTALW. Sequencing produced 2'456'795 150bp-paired-end reads that were assembled into 145 contigs, with 84 contigs of >1000bp. After removal of contigs of <300bp, the total contigs size is 2'655'628bp for a G+C content of 59.0%. RAST system identified 9 putative CDSs for multidrug resistance efflux pumps that could be involved in various antibiotic resistances. However, we observed a high sensitivity to ceftriaxone and gentamicin on the antibiotic sensitivity test and no other resistance genes were found with ResFinder. Interestingly, we noticed a gene encoding for an adhesin, sharing 88.6% identity with other gamma-proteobacteria and 45.9% identity with E. corrodens, another agent of infective endocarditis. Three incomplete phage sequences were found and one of them might still be active because an integrase is located at a distance of 5 open reading frames (=1884bp) from the last phage-associated protein. To conclude, this draft genome sequence will be helpful for further genomic comparison aiming at better understanding C. hominis biological and pathogenic features.

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MSR(A)-LIKE GENE IN MACROLIDE-RESISTANT STAPHYLOCOCCUS ARLETTAE FROM BOVINE MASTITIS MILK

SN ANDREIS[1], V PERRETEN[1], N WOHLWEND[2], S SCHWENDENER[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]labormedizinisches zentrum Dr Risch, Bern-Liebefeld, Switzerland

Staphylococcus arlettae is a coagulase-negative Staphylococcus (CoNS) which belongs to the normal flora of humans and animals. It is also sporadically associated with mastitis in cows. S. arlettae M1670 was isolated from bovine mastitis milk in Switzerland and analysed for antibiotic resistances by minimal inhibitory concentration (MIC) measurement, PCR and microarray. Strain M1670 exhibited resistance to erythromycin (MIC >8µg/ml), clindamycin (MIC 2µg/ml), fusidic acid (MIC >4µg/ml) and penicilline (MIC 0.5µg/ml). However, no known resistance genes were detected. Whole genome sequencing using Illumina MiSeq revealed the presence of a msr(A)-like gene which shares 87% nucleotide sequence identity with the msr(A) gene from S. epidermidis strain 968. It encodes a Msr(A)-like protein of 488 amino acids that contains the ATP-binding cassettes (ABC) transporter signature pattern and two ABC domains typical for class 2 ABC proteins. The msr(A)-like gene was located downstream of a putative virulence factor encoding a lipase. Comparative sequence analysis of the M1670 draft genome with the genome of S. arlettae strain CVD059 indicated that the msr(A)-like variant and lipase are inserted into the accessory region of the S. arlettae chromosome. This discovery shows that diverse genomic fragments containing msr(A)-like macrolide resistance genes are present in S. arlettae from animal origin.

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CALCIUM AFFINITY TO EXOPOLYSACCHARIDES OF CARIOGENIC SPECIES

M. ASTASOV-FRAUENHOFFER[1], T. WALTIMO[1], O. BRAISSANT[2]

1Clinic for Preventive Dentistry and Oral Microbiology, UZB - University Center for Dental Medicine, University of Basel. Hebelstrasse 3, 4056 Basel, Switzerland, 2Center of Biomechanics & Biocalorimetry, c/o Department Biomedical Engineering (DBE), University of Basel. Gewerbestrasse 14, 4123 Allschwil, Switzerland

In cariogenic biofilms, exopolysaccharides (EPS) provide an abundance of primary binding sites with different chemical functional groups such as carboxylic acids, phosphates and amines and form the core of the matrix-scaffold. Among these groups carboxylic acid and phosphates are known to bind calcium and other biologically important cations like magnesium, iron or zinc. During caries development calcium gets dissolved from enamel, however little is known whether this dissolved calcium is bound within the cariogenic biofilm. The aim of the study was to investigate and refine the role for the acid-base and calcium binding properties of the EPS of Steptococcus mutans, Lactobacillus rhamnosus, and Candida albicans. Acid-base titration of the EPS revealed that it contained mostly acidic proton binding sites. Some binding sites near neutral and alkaline pH were observed, however in much lower concentrations. Isothermal titration calorimetry (ITC) showed that EPS from bacterial strains had a binding affinity one order of magnitude higher to calcium than to lactic or citric acid. However, for EPS purified from C. albicans, titration data revealed relatively weak binding thereby not allowing estimation of an accurate binding affinity. In conclusion, the study reveals that within the biofilm matrix EPS can bind calcium with a higher affinity than organic acids thus, providing the cariogenic species with a possible mechanism to withstand higher concentrations of calcium ions which unbound could have toxic effects.

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DETERMINANTS OF BIOFILM FORMATION AND CLEANABILITY OF TITANIUM SURFACES

LK ZAUGG[1], M ASTASOV-FRAUENHOFFER[2], O OLIVIER BRAISSANT[3], I HAUSER-GERSPACH[2], T WALTIMO[2], NU ZITZMANN[1]

[1]Department of Periodontology, Endodontology and Cariology, University Center for Dental Medicine Basel, University of Basel, Hebelstrasse 3, 4056 Basel, [2]Clinic for Preventive Dentistry and Oral Microbiology, University Center for Dental Medicine Basel, University of Basel, Hebelstrasse 3, 4056 Basel, [3]Center of Biomechanics & Biocalorimetry, c/o Department Biomedical Engineering (DBE), University of Basel, Gewerbestrasse 14, 4123 Allschwil

Peri-implant diseases are primarily related to biofilm formation along the mucosal margin of the peri-implant tissues. Adequate oral hygiene and professional cleaning contribute to the prevention of peri-implantitis. However, thorough biofilm removal is challenging, and the remaining biofilm, particularly in marginal and interproximal regions, enhances the risk of peri-implant disease development.

The present study analysed in vivo biofilm formation and the cleanability on titanium discs with 4 different surfaces (M, machined TiZr alloy; modMA, machined and acid etched TiZr alloy; modSLA, machined, sand blasted and acid etched TiZr alloy; TAV MG, micro-grooved titanium aluminium vanadium alloy) in an experimental model.

Custom-made discs were mounted in individual intraoral splint housings and worn by sixteen volunteers for 24 h. Cleanability of the materials was performed by a standardized cleaning device. Safranin staining assay, isothermal microcalorimetry (IMC) and scanning electron microscopy (SEM) were used for microbiological assessments.

The hydrophilic surfaces modMA and modSLA with greater surface micro-roughness exhibited significantly more biofilm than the hydrophobic surfaces TAV MG and M. The standardized cleaning procedure substantially reduced the biofilm mass on all surfaces. After cleaning, the IMC analyses demonstrated a longer lag time of the growth curve on TAV MG compared to modSLA. Inter- and intraindividual variations in biofilm formation on the titanium discs were evident throughout the study.

The current investigation demonstrated that hydrophilicity and surface roughness drove the initial biofilm adhesion and formation in vivo. Surface topography was the most influential factor for surface cleanability, with the TAV MG surface retaining larger amounts of the initial biofilm hidden within the micro-grooves. The machined surface without grooves was easier to clean, but recolonization indicated by increased metabolic activity (growth rate) in IMC occurred despite mechanical biofilm removal.

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COMPLETE NUCLEOTIDE SEQUENCE OF AN INCF PLASMID CARRYING EXTENDED-SPECTRUM B-LACTAMASE AND AMINOGLYCOSIDE ACETYLTRANSFERASE GENES FROM EQUINE E. COLI

S. SCHWENDENER[1], D. VOGT[1], S. N. SEIFFERT[2], A. ENDIMIANI[2], A. CARATTOLI[2], V. PERRETEN[1]

[1]Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland, [2]Institute of Infectious Diseases, University of Bern, Bern, Switzerland

Multidrug-resistant (MDR) E. coli producing extended spectrum β-lactamases (ESBL) have been increasingly isolated in hospital, community and animals. The ESBL genes are frequently located on plasmids which can be easily transferred between bacteria. To date, only few ESBL-carrying plasmids have been completely sequenced from E. coli isolated from horses. A MDR E. coli sequence type (ST) 744 was isolated from equine wound in 2011. The E. coli strain carried the β-lactamase gene blaTEM-1 and the ESBL gene blaCTX-M-14 and nine additional acquired genes conferring resistance to chloramphenicol (catA1), trimethoprim (dfrA17), tetracycline [tet(A)], sulphonamide (sul1) and aminoglycosides [aac(3)-IIc, aadA4, aph(3')-Ia, strA and strB]. It also contained mutations in the quinolone resistance determining region of the DNA gyrase subunit A (GyrA: S83L, D87N). The β-lactamases (blaTEM-1 and blaCTX-M-14) and the aminoglycoside transferase [aac(3)-IIc] genes were solely transferred to a sensitive E. coli recipient strain by conjugation. Whole plasmid sequencing revealed the presence of these genes on a IncFII (F2:A-:B-) plasmid pKM811 with a size of 78,976 bp and an average GC content of 52%. The plasmid scaffold consists of a continuous 18,346-bp MDR region (MRR) and a 60,630-bp backbone coding for characteristic IncF plasmid functions that shared over 99% nucleotide sequence identity with the reference IncF plasmid R100. The MRR of pKM811 harbours ISEcp1-blaCTX-M-14, blaTEM-1 carried by a Tn3 fragment and a IS26 flanked aac(3)-IIc gene. The region represents a newly combined structure carrying several complete and fragmented mobile elements including four intact IS26. Identical modules containing ISEcp1-blaCTX-M-14 and a IS26-aac(3)-IIc were found on other plasmids suggesting exchange of resistance gene module by transposase and recombination between MRR of different plasmids. These resistance genes may have been selected and maintained in E. coli from equine environment where β-lactam and aminoglycoside antibiotics are frequently used for treatment.

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TWO GENOMIC DIAGNOSTIC APPROACHES FOR PATIENTS SUSPECTED WITH WHOOPING COUGH

D. BANDEIRA[1], L. BERTAIOLA-MONNERAT[2], M.-L. TRITTEN[1], H. H. SIEGRIST[1], R. LIENHARD[1]

[1]ADMed Microbiologie, 2300 la Chaux-de-Fonds, Suisse, [2]HFR Hôpital cantonal, 1708 Fribourg, Suisse

DIAGNOSTIC APPROACHES FOR PATIENTS SUSPECTED WITH WHOOPING COUGH

D. BANDEIRA[1], L. BERTAIOLA-MONNERAT[2], M.-L. TRITTEN[1], H. H. SIEGRIST[1], R. LIENHARD[1]

[1]ADMed Microbiologie, 2300 la Chaux-de-Fonds, Suisse, [2]HFR Hôpital cantonal, 1708 Fribourg, Suisse

Two genomic tests for the rapid identification of the agent of whooping cough were compared. Eighty-nine nasopharyngeal aspirates or swabs from patients and 14 external quality controls from QCMD were analysed. Native samples were tested on the Biofire FilmArray Respiratory Panel (FA) (bioMérieux, Marcy-l'Etoile, F) to detect Bordetella pertussis and 21 other pathogens. The same samples were then extracted by Nuclisens®easyMag and tested with the RIDA®GENE Bordetella real-time multiplex PCR (RG) (r-biopharm, Darmstadt, D) using the RotorGene Q to detect B. pertussis, B. parapertussis and B. holmesii. The comparison of results from patient swabs showed several discrepancies. Ten B. pertussis were found by FA versus 17 with RG. None B. parapertussis or B. holmesii were detected by RG. In the 10 samples positive for Bordetella with FA co-infections were detected in 3 samples with rhino/enterovirus (RV/EV) and in 2 with RV/EV and adenovirus (ADV), 5 were positive for Bordetella only. From 79 negative samples only 22 (27.8%) were totally negative. All the others were positive for at least one pathogen; 36 (45.6%) for RV/EV, 9 (11.4%) for respiratory syncytial virus, 7 (8.9%) for parainfluenza virus, 6 (7.6%) for human metapneumovirus, 5 (6.3%) for influenza virus, 5 (6.3%) for C. pneumoniae, 4 (5.1%) for M. pneumoniae, 3 (3.8%) for adenovirus, 2 (2.5%) for coronavirus. Testing EQC samples (QCMD) out of 6 positive B. pertussis 5 were found by RG versus only 3 by FA. Furthermore, RG correctly identified 1 B. parapertussis, 2 B. holmesii and the 4 negatives (2 B. bronchiseptica and 2 Bordetella spp. negatives). FA gave two false positive results for B. bronchiseptica revealing a specificity problem with the primers. Due to its lack of sensitivity for samples with low pathogen concentration, FA appears less effective than RG to diagnose the aetiology of pertussis cough. However, despite its lack of sensitivity and specificity; the broad spectrum approach of FA gives useful information about the aetiology of respiratory problems and remains a rapid and easy to use test to diagnose respiratory infections without specific clinical symptoms of pertussis.

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Wednesday, June 15th

EVALUATION OF THE BIOFIRE FILM ARRAY® GASTROINTESTINAL PANEL TEST FOR THE SCREENING OF PATHOGENS IN STOOLS

M.-L. TRITTEN [1], J. SCHMID[1], H.H. SIEGRIST[1], R. LIENHARD[1]

[1]ADMED Microbiologie, 2300 La Chaux-de-Fonds, Switzerland

Diagnostic laboratories need sensitive tools to detect pathogens in patients' stools in order to give rapid and reliable results to clinicians. For this purpose, we evaluated a commercial rapid multiplex PCR device, the Biofire FilmArray®Gastrointestinal (GI) panel test (BioMérieux) which detects 13 bacteria, 5 viruses and 4 parasitic protozoa in approximately 1 hour. Two hundred and forty seven stools were analysed by the Biofire GI test and the results compared with those obtained by our routine procedures (bacterial cultures, standard microscopy for parasites, PCR or culture for toxin producing C.difficile) according to the requests of our clinicians. One or more pathogens were detected by PCR in 184 stools and 63 specimens were negative. Among all those samples, 172 were tested for bacteria (Salmonella spp., Shigella spp., Campylobacter spp.) by routine culture. Biofire yielded 38 Campylobacter sp. (culture 34), 11 Salmonella sp. (culture 12) and 6 Shigella sp. (culture 4). Of 35 samples tested for toxigenic Clostridium difficile with Biofire GI and culture 15 yielded a positive result with Biofire, 4 of which remained culture negative. We also tested 75 stools for intestinal protozoa (Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum and Cyclospora cayetanensis). Forty samples were positive with Biofire, 9 of these (3 Cryptosporidium and 6 Giardia) remained negative with microscopy and 1 Giardia was not detected by PCR. Several other bacteria and viruses were detected by Biofire in 74 stools. The Biofire GI test is an interesting and sensitive tool to detect a large panel of pathogens in a short time and thus ideal for emergencies. However, it is important to obtain good clinical details from clinicians as the panel does not include some important pathogens. Moreover, cultures remain essential to recover strains in order to perform typing and antibiogrammes.

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Wednesday, June 15th

PREVALENCE AND CHARACTERIZATION OF NASAL METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM HUMAN AND VETERINARY SURGEONS

V. POST[1], L.G. HARRIS[2], M. MORGENSTERN[3], R.G. RICHARDS[1], T.F. MORIARTY[1]

[1]AO Research Institute, AO Foundation, Davos Switzerland, [2]University of Swansea, Swansea, United Kingdom, [3]BGU Murnau, Murnau Germany

Staphylococci are common opportunistic pathogens colonizing the human population. The data available to date on nasal colonization in surgeons has been limited. A prospective study was undertaken in late 2013. The aim was to identify the prevalence of staphylococci (S. aureus/methicillin-resistant S. aureus (MRSA) and coagulase-negative staphylococci (CoNS/MRCoNS)) in an international cohort of surgeons. Nasal swabs and basic demographic data were collected from participants at an international educational event, on an anonymous and voluntary basis. MRSA isolates were subjected to agr-, spa- and MLST typing, and the presence of 22 virulence factors was screened for by PCR. Additionally, biofilm-forming ability, haemolytic activity, staphyloxanthin production and antibiotic resistance were determined for these MRSA isolates. The genome of a rifampicin resistant MRSA was sequenced, due to the importance of this antibiotic in patients served by orthopedic surgeons. Amongst the 1,166 human surgeons, the average overall S. aureus nasal colonization rate was 28% and MRSA rate 2% while the MRSA rate for veterinary surgeons was 5%. MRCoNS nasal carriage rate was 21%. The 26 MRSA displayed no remarkable virulence gene pattern and 35% of the isolates carried at least one of the Panton-Valentine leukocidin lukFS-PV, the exfoliative toxin eta or the toxic shock syndrome tst genes. All isolates were resistant to multiple antibiotics. Resistance to rifampicin was due to 3 mutations in the rpoB gene. Half of the isolates belonged to well-described clonal lineages, ST1, ST5, ST8, ST45 and ST59 that are associated with severe infections and increased patient mortality. Two of the 3 veterinarian MRSA belonged to epidemic livestock-associated MRSA clonal lineages ST398 and ST8 associated with high transmission potential between animals and humans. In conclusions, surgeons are colonized by S. aureus and MRSA at broadly equivalent rates to the general population. Twenty-six surgeons were colonized with multiply antibiotic resistant MRSA and belonged to clonal lineages with high transmission potential and causing increased infection severity.

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PHAGE THERAPY AND CIPROFLOXACIN ARE HIGHLY SYNERGISTIC AGAINST EXPERIMENTAL ENDOCARDITIS DUE TO PSEUDOMONAS AERUGINOSA

F. OECHSLIN[1], P. PICCARDI[1], M. GIDDEY[1], S. MANCINI[1], J. GABARD[2], P. MOREILLON[1], J.M. ENTENZA[1], G. RESCH[1], Y-A. QUE[3]

[1]Dept. of Fundamental Microbiology, University of Lausanne, Switzerland, [2]Pherecydes Pharma, Romainville, France, [3]Dept. of Intensive Care Medicine, Bern University Hospital, Switzerland

The continuing development of antibiotic resistance stresses the need for alternative treatment. Infective endocarditis due to P. aeruginosa is an archetype of highly lethal valve infection in human. Therefore we used in vitro and in vivo models of P. aeruginosa experimental endocarditis (fibrin clots and rats with catheter-induced aortic vegetations, respectively) to study the efficacy of an anti-pseudomonas phage cocktail and ciprofloxacin administered alone or in combination.

In fibrin clots, phage therapy decreased bacterial density by 6 log10 CFU/g in 6h. Bacterial regrowth due to phage resistance was observed after 24h, but was prevented by addition of ciprofloxacin (2x MIC). In rats, phage therapy alone decreased vegetation bacterial density by 2.5 log10 CFU/g after 6h ($P<0.001$), compared to 2.2 log10 CFU/g with ciprofloxacin ($P<0.05$). Moreover, combining phages with ciprofloxacin appeared highly synergistic with a >6 log10 CFU/g decrease in 6h, and successful treatment in 64% (7/11) of the animals.

Phage-induced killing correlated with in situ phage multiplication - as also confirmed by histology and transmission electron microscopy examination of the vegetations - and cytokines production compared to antibiotherapy alone. Importantly, no phage-resistant mutants were detected in vivo, which was most likely due to altered fitness. Indeed, two phage-resistant clones isolated in vitro were 50-70% less infective in rats with experimental endocarditis ($P<0.01$ versus parent strain). This infectivity decrease was either due to a 362 kb deletion encompassing the galU gene, resulting in impaired LPS synthesis, or a 15 bp deletion in the pilT gene resulting in impaired motility.

In conclusion, phage therapy significantly reduced P. aeruginosa experimental endocarditis and was highly synergistic with ciprofloxacin. Phage-resistant mutants selected in vitro resulted in impaired infectivity, due to reduced in vivo fitness. Phage therapy alone or combined with antibiotics represents a promising alternative in the treatment of P. aeruginosa infections and merits further consideration.

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Tuesday, June 14th

GENOME OF THE CARBAPENEMASE-PRODUCING CLINICAL ISOLATE ELIZABETHKINGIA MIRICOLA CHUV: EVIDENCE FOR INTRINSIC RESISTANCE TRAIT OF AN EMERGING PATHOGEN

O OPOTA[1], S-M DIENE[2], G PROD'HOM[1], P ECKERT[3], G GREUB[1]

[1]Institute of Microbiology, University Hospital of Lausanne, Lausanne, Switzerland, [2]Genomic Research Laboratory, Service of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland, [3]Service of Adult Intensive Care Medicine, University Hospital of Lausanne, Lausanne, Switzerland.

Background: Whole genome analysis has emerged as an opportunity for clinical microbiologists to collect, early after description, informations on bacteria traits for newly characterized species of potential medical interest. Elizabethkingia miricola, a recently described ubiquitous non-fermenting Gram negative rod of the Flavobacteriaceae family, is an emerging pathogen causing severe infections in humans including pulmonary abscess and sepsis. We recovered the strain E. miricola CHUV, a multidrug resistant carbapenemase-producing isolate, from the respiratory secretions of a patient who died from a septic choc with a pulmonary origin soon after admission in the intensive care unit of our hospital. We report the complete genome of this bacterium and the functional/comparative analysis to increase our understanding of its pathogenic potential.
Material/methods: The genomic DNA of E. miricola CHUV was extracted and subjected to whole-genome shotgun sequencing by using the MiSeq technology (Illumina, San Diego CA) from a single library of paired-end sequencing reads of 2x150 bp. Genome assembly was performed using the SPAdes v3.5.0 program.
Results: The genome of E. miricola CHUV consists of a 4'286'503 bp chromosome and a plasmid of 176’107 bp, which corresponds to 4006 predicted coding sequences. The strain E. miricola CHUV was resistant to several major classes of antibiotics including carbapenem through the production of a metallo-β-lactamase with carbapenemase activity. The resistome analyses revealed the presence of a high number of resistance genes. Interestingly, we 1) identified only a limited number of mobile elements with only two of them located in the proximity of resistance genes, 2) failed to detect prophage-related sequences and 3) identified 10 restriction-modification system genes in this genome suggesting a very limited rate of DNA exchange with other bacteria.
Conclusions: These findings suggest that multi-drug resistance, a keystone of the pathogenic potential of E. miricola, is an intrinsic trait due to the presence of a high number of resistance genes within the bacterial core genome and is not due to DNA exchanges with other bacteria.

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Wednesday, June 15th

WADDLIA CHONDROPHILA: A NOVEL AGENT OF MALE INFERTILITY?

M. STOJANOV[1], J. GYGER[1], V. CASTELLA[2], N. VULLIEMOZ[3], D. BAUD[1]

[1]Materno-fetal and Obstetrics Research Unit, Department "Femme-Mère-enfant", Maternity, University Hospital, Lausanne, Switzerland, [2]University Center of Legal Medicine, University Hospital, Lausanne, Switzerland, [3]Reproductive Medicine Unit, Department "Femme-Mère-enfant", Maternity, University Hospital, Lausanne, Switzerland

Introduction: Intracellular bacteria, which fail to grow on media used routinely to isolate human pathogens, could represent yet unrecognized agents of infertility and miscarriages. Similarly to Chlamydia spp., Waddlia chondrophila is an emerging intracellular pathogen that has been associated to adverse pregnancy outcomes both in humans and animals. Our recent observations indicate that seroprevalence of anti-Waddlia antibodies is higher in male patients from couples with infertility problems, suggesting that this bacterium might play impact reproduction in humans.

Material and methods: We established an in vitro model of infection of human spermatozoa by Waddlia. Controls included filtrate of cell culture used to grow Waddlia (mock) and latex beads (0.6 µm diameter). Attachment to spermatozoa and internalization of Waddlia was monitored by confocal microscopy using a specific antibody. Viability of spermatozoa was assessed with flow cytometry using the LIVE/DEAD Sperm Viability Kit. The amount of bacteria was monitored with a specific quantitative RT-PCR.

Results: Using confocal microscopy we observed that, despite multiple washing steps, Waddlia was able to attach to spermatozoa. However, internalization and replication of bacteria were not observed. Presence of Waddlia decreased significantly viability of spermatozoa, with almost a 2-fold increase compared to control at 72 h post-infection (32% versus 18%). Decrease of viability was not observed for spermatozoa incubated with mock or latex beads.

Conclusions: We showed in this study that Waddlia had a negative impact on spermatozoa viability. Bacteria were observed in close contact with spermatozoa membrane, suggesting the presence of specific interactions. Bacterial replication was not observed. Future analysis will focus on the impact of Waddlia on spermatozoa motility, a key parameter for the evaluation of sperm quality. Moreover, specificity of the Waddlia-spermatozoa interaction will be analysed at the molecular level, with the goal to determine specific receptors involved in the attachment process. Taken together, our data suggest that Waddlia might be associated with fertility impairment in men.

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Tuesday, June 14th

EVALUATION AND INTRODUCTION OF THE BD MAX ENTERIC PANEL PCR ASSAY IN THE ROUTINE PRACTICE OF A DIAGNOSTIC LABORATORY

G. JOST[1], N. LIASSINE[1], F. DE MARVAL[1]

[1]Laboratoire Dianalabs, Geneva, Switzerland

Objective: This study was aimed to evaluate the BD MAX Enteric Bacterial Panel (EBP) PCR assay, in comparison with the standard stool culture.
Methods: Stool culture was performed using standard procedures. BD MAX EBP (BD, Sparks, MD) is a multiplex real-time PCR assay which targets Campylobacter jejuni/coli, Salmonella spp., Shigella spp./EIEC and EHEC.
Results: Shigella spp./EIEC: a total of 157 stools were tested: 150 were negative and 2 were positive both by PCR and culture; 5 specimens were positive by PCR but negative by culture, and therefore presumed to be EIEC. Campylobacter spp.: of the 3259 specimens tested, all C. jejuni/coli detected by culture were positive by PCR, whereas 9 Campylobacterales, other than C. jejuni/coli, were diagnosed only by culture (8 Arcobacter spp., 1 Helicobacter pullorum). Discrepant results were observed in 110 (3.4 %) specimens which were tested positive by PCR and remained negative by culture. Salmonella spp. : A total of 3259 specimens were analysed, of which 67 (2%) were Salmonella spp.-positive and 3174 were Salmonella spp.-negative by culture and PCR. Eleven specimens that were positive by culture for Salmonella spp. remained negative by PCR, whereas 7 PCR-positive Salmonella spp. remained negative by culture. EHEC: 45 out of 3259 stool specimens (1.4%) were tested positive by PCR; 28 of these specimens were sent to the NENT for confirmation, 20 of which were confirmed as EHEC.
Conclusion: Introduction of PCR allows a more rapid and sensitive diagnosis. In the present study, maximum benefit was obtained for the detection of C. jejuni/coli and Shigella spp./EIEC. However, the culture allows a more extended spectrum of Campylobacterales than PCR. Surprisingly, culture detected more often Salmonella spp. than PCR in our routine practice. These features prompt us to maintain the culture of Salmonella spp. and Campylobacter spp. The multiplex PCR assay allows the routine detection of pathogens (EIEC and EHEC) that were formerly investigated only in particular situations, which leads to more positive results; this can be disturbing for clinicians and epidemiological analysis.

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TRANSFER OF AN ESTABLISHED HOME-MADE REAL-TIME PCR TARGETING PNEUMOCYSTIS JIROVECII ON BDMAX PLATFORM

JG DISERENS[1], A CROXATTO[1], R BROUILLET[1], P.M HAUSER[1], G GREUB[1], K JATON[1]

[1]Institute of Microbiology, University Hospital and University of Lausanne, 1011 Lausanne, Switzerland

BACKGROUND: Pneumocystis jirovecii (PCP) is an opportunistic pathogen causing infection in both AIDS and immunocompromised patients. The examination of respiratory sample for the detection of PCP is conventionally performed by microscopy. However, PCR exhibits a higher sensitivity than microscopy and allows accurate quantification. Thus, a quantitative real-time Taqman PCR was developed in our platform with a time to results of 4 hours, but this test is only available from Monday to Friday. Therefore, we decided to transfer our Taqman PCR to a rapid PCR format i.e. the BD MAXTM system (BD Diagnostics) that provides results in about 100 minutes without laborious hands-on work.
METHODS: The BD MAX system was compared with automatic MagNA Pure DNA extraction and conventional real-time PCR using 200µl of BAL. The same primers and probe, with different concentrations, and the same master mix (Applied Biosystem) were used with the two systems. Intra- and inter- run assays were performed to test the robustness of the PCR and various concentration of plasmids carrying the PCP DNA sequence were used to measure the quantification accuracy. A total of 25 frozen BAL (15 positive and 10 negative) were analysed with both molecular assays in a retrospective study. The Ct were compared to quantify the results.
RESULTS: For the BD MAX, optimal concentrations of 0.6µM of primers and 0.3µM of probe were used and allowed a detection of 100% of the 2E5 and 2E4 copies/ml of plasmidic PCP DNA and 60% of 2E3 copies/ml. In the retrospective study, the BD MAX system showed a 100% qualitative correlation with the conventional TaqMan assay. For positive results, a good quantification correlation of the Ct values between the two systems was observed with an average of 1 Ct difference. Moreover, no cross contamination were observed with the BD MAX.
CONCLUSION: The transfer of a home-made PCR on the BD MAX system was relatively easy to perform and allows the detection and quantification of Pneumocystis DNA. The new rapid molecular BD MAX test showed equal performance and good quantitative correlation with our home-made real-time PCR allowing a significant decrease of the time to results.

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Wednesday, June 15th

COMPARISON OF FOUR COMMERCIAL MOLECULAR METHODS FOR THE TYPING OF CARBAPENEMASES

E COTTER[1], L TISSIÈRES LOVEY[1], A DUMOULIN[1], G PRAZ[1]

[1]Hôpital du Valais - Institut Central - Service des Maladies Infectieuses

During the last decade Carbapenemase-producing Enterobacteriaceae (CP-CRE) became a clinical and epidemiological challenge. Their accurate, rapid detection and typing in the clinical microbiology laboratory has therefore gained in importance. Several molecular methods for detection and typing of carbapenemases are available, differing in detected subtypes, ease of use, and cost. In this study, we compared the performance of the CheckMDR CT103 (Check-Points), Xpert Carba-R (Cepheid), eazyplex Superbug CRE (Amplex) and Genspeed Superbug CR (Greiner bio-one) by testing 13 CP-CRE strains and 11 non CP-CRE strains obtained from a reference laboratory. Additionally, the strains were tested with the phenotypic Rapidec Carba NP assay (bioMérieux). All methods overall exhibited a high degree of correlation. For 11 of the 13 CP-CRE strains, results were concordant. For one strain, the CheckMDR assay detected a non-NDM-type CRE, whereas the other assays reported a NDM-type CRE. Another strain was reported as a NDM-type CRE by three molecular assays, but was negative with the Genspeed assay and the Rapidec NP phenotypic assay. Additional testing is ongoing to resolve the discordant results. The 11 non CP-CRE strains were reported correctly by all methods. The Xpert Carba-R assay appeared to be the most convenient and easy to use, detecting the most common carbapenemase types. The CheckMDR assay covers the largest panel of carbapenemases, but requires more hands-on time. The Easyplex and the Genespeed are good alternatives, covering the most frequent CRE types, with a moderate hands-on time.

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PNEUMOCYSTIS JIROVECI PNEUMONIA: DISCREPANCIES BETWEEN CLINICAL AND LABORATORY DIAGNOSIS

M. OBERLE[1], C. OTIGER[1], H. FANKHAUSER[1], G. WYSS[2], R. ZWEIFEL[3], Ch. FUX[2], A. CONEN[2]

[1]Institut für Labormedizin, Kantonsspital Aarau, Aarau, [2]Abteilung für Infektiologie und Spitalhygiene, Kantonsspital Aarau, Aarau, [3]Institut für Pathologie, Kantonsspital Aarau, Aarau

Background: Pneumocystis jiroveci, an environmental fungus, causes various pulmonary infections in immunocompromised patients. Diagnosis of P. jiroveci pneumonia (PcP) includes host factors, clinical and radiological parameters as well as the detection of cysts or trophic forms in respiratory specimens by staining, DNA amplification (PCR) or a combination of them. An ambiguous clinical presentation thereby often correlates with negative staining but positive PCR, making a definitive diagnosis difficult. Here we compare microbiological tests with the presumptive clinical diagnosis and discuss the significance of quantitative PCR (qPCR).

Material/methods: Respiratory specimens were analyzed for the presence of P. jiroveci by qPCR (mt large subunit rRNA, RIDA Pneumocystis kit, rBiopharm, Germany) and Toluidine-blue staining (Toluidine). Papanicolaou-staining (Pap) was done additionally with most of the specimens. The clinical assessment included body temperature, blood pressure, respiratory rate, oxygen saturation, arterial blood gas analysis, and a chest X-ray. Data on immunosuppressive treatments and co-morbidities were collected. Treatment was initiated upon a presumptive clinical PcP diagnosis.

Results: One hundred and nineteen specimens of 104 patients were analyzed by qPCR and Toluidine; 70% were additionally analyzed by Pap. Three cases were positive with Toluidine (positivity rate 3%) and with Pap (5%); twenty-four specimens were positive with qPCR (20%; median 4100 copies/ml, 150 – 3.6x106 copies/ml). Low qPCR (< 5000 copies/ml) resulted more often to discrepancies with the clinical PcP diagnoses whereas high copy numbers (> 10’000 copies/ml) rather confirmed the clinical investigation.

Conclusions: Here we summarize analyses for PcP of more than one hundred patients and compare clinical parameters with laboratory results. qPCR had the highest sensitivity for the detection of P. jiroveci, but its suitability for PcP-diagnosis in clinical practice is controversial. Discrepancies between clinical severity and laboratory results may lead to inconclusive diagnosis; such cases are discussed in the paper.

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Tuesday, June 14th

MONITORING CLONAL DIVERSITY OF EXTENDED-SPECTRUM CEPHALOSPORIN-RESISTANT ENTEROBACTERIACEAE COLONIZING THE GUT 6 MONTHS AFTER TRAVELING TO INDIA

J PIRES[1], E KUENZLI[3], S KASRAIAN[1], R TINGUELY[1], H FURRER[5], M HILTY[1], C HATZ[3], A ENDIMIANI[1]

[1]Institute for Infectious Diseases, University of Bern, Bern, Switzerland, [2]Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, [3]Swiss Tropical and Public Health Institute, Basel, [4]Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, [5]Department of Infectious Diseases, Bern University Hospital, University of Bern, Bern, Switzerland

Background: Traveling to high prevalence areas of extended-spectrum cephalosporin-resistant Enterobacteriaceae (ESC-R-Ent) is linked with the colonization of the human gut by these MDR bacteria. However, factors modulating the acquisition and persistence of ESC-R-Ent in the gut are unknown. We aim to monitor this condition in volunteers travelling to India.
Methods: Thirty-eight volunteers living in Switzerland underwent screening before and after their trip, and also 3 and 6 months after traveling. Native stools were enriched in LB broth and plated on selective plates (BLSE, ChromID ESBL, and Supercarba). Species identification was obtained using MALDI-TOF MS. At least 5 Enterobacteriaceae colonies were analyzed. Double-disk synergism test was performed and clonal relatedness in E. coli was assessed using rep-PCR, Phylogenetic group (PhG) analysis and MLST. Check-Points CT103XL microarray and PCR/DNA sequencing were implemented for β-lactamases characterization.
Results: Colonization before traveling was low (8%), rising to 76% after the journey. Colonization decreased over time, reaching 33% and then 31% at 3 month and 6 months after traveling, respectively. Low β-lactamase diversity was identified at all time points, being CTX-M-15-like the most prevalent after traveling (~80%). Surprisingly, rep-PCR and MLST revealed that E. coli diversity varied from 1 to 5 clones when volunteers returned to Switzerland. These clones were mostly from PhG A (~60%) and B1 (~18%) and diverse non-epidemic STs. This polyclonal diversity decreased over time as when colonization was persistent at 3 months and 6 months, usually only one clone was identified.
Conclusion: When traveling to high ESC-R-Ent prevalence areas, a polyclonal acquisition of ESC-R-Ent in the gut occurs. Over time, this phenomenon is resolved either by colonization clearance or by single clonal persistence. This initial acquisition of ESC-R-Ent might be linked to non-human sources (e.g., food-chain, environment), since the isolates belong to a multiplicity of genetic backgrounds that are usually linked with these settings. This work was supported by SNF grant No. 153377 due to AE.

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Wednesday, June 15th

BACTERIOPHAGE MS2 AND ECHOVIRUS 11 MUTANTS WITH INCREASED RESISTANCE TO COMMON WATER DISINFECTANTS

Q ZHONG[1], A CARRATALÀ [1], T KOHN[1]

[1]Environmental Chemistry Laboratory, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

Common water disinfectants like chlorine have been reported to select for resistant viruses. Yet, only little attention has been devoted to characterizing disinfection resistance. The goal of this study was to produce disinfectant-resistant viruses and to characterize the underlying resistance mechanisms. Resistant virus populations of echovirus 11 and MS2 coliphage were established by experimental evolution. Specifically, viruses were repeatedly inactivated by ClO2 and subsequently regrown. In parallel, control experiments were conducted in which virus numbers were reduced by dilution rather than ClO2 exposure. Virus inactivation kinetics were quantified to capture the emergence of resistance. Once a resistant population was established, their genomes and structures were studied and the changes were linked to modifications in vital viral functions. Furthermore, we investigated changes in the replicative fitness, and the cross-resistance towards other disinfectants. Both MS2 and echovirus 11 exhibited resistance after repeated disinfection-regrowth passages. Interestingly, resistance also emerged without exposure to ClO2, indicating that cell passaging rather than ClO2-exposure drives resistance development. The resistant populations exhibited several fixed mutations that caused the substitution of ClO2-labile by ClO2-stable amino acid. These mutations imply a greater protein stability toward oxidation by ClO2. We thus propose that one of the mechanisms underlying resistance is the protection of the host attachment site from oxidation. Alternatively, host attachment may be maintained by switching to a different attachment site. Cross-resistance to other disinfectants was shown to depend on the disinfectants' main viral target: the resistant populations also exhibited resistance against free chlorine, which acts on both viral proteins and genome. In contrast, no cross-resistance was observed for UV light, which exclusively targets the viral genome. Finally, the mutants were inactivated more readily by heat, which indicates that the protection of the viral proteins from oxidation came at the cost of lower thermal stability.

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PLASMID-ENCODED COLISTIN RESISTANCE IN SOUTH AFRICA

N. KIEFFER[1], L. POIREL[1], A. BRINK[2], J. COETZE[2], A. JAYOL[1], P. NORDMANN[1]

[1]Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland, [2]Department of Clinical Microbiology, Ampath National Laboratory Services, Milpark hospital, Johannesburg, South Africa

Emergence of multidrug-resistant (MDR) Enterobacteriacae is increasing worldwide. The ultimate drug to treat patients infected by these MDR Gram negative rods is polymyxins. Polymyxin resistance has been described being associated with mutations in the chromosome, in particular in genes involved in the pathway of lipopolysaccharide (LPS) modifications. The plasmid-born mcr-1 gene was very recently identified to be responsible of a transferable low-level resistance toward colistin and is already reported worldwide. Here we report seven clonally-unrelated Escherichia coli harboring mcr-1 from South Africa. Two plasmids fully sequenced were assembled and the study of the genetic context revealed an identical 2600bp-long DNA sequence defining a mcr-1 cassette. Promoter sequences responsible for the expression of mcr-1 were characterized, including an additional hybrid promoter brought by the insertion sequence ISApl1. These findings describe the diversity of plasmids carrying mcr-1 and highlight the impact of this new resistance gene in human medicine.

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RAPID DETECTION OF COLISTIN RESISTANCE IN ENTEROBACTERIACEAE

P. NORDMANN[1], A. JAYOL[1], L. POIREL[1]

[1]Medical and Molecular Microbiology Unit, Department of Medicine, University of Fribourg

Enterobacterial strains resistant to colistin are increasingly reported worldwide. Currently available colistin susceptibility methods are fastidious, time-consuming (24 h) and some methods are not reliable. Therefore, we have developed the Rapid Polymyxin NP test which is based on the detection of bacterial growth in presence of a defined concentration of colistin in a well-defined medium. Growth is evidenced by acid formation related to glucose metabolism observed through a color change (orange to yellow) of a pH indicator (red phenol). A total of 200 enterobacterial strains from varied species were used to evaluate the performance of the Rapid Polymyxin NP test. Five strains were intrinsically resistant to colistin, 130 strains had an acquired mechanism of resistance to colistin (chromosomally-encoded, plasmid-mediated MCR-1 and not yet known), and 65 strains were susceptible to colistin. MICs of colistin were determined using the reference broth microdilution (BMD) method and results were interpreted according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing. The sensitivity and the specificity of the Polymyxin NP test were excellent, being 99.3 and 95.4 %, respectively, as compared to the BMD method taken as the gold standard. It was rapid (less than 2h) and reproducible. The Rapid Polymyxin NP test was easy to perform, rapid, reliable, cost-effective, sensitive, and specific. It detects colistin-resistant enterobacterial strains from any species regardless the molecular mechanism of resistance to colistin (intrinsic, chromosomic and/or plasmid-mediated). It is a very useful tool for preventing spread with colistin-resistant strains and will change the overall management of those infected / colonized patients.

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A MULTI-CENTER EVALUATION OF THE NEW FILMARRAY MENINGITIS PANEL (BIOFIRE) FOR RAPID PCR-BASED DIAGNOSTICS

D. MEINEL[1], C. OTTIGER[2], O. DUBUIS[3], V. HINIC[1], C. STRAUB[1], A. EGLI[1]

[1]Clinical Microbiology, University Hospital Basel Basel, [2]Institute for Laboratory Medicine, Cantonal Hospital Aarau, Aarau, [3]Clinical Microbiology, Viollier AG, Allschwil, Switzerland

Background: Immediate and effective treatment in patients with meningitis or encephalitis is associated with improved clinical outcomes. However, the diagnostic prodcedure on cerebrospinal fluid (CSF) is time-consuming, labor-intensive, and due to pre-treatment often unsuccessful. Hence, we aimed to assess the performance of the Filmarray (FA) ME panel (Biofire, Salt Lake City, USA), a new rapid broad panel PCR assay.
Material/methods: The performance of the FA ME panel was compared with routine single PCR assays for viruses, and standard culture procedures for bacteria and yeasts. The FA panel is a nested-PCR and covers 6 bacterial (E. coli K1, H. influenzae, L. monocytogenes, N. meningitidis, S. agalactiae, and S. pneumoniae), 7 viral species (CMV, enterovirus, HSV1, HSV2, HVV6, hum. parechovirus, and VZV), and Cryptococcus neoformans/gattii. A total of 42 CSF samples were examined to determine the overall sensitivity and specificity. Six CSF samples with not-covered bacteria (e.g. S. aureus) were included as specificity controls. The limit of detection was determined by serial dilutions of bacteria and viruses.
Results: 23 samples were tested positive by FA, with21being confirmed by routine diagnostic. Two were culture-negative, however the discrepant FA results were confirmed with an in-house S. pneumoniae PCR and with positive blood-cultures (H. influenza). 19 culture- and PCR-negative samples were also negative with the FA panel. Overall, the sensitivity and specificity was 100%.
In serial dilutions S. pneumoniae, S. agalactiae, L. monocytogenes, N. meningitidis, E. coli K1, and C. neoformans were detected at 60, 7’500, 200, 10, 2’000, and 10 CFU/mL, respectively. For HSV1, HSV2, and VZV the dilutions showed detection limits of 42, 53, and 120 copies/mL, respectively.
Conclusions: The FA ME panel provides a sensitive and rapid PCR-based method for the detection of most common bacterial and viral pathogens associated with meningitis and encephalitis.

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OPTIMIZING THE EXTRACTION OF EXTRACELLULAR DNA OF AEROBIC GRANULAR BIOFILMS

Katarzyna BIALEK[1], Christof HOLLIGER[1]

[1]Laboratory for Environmental Biotechnology, School for Architecture, Civil and Environmental Engineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Formation and properties of aerobic granules for enhanced biological phosphorus removal (EBPR) from sequencing batch reactor (SBR) strongly depend on the extracellular polymeric substances (EPS) in which the microorganisms are embedded. The composition of EPS varies between different species but mostly it is made up of proteins, polysaccharides and extracellular DNA (eDNA), that has been recently recognized as an important structural component. Although great amount of literature on the EPS extraction is available, till now, there is no standardized protocol for EPS extraction. Extraction methods involving multi step processes seem to be the most promising, although too harsh extraction procedures can lyse the cells and release intracellular compounds, which contaminate the EPS samples creating bias. Commonly employed intracellular markers of cell lysis i.e. Glucose-6-phosphate dehydrogenase (G6PDH) or 2-keto-3-deoxyoctonate (KDO) are not always applicable, especially in systems where glycolysis does not occur and in communities with gram-positive bacteria, respectively. Therefore a new method to measure cell lysis is needed. The activity of isocitrate dehydrogenase (ICDH), an ubiquitous enzyme found in all living organisms utilizing the Krebs (citric acid) cycle, has been assessed as intracellular marker of cell lysis. Initial assays using this enzyme activity during EPS extraction revealed to be very sensitive to detect cell lysis in samples of microbial communities present in granular biofilms cultivated in SBR. Preliminary results of EPS extraction using multi-step method and enzymatic extraction as well as selection of appropriate dyes to visualize and distinguish intracellular and extracellular DNA in granular biofilm samples will be discussed.

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COMPARISON BETWEEN PHYLOGENETIC TREES AND MALDI-TOF MASS SPECTROMETRY FOR THE IDENTIFICATION OF FLAVOBACTERIUM PSYCHROPHILUM

N STREPPARAVA[1], T WAHLI[1], H SEGNER[1], A CAMINADA[2], M TONOLLA[2], O PETRINI[3]

[1]Department of infectious diseases and pathobiology, Centre for Fish and Wildlife Health, Bern, Switzerland, [2]Department of Environment Building and Design, Laboratory of Applied Microbiology, Bellinzona, Switzerland, [3]POLE Pharma Consulting, Breganzona, Switzerland

Flavobacterium species are widespread in natural and artificial ecosystems. These species are ubiquitous, opportunistic pathogens that may infect gills, skin, fins and internal organs of a range of injured or immunologically compromised or stressed individuals that are particularly susceptible. Infection often leads to high mortality rates, with large economic losses for fish farmers. Their taxonomy and phylogenetic relationships, however, are still unclear. MALDI-TOF MS is increasingly used in clinical microbiology laboratories to identify bacteria. This study aimed to assess the reliability of MALDI-TOF MS for the identification of Flavobacteria, using the fish pathogenic species F. psychrophilum as an example. We studied 119 Flavobacteriaceae samples including 115 Flavobacterium spp. isolates collected in fish farms in Switzerland and reference strains of F. psychrophilum, F. columnare, F. branchiophilum, F. johnsoniae, F. succinicans, F. frigidimaris, F. pectinovorum, F. aquidurense, F. hercynium, F. fryxellicola, F. limicola, F. psychrolimnae and F. aquatile obtained from culture collections. A dendrogram was prepared using MALDI-TOF MS data and was compared to phylogenetic trees constructed using the 16S rRNA and rpoC gene sequences. The MALDI-TOF MS dendrogram reflected the relationships seen in the 16S rRNA- and rpoC-based phylogenetic trees. Importantly, the MALDI-TOF analysis resulted in an unequivocal grouping of the F. psychrophilum isolates. MALDI-TOF MS can be used to identify Flavobacterium psychrophilum out of complex isolates. Still, the inclusion of additional spectra of well-characterized isolates of other fish pathogenic and environmental species in the MALDI-TOF database is needed. Flavobacterium environmental species are not yet well represented in the different databases, however, use of MALDI-TOF MS technology would implement fast and reliable identification saving time and improving fish management in both wild and fish farm environment.

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Wednesday, June 15th

DISTRIBUTION OF VIRULENCE FACTORS IN ESBL-PRODUCING ESCHERICHIA COLI ISOLATED AT THE ENVIRONMENT, LIVESTOCK, FOOD AND HUMAN INTERFACE.

M NÜESCH-INDERBINEN[1], A MÜLLER[1], R STEPHAN[1]

[1]Institute for Food Safety and Hygiene, University of Zurich, Winterthurerstr. 272, 8057 Zurich, Switzerland

In this study, extended-spectrum ß-lactamase (ESBL)-producing E. coli isolates recovered from the following sources were characterized with regard to the occurrence and distribution of uropathogenic and enteric pathogenic virulence factors: surface waters (rivers and lakes), the intestines of freshwater fish, fresh vegetables, retail poultry meat and the fecal samples of livestock, healthy humans and primary care patients. Among the 207 isolates, 82% tested positive by PCR for one or more of the virulence factors (VF) that predict uropathogenicity, TraT, fyuA, chuA, PAI, yfcv or vat. Uropathogenic Escherichia coli (UPEC) was detected in each of the analyzed sources. Regarding virulence factors for intestinal pathogenic E. coli, these were found to be rarer and predominantly associated with the aquatic environment, with aagR (EAEC) found in isolates from surface waters and STp and LT associated with isolates from fish. Aggregate VF scores were lowest among isolates belonging to phylogenetic group B1 and highest among group B2. Clustering of the isolates by phylogenetic group, multilocus sequence type (MLST) and ESBL-types detected clonal overlaps of A:ST10(CTX-M-1) and D:ST350(CTX-M-1) between the sources of livestock, poultry meat and healthy humans, suggesting animal food, in particular poultry, represents a potential reservoir for these particular UPEC clones. The clones A:ST10(CTX-M-55) and B2:ST131(CTX-M-27), harbouring uropathogenic virulence factors were significantly associated with fresh vegetables and with fish, respectively. Further clonal complexes with source overlaps included D:ST38(CTX-M-14), D:ST69(CTX-M-15), D:ST405(CTX-M-15) and D:ST648(CTX-M-15), which were found in surface water and healthy humans. Identifying potential reservoirs of UPEC in the environment, animals, food and humans is important in order to assess routes of transmission and risk factors for acquiring UPEC.

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Tuesday, June 14th

DETECTION OF CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI IN BROILERS ANTE MORTEM BY QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION

K HAAS[1], G OVERESCH[1], P KUHNERT[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

Human Campylobacteriosis is a major public health concern in Europe, including Switzerland with 9 million estimated cases per year. As known from other countries Campylobacter (C.) jejuni and C. coli from poultry is the main source of human cases in Switzerland as well (1). Contamination of broiler carcasses occurs at the slaughterhouse by the incoming flock and remains on the meat up to retail level and thus reaches the consumer (2, 3). Therefore, identification of Campylobacter-positive broiler herds before slaughter is essential for taking measures to avoid carry-over of pathogens via the slaughter process into the food chain.
However, appropriate methods and their validation to test poultry flocks ante mortem are missing. A quantitative real-time PCR (qPCR) that allows simultaneous detection, quantification and differentiation of C. jejuni and C. coli was recently developed (4). We optimized and adapted this qPCR to serve an easy, sensitive and quantitative method for Campylobacter detection in poultry flocks ante mortem by analysis of boot socks. An adequate correlation was shown between qPCR and culture, as well as between boot socks and caecal samples, regarded as gold standard. Thereby, our method allows quantifying the Campylobacter load of a flock shortly before slaughter by simply walk through the chicken house for taking boot sock samples. It further allows a categorization of the broiler herds into negative, low, moderate or high Campylobacter colonization.
Based on the results of this new qPCR, risk assessment models like evaluating the possible effect of sorting flocks before slaughter can be easily implemented. Similarly, targeted identification of highly colonized flocks for improvement of biosecurity measures on the farm level will become feasible presenting an opportunity to increase the food safety.

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Wednesday, June 15th

ENDOSPORE DNA AS A PROXY OF ANTIBIOTIC RESISTANCE GENES IN SEDIMENTS OF LAKE GENEVA CONTAMINATED WITH HEAVY METALS

Z. BAYRYCHENKO[1], C. PAUL[1], L. MADUENO[2], K. BECK[3], H. BUERGMANN[3], P. JUNIER[1]

[1]Laboratory of Microbiology, University of Neuchâtel, Neuchâtel, Switzerland, [2]Centro de Investigación y Desarrollo en Fermentaciones Industriales, UNLP-CCT CONICET, La Plata, Argentina, [3]Department of Surface Waters – Research and Management, Eawag, Kastanienbaum, Switzerland

Antibiotics resistance in pathogenic microbes is a pressing problem worldwide. Research over the last decades has shown that aquatic environments play a role as reservoir of antibiotic resistance genes (ARG) and thus as a pathway of dissemination of resistant organisms. There is also evidence of co-occurrence of ARG and heavy metals in aquatic sediments, but the reason for this is still unknown. Vidy Bay in Lake Geneva (Switzerland) receives treated and partly untreated sewage from the city of Lausanne, which results in high levels of sediment contamination. Previous studies have shown that Lake Geneva sediments are polluted with multi-resistant bacteria and ARG, as well as with heavy metals. Therefore these sediments are ideal to investigate the co-occurrence of ARG and heavy metals. Also the sediments with the highest levels of heavy metal contamination are enriched with Clostridium spp, which are endospore-forming Firmicutes commonly found in the human intestinal tract. Endospores are very resistant cell forms able to survive harsh environmental conditions. Unlike total bacterial DNA, which decreases with depth, endospore DNA remain constant with depth and is thus expected to better preserve information from the time of deposition. The aims of our study are to assess the potential of using endospores as archives for the spatial-temporal distribution and origin of ARG in lake sediments. In addition, the relationship between the distribution of ARG, heavy metals and endospores will be analyzed. Our preliminary analysis has shown that ARG tetW and sul1 are detected in endospore-DNA and that compared to the total DNA, endospore-DNA is enriched in ARG. We hypothesize that endospores is a useful tracer to understand the co-occurence of ARG and heavy metals in the environment and that they could clarify the origin of ARG in lake sediments. We are currently evaluating the distribution of tetW and sul1 genes in endospore-enriched DNA extracted from Lake Geneva sediments contaminated with heavy metals.

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Tuesday, June 14th

THE ROLE OF EISENIA FETIDA IN THE GERMINATION, SPORULATION AND DISPERSION OF BACILLUS SUBTILIS

M. STRAGIOTTI[1], C. LE BAYON[2], P. JUNIER[1]

[1]Laboratory of Microbiology, University of Neuchâtel, Switzerland. , [2]Laboratory of Functional Ecology, University of Neuchâtel, Switzerland

Interactions between microorganisms and earthworms are known under the theory dubbed the Sleeping Beauty Paradox. In this theory, earthworms have developed a mutualistic interaction with soil microbiota. The intestinal mucus of earthworms activates microbial communities usually present in soil in a dormant state. This soil microbiota digests organic matter, making it available to the earthworm. Consequently, earthworms play a role in the regulation of the abundance and the diversity of microbial communities. However, one aspect that is poorly understood is the role of earthworms in the active dispersal of microorganisms. Soil is a heterogeneous habitat, particularly in the distribution of resources. Faced with nutrient depletion or other environmental stressors, some bacteria have the ability to form endospores, which are differentiated cells in a dormant metabolic state. Endospores are poorly mobile and thus their dispersal might be limited. However, their resistance might offer a way to reverse this, by dispersing within the intestinal tract of earthworms. In such context, the purpose of this research is to study the influence of the epigeic earthworms Eisenia fetida on the metabolic state of the endospore-forming bacteria Bacillus subtilis and its role in the active dispersal of this bacterium. We hypothesize that 1) B. subtilis endospores survive through the digestive tract of earthworm and germinate in its casts and 2) B. subtilis are spread by E. fetida. In order to address the first hypothesis, microcosms will be inoculated with GFP-labelled B. subtilis strains. Cultural methods will be used to quantify the microbial load of earthworms' digestive content. In order to verify the second hypothesis, earthworms fed on a substrate inoculated with bacteria will be introduced in microcosms free of the studied strains. Microcosms will be harvested and the bacteria quantified as previously described. This study contributes to the understanding of soil functioning and microbial spatial distribution in soils and the role of earthworms as a vector of colonisation of new ecological niches by microorganisms.

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Wednesday, June 15th

INFLUENCE OF ECOLOGICAL FACTORS ON ARBUSCULAR MYCORRHIZAL FUNGI COMUNITIES OF BOTRYCHIUM LUNARIA

F.A SANDOZ[1], V HERVE[1], V HERVE[3], B DAUPHIN[2], J.R GRANT[2], S BINDSCHEDLER[1]

[1]Laboratory of Microbiology University of Neuchâtel, Neuchâtel, Switzerland, [2]Laboratory of Evolutive Botany, University of Neuchâtel, Neuchâtel, Switzerland, [3]Laboratory of Biogeosciences, Institute of Earth Sciences, University of Lausanne, Lausanne, Switzerland

Botrychium lunaria (Ophioglossaceae family), is a widespread fern that is completely dependent of a symbiosis with arbuscular mycorrhizal fungi (AMF) during all stages of its life cycle. Both the germination of spores and the formation of the achlorophyllous gametophytes occur entirely underground. Moreover, sporophytes produce their first emergent leaves only after 8 years. As a result, B. lunaria is under the total dependence of AMF communities in terms of carbon sources for a rather long period of time. In addition, the symbiosis remains important even after the emergence of mature sporophyte because of the particular root architecture that prevents for efficient foraging of nutrients in soils. This project aims at identifying the AMF communities associated to the roots of B. lunaria and the influences that biotic and abiotic factors have on the composition of these communities. We hypothesize that the alpha diversity of AMF communities of B. lunaria roots are related to the amount of carbon, nitrogen and phosphorous present in the rhizospheric soil of B. lunaria, but also to the alpha diversity of neighboring plants. In addition, we hypothesize that B. lunaria does not impose a selection for its AMF partners. Finally, we hypothesize that the four orders making up the Glomeromycota phylum are present in the roots of B. lunaria. In order to address these hypotheses, 72 soil samples without the presence of B. lunaria sporophytes and 72 B. lunaria sporophytes with their roots and their rhizospheric soil were sampled in four B. lunaria populations at the same altitude (2500 m a.s.l.). In total, 122 amplicons of the 18S rRNA gene (50 from soil samples and 72 roots samples from sporophyte root samples) were sequenced. In parallel, organic carbon, total nitrogen, total phosphorous, and pH were measured on rhizospheric soil samples. Preliminary results show that in total, 335 OTUs have been identified. Moreover, the sporophyte of B. lunaria is colonized by species of Glomerales, Diversisporales and Archaeosporales, with Glomerales OTUs being the most abundant ones. This study represents the first inventory of the AMF diversity of B. lunaria.

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Wednesday, June 15th

IDENTIFICATION OF MICROORGANISMS IN AEROBIC GRANULAR SLUDGE ACTIVELY INVOLVED IN BIOLOGICAL PHOSPHORUS REMOVAL

A. GELB[1], J. MAILLARD[1], C. HOLLIGER[1]

[1]Ecole Polytechnique Fédérale de Lausanne (EPFL), Civil and Environmental Engineering, Laboratory for Environmental Biotechnology, Lausanne, Switzerland

One of the aims of the treatment of domestic and industrial wastewater is the removal of phosphorus prior to discharge into the environment. Since phosphorus concentrations in wastewater exceed the requirement of bacterial growth, biological phosphorus removal is based on the ability of a group of microorganisms, named “Polyphosphate Accumulating Organisms” (PAO), to form large quantities of polyphosphate in their cells.

In this study, we are focussing on the bacteria actively involved in the phosphorus removal in a lab scale bioreactor operated with aerobic granular sludge technology. This process based on dense microbial biofilms is a cost-effective and land-saving alternative to the conventional wastewater treatment with activated sludge.

The identification of the microorganisms belonging to PAO relies on their distinct phenotype assessed with fluorescent probes targeting the intracellular polyphosphate structures. Sequencing of the 16S rRNA gene amplicons of PAO selected with flow-cytometry will be used to reveal the phylogenetic affiliation of the different actors involved in the phosphorus removal. This is of particular significance since low abundant microorganisms may play an important role in the phosphorus removal performance of aerobic granular sludge. We hypothesize that the maintenance of different populations involved in the same functional process is explained by multiple ecological niches present in such biofilm structures.

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Tuesday, June 14th

MEASURING MICROBIAL INTERACTIONS IN SOIL IN HIGH THROUGHPUT

M DUBEY[1], J R VAN DER MEER[1]

[1]Department of Fundamental Microbiology, University of Lausanne, Quartier Unil-Sorge, Biophore building, 1015 Lausanne, Switzerland

Microbial ecosystem engineering approaches often rely on the introduction of one or more selected species into an existing microbial community. The success of introduced species to a large extent depends on the types of interactions that it is developing with other existing microbes, such as neutralism, commensalism, syntrophism or competition. Deciphering the rules governing microbial species' interactions is a strenuous task.

Our project is focused on better understanding the principles of success of establishing pure cultures in complex microbial ecosystems such as contained within soil. Here we develop a high-throughput co-cultivation approach that might enable us to study the species' "interactome", the identification of favourable and non-favourable species combinations that decide on the survival of the inoculant in the community. We use agarose micro-beads as growth chambers to randomly combine soil community members with or without pure culture inoculants. Growth of co-cultured species members is followed by microscopy and enables a global overview of potentially positive effects of the inoculant on growth of the members in the soil on specific carbon substrates. In addition, the “interactome” study allows detecting possible species pairs, negative or positive, that can be recovered and identified in detail. The resulting knowledge not only provides ample data in designing functional synthetic communities but also construct new avenues for “synthetic ecology”.

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Tuesday, June 14th

IMPACT OF ABIOTIC FACTORS IN THE INTERACTION OF THE ECTOMYCORRHIZAL AND SAPROPHYTIC FUNGUS MORCHELLA CRASSIPES AND ITS ENDOBACTERIUM MASSILIA SP.

Y VENTURA[1], A LOHBERGER[1], A LOHBERGER[2], S BINDSCHEDLER[1]

[1]Laboratory of Microbiology, University of Neuchâtel, Neuchâtel, Switzerland, [2]Laboratory of Biogeosciences, IDYST, University of Lausanne, Lausanne, Switzerland

Fungi are involved in many type of interactions with other living organisms. An example of those are bacterial-fungal interactions (BFI), which are essential for soil functioning. In some cases, the presence of endobacteria inside fungal cells affects the fitness and pathogenicity of fungi interacting with plants. However, in these studies mostly the function of the endosymbiotic bacterium has been considered, while the role of the fungal host is still poorly understood. In this study we analyse the interaction between the ectomycorrhizal and saprophytic fungus Morchella crassipes and Massilia sp., an endobacterium isolated from this fungal species. The first aim of this study was to establish the type of interaction (i.e. positive or negative) between these two organisms. We hypothesized that Massilia sp. helps its host to cope with stressing environmental conditions. In order to address this hypothesis, the wild type fungus was cured by an antibiotic treatment to remove endobacterial populations. A co-culture was then performed in order to reintroduce the bacterium (wild type and GFP labelled) into the fungus. The effect of curing the fungus on fitness will be measured by comparing fungal biomass. Finally, the cured fungus with the reintroduced bacterium will be cultured under different varying growth conditions and the presence of the endobacterium will be assessed by qPCR of the 16S rRNA gene. We expect observing that the physiology of the wild type fungus is re-established by the reintroduction of its endobacterium. However, under extreme abiotic conditions for the fungus, we expect the endobacterium to be released in the medium. Based on these expected results and initial observations so far, the interaction between the fungus M. crassipes and its endobacterium Massilia sp. seems to be a positive symbiotic interaction according to Koch's postulates. However, the fungus seems not to need its endobacterium under all environmental situations, suggesting that this interaction follows a trade-off suiting both organisms. This study represents an essential step towards understanding the close relationship existing between a fungus and its endobacterial partner.

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Tuesday, June 14th

CHARACTERIZATION OF A LARGE 14-KB OPERON INVOLVED IN CONJUGATIVE TRANSFER OF ICECLC OF PSEUDOMONAS KNACKMUSSII B13

A. VUCICEVIC[1], S.D. SULSER[1], F. DELAVAT[1], V. SENTCHILO[1], J.R. VAN DER MEER[1]

[1]UNIL, FBM, DMF - Quartier Sorge, Batiment Biophore CH-1015 Lausanne, Switzerland

Integrative and conjugative elements (ICEs) are mobile DNA elements integrated in the bacterial host chromosome, but able to excise and transfer themselves into a new host cell. ICEclc of Pseudomonas knackmussii B13 is a model ICE for a family of elements widespread in Gamma- and Betaproteobacteria. Despite several studies, the role of most genes encoded on ICEclc in transfer remains obscure. One such example is comprised by a 14 kb cluster on ICEclc (provisorily named the 81655 operon), whose expression is among the highest detected so far for ICEclc genes. Here we investigate the role of the 81655 operon on ICEclc transfer and try to understand the nature of its extremely high expression. Using gene replacement techniques, we find that several of the genes within the 81655 operon are directly involved in ICEclc transfer, albeit deletion of others (notably of the first gene of the operon itself) has no discernable effect on transfer rates. Expression of the 81655 operon studied by micro-array hybridizations and RNA sequencing indicated specific high transcription in stationary phase but not in exponentially growing cells. Reporter gene fusions to the 81655 promoter region introduced in Pseudomonas putida with or without ICEclc showed expression only in cells containing ICEclc and only in a small fraction of cells. Expression coincided in cells which simultaneously expressed other ICEclc promoters, such as the intB13 integrase promoter.

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Wednesday, June 15th

NEW BIODEGRADATION TESTS FOR CHEMICAL COMPOUNDS AT LOW ENVIRONMENTAL RELEVANT CONCENTRATIONS

B OZEL DUYGAN[1], S BEGGAH[1], S REY[2], M SEYREFIELD[2], J VAN DER MEER[1]

[1]Department of Fundamental Microbiology, University of Lausanne, Switzerland, [2]Biotechnology Department, Firmenich SA, Switzerland

Existing biodegradation methods such as OECD tests are typically carried out at relatively high substrate concentrations such as 20 mgC/L that may result in under- or over- estimation of observed substrate utilization kinetics at environmentally relevant low concentrations. Furthermore, although well established, OECD guidelines are largely not suitable for testing hydrophobic and volatile compounds such as fragrances. Our main goal is to develop a standardized and validated growth-linked biodegradation test as an alternative method to existing biodegradation tests in the range of 0.1 - 10 mgC/L. Our methodological concept is based on comparative evaluation of cell density measurement by flow cytometer and substrate disappearance measurement by CO2 evolution and gas chromatography under assimilable organic carbon restricted conditions. We conduct our experiments with lake water microbial communities at starting cell density of 10E4 and 10E5 cells/mL in a defined mineral medium. Sodium benzoate, 1-octanol, anthraquinone and phenol are selected as primary positive controls for readily biodegradable compounds. We observed step-wise increase in the lake community size (at initial cell density of 10E4 cells/mL) at the expense of added positive control substance at concentrations of between 0.1 – 2 mgC/L and 1–2 mgC/L, respectively. Yield approximations from the observed community growth was in line with CO2 evolution test results for the target compounds. For accurate mass balance between compound and community size, we will further simultaneously measure lake water community growth and compound disappearance by gas chromatography. Lake water communities that enable biodegradation of the test compounds will be analyzed for diversity changes, and we will also further isolate and characterize degrader bacteria.

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Tuesday, June 14th

MODELING GENETIC CIRCUITS OF ARSENIC BIOREPORTERS

Y BERSET[1], D MERULLA[2], A JOUBLIN[2], V HATZIMANIKATIS[1], J R VAN DER MEER[2]

[1]Laboratory of Computational Systems Biotechnology, EPF Lausanne (CH); , [2]Department of Fundamental Microbiology, University of Lausanne (CH);

Bioreporters are living cells that have been genetically engineered to reveal the presence of a specific chemical compound. Because they are cheap to produce and can potentially be integrated in microfluidics devices,
bioreporters gathered interest as an alternative to chemical analysis of toxic compounds. In order to improve the reliability of arsenic bioreporters, we developed a detailed mechanistic model describing the gene regulatory networks – feedback or uncoupled – involved in bioreporter strains (1). The model includes the binding steps between the regulatory protein ArsR, its specific and non-specific DNA sites and arsenic, as well as the transcription, translation and maturation of the reporter gene (GFP).
We used a part of the experimental data to calibrate the model, i.e. to find sets of model parameters that best fit the data. The parameter search was performed by a population based method and concluded by a gradient method. With the obtained parameters, we tested the ability of the model to simulate the remaining experimental data consisting of allelic variations and deletions.

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Tuesday, June 14th

INTERROGATING THE FUNCTION OF UNANNOTATED CANDIDA ALBICANS ORFs EXPRESSED IN VIVO

P. BERRA[1], S. AMORIM-VAZ[1], H. BESANÇON[1], F. ISCHER[1], D. SANGLARD[1], A.T. COSTE[1]

[1]CHUV-Institut de Microbiologie

A large proportion of Candida albicans open reading frames(ORFs) have still unknown functions. One useful approach to unveil their function is to obtain transcriptional data of these ORFs in specific conditions. We have used an RNA enrichment approach to identify genes that are expressed in vivo in two different animal models (mice, Galleria mellonella larvae) (1). Among the most upregulated genes in vivo, 2 ORF with unknown functions were present (orf19.1363 and orf19.7455) and are the subject of the present study.

In this work, these 2 ORFs were characterized by reverse genetics. We investigated the phenotypes of the respective null mutants in vivo, but also their phenotypes on several in vitro conditions mimicking the conditions of the host environment such as oxidative-, pH-, CO2-, temperature-stresses, use of alternative carbon sources, iron and zinc deprivation and filamentation conditions. In addition, we used several drug stresses, biofilm and adhesion on plastic surface assays and co-cultures with immune cells.

This large in vivo/in vitro screen allowed us to determine that orf19.1363 was required to survive and invade the host. Indeed, the fungal burden following infection in G. mellonella was significantly lower than for the wild type strain. Plus, the mutant was less efficiently uptaken by murine macrophages than the wild type. No significant in vitro phenotype which could help to determine its exact role was identified, however the experiments highlighted a more specific role in vivo.

The study of two independent orf19.7455 deletion mutants revealed a role of this gene in virulence in G. mellonella, since the larvae survive slightly longer when infected by the mutants than by the wild type or revertant strains. These mutants also showed decreased biofilm formation efficiency in vitro and a specific rate of filamentation, with a faster initiation of filamentation decreasing with time.

Although the characterization of both mutants is not complete yet, this study highlights a framework to assess the function of the numerous remaining uncharacterized C. albicans genes specifically expressed in vivo.

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Tuesday, June 14th

SHEDDING LIGHT ON LOCAL KINASE ACTIVITY USING A BIMOLECULAR SYNTHETIC KINASE ACTIVITY RELOCATION SENSOR

N. MIRA[1], S. PELET[1]

[1]Quantitative Signaling Group, Department of Fundamental Microbiology, University of Lausanne, UNIL-Sorge District, Biophore Building CH-1015 Lausanne, Switzerland

Studies of signaling pathways have focused on the quantification of global changes of MAPK activity. However, localized activity of MAPK may play a crucial role in many signal transduction cascades. For instance, in the yeast mating process, Fus3 MAPK and other signaling components are enriched at the tip of the mating projection. To quantify this local activity, we sought to develop a Bimolecular Synthetic Kinase Activity Relocation Sensor (Bi-SKARS), which consists of two synthetic modules: the docking module includes a MAPK docking site, a phosphorylatable sequence and a membrane targeting sequence, while the translocating module bears a phospho-binding domain as well as a fluorescent protein to track its sub-cellular location. The MAPK binds to the docking site upon pheromone stimulation and phosphorylates the Ser/Thr-containing motif. The recruitment of the translocating module via the phospho-Ser/Thr binding domain results in a change in fluorescence distribution.
Using this sensor, we observed a robust membrane enrichment of the sensor at the plasma membrane 4 min after stimulation of cells with pheromone. The specificity of the system to Fus3 MAPK was confirmed by the absence of recruitment with a non-docking version of the biosensor. Our next experiment will be to study the dynamic response of the biosensor using a fus3-as allele, which can be inhibited by an ATP analog. We will also verify the specificity of interaction between the two modules using a non-phosphorylatable and a phosphomimetic Bi-SKARS. The quantification of MAPK local activity will allow us to gain new insights into the conversion of Fus3 MAPK activation from an isotropic response to a polarized signal at the shmoo tip and its contribution to the establishment of a stable polarity site.

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FIRST DETECTION OF TR34/L98H MUTATION IN ASPERGILLUS FUMIGATUS ISOLATES FROM ENVIRONMENT SAMPLES IN SWITZERLAND

A RIAT[1], F ISCHER[3], JC SCHRENZEL[2], K GINDRO[4], D SANGLARD[3]

[1]Bacteriology Laboratory, University of Geneva Hospitals, , [2]Genomic Research Laboratory, Service of Infectious Diseases, University of Geneva Hospitals and Faculty of Medicine; Geneva (Switzerland), [3]Institute of Microbiology, University Hospital Lausanne and University Hospital Center, CH-1011 Lausanne, Switzerland, [4]Agroscope, Institute for Plant Production Sciences IPS, Mycology and Biotechnology, 1260 Nyon, Switzerland.

Abstract
Aspergillus fumigatus isolates resistant to azole drugs have been reported in several countries, however no recent data exist in Switzerland.
Snelders and colleagues (1) have described azoles resistance in A. fumigatus isolated from clinical samples for the first time in 2008. The mutations found in the azole-resistant isolates were associated with the gene CYP51A, whose product is involved in ergosterol biosynthesis. Different codon substitutions in CYP51A have been identified from A. fumigatus in clinical samples as well as samples from the environment. The presence of A. fumigatus with a single substitution in CYP51A is mainly encountered in patients after long-term azole therapy. In addition to single mutations, a tandem repeat in the promoter region of the CYP51A has been identified and was found to confer multi-azole resistance (TR34/L98H and TR46/Y121F/T289A)(2). Different studies have suggested an environmental origin of the TR34/L98H and TR46/Y121F/T289A mutations with a predominance of TR34/L98H (3, 4).
Here, we describe the first environmental A. fumigatus isolates, which harbored CYP51A mutations that confer azole resistance. Sixty-nine samples were collected in the Geneva Lake area and four of them showed resistance to azoles drugs. Three isolates possessed the TR34/L98H mutation and one isolate had a single substitution at the position 54 (G54R) of CYP51A. In addition, 20 A. fumigatus isolated from cystic fibrosis patients during the year 2011 and 2015 were phenotypically tested for azole resistance, however all of them were susceptible to itraconazole.
In conclusion, the occurrence of azole resistance from environmental origin in Switzerland is in agreement with reports from other countries. This situation needs further systematic surveillance, since transmission of azole-resistant isolates to patients is possible.

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EFFICIENT ARTHRODERMA BENHAMIAE EXPRESSION SYSTEM TO PRODUCE DERMATOPHYTE SECRETED ANTIGENIC PROTEINS

N DE COI[1], T YAMADA[2], K SALAMIN[1], M MONOD[1]

[1]Department of Dermatology, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland., [2]Teikyo University Institute of Medical Micology, Hachioji, Tokyo, Japan

Background: We obtained a complete gene expression profile of the genes coding for proteins secreted by A. benhamiae (dermatophyte) during infection using RNA-seq analysis. The expression profile of genes encoding secreted proteins was found to be very different from those during in vitro growth using various substrates. In particular, the most upregulated genes encoding secreted proteases during infection were those encoding subtilisins (SUB6, SUB7, SUB8 and sUB10), which have never been detected in all in vitro conditions tested. SUB6 was known to be a major allergen in the related dermatophyte Trichophyton rubrum.
Objective: The objective of this work was to characterize SUB6 and SUB7 as recombinant proteins. Recombinant SUB6 and SUB7 could not be obtained using either Escherichia coli, or Pichia Pastoris or Aspergillus expression systems. Therefore, we developed an Arthroderma benhamiae system to produce dermatophyte secreted proteins for further characterization and antigen tests.
Methods: A plasmid pNDC1 was designed for the production of dermatophyte secreted proteins under the control of the Translation Elongation Factor 1 (TEF1) promoter, and Agrobacterium mediated transformation was used.
Results: Transformants grown in minimal liquid medium (MM) were tested for proteolytic activity using N-Suc-Ala-Ala-Pro-Phe-pNA as a substrate. Activity varied in a 1 to 10 ratio from one clone to another. The transformants with maximum SUB6 or SUB7 activity were retained for further investigations. Western blot analyses revealed that SUB6 or SUB7 were indeed secreted by the selected transformants as strong signals were obtained with antibodies. A yield of 10µg/ml culture supernatant was obtained for both Sub6 and SUB7. Characterization of individual recombinant SUBs is underway.
Conclusion: The developed Arthroderma benhamiae expression system revealed to be efficient to obtain dermatophyte secreted proteins which could not be produced using heterologous expression systems.

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TRANSCRIPTOMIC APPROACH TO DETERMINE THE INTERACTION BETWEEN CANDIDA ALBICANS AND THE HOST MUCOSA IN VIVO

S ALTMEIER[1], F SCHÖNHERR[1], S AMORIM-VAZ[2], Vdu T TRAN[3], AT COSTE[2], D SANGLARD[2], S LEIBUNDGUT-LANDMANN[1]

[1]Section of Immunology, Vetsuisse Faculty, University of Zürich, Winterthurerstrasse 266a, 8057 Zurich, Switzerland, [2]Institute of Microbiology, University of Lausanne and University Hospital Center, Rue du Bugnon 48, 1011 Lausanne, Switzerland, [3]Swiss Institute of Bioinformatics, University of Lausanne, CIG - Quartier Sorge, Bâtiment Génopode, 1015 Lausanne, Switzerland

Candida albicans is one of the most important disease-causing fungi in humans. Infections of the oral and vaginal mucosa are by far the most frequent disease manifestations affecting millions of people worldwide. While C. albicans lives as a commensal on mucosal surfaces of most healthy individuals, it can become pathogenic under certain circumstances. Predisposing conditions include immunosuppression and acquired or congenital immunodeficiencies. Disease development may also be influenced by the variation in fungal virulence that exists among C. albicans strains in the population. Here, we used a genome-wide transcriptomic approach in combination with a mouse model of oropharyngeal candidiasis to study the mutual response of the fungus and the host within the infected tissue. The host expression profiles reveal a dynamic regulation of the host response pathways and uncover novel candidate genes involved in the response to C. albicans. Applying this approach to mice infected with different fungal isolates of high and low pathogenicity revealed major differences in the transcriptional response to these isolates that reflect their disparate capacity to persist in the host mucosa. Together, these data advance our current understanding of the interaction between C. albicans isolates and the mucosal epithelium, which is of paramount importance for understanding the factors that determine the fine balance between commensalism and infection.

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ANALYSIS OF THE MYCOBIOTA PRESENT IN ABORTION MATERIAL FROM CATTLE BY ITS AMPLICON SEQUENCING

S VIDAL[1], V PERRETEN[1], S RODRIGUEZ CAMPOS[1]

[1]Institute of Veterinary Bacteriology - Länggass-Str. 122 3001 Bern

Mycotic abortion is a sporadic reproductive problem of dairy cattle all over the world. It can be caused by different fungi, which are also present in the environment. However, the transmission and epidemiology of abortion-associated fungi has not yet been widely studied. In Switzerland, only few infectious agents are examined in routine abortion diagnosis due to the high costs associated with extended spectrum analysis. In order to get new insight into agents causing abortion in cattle, we used next generation sequencing (NGS) to study the population of fungi associated with abortion. Fungal microbiota of 95 samples of abortion material from cattle [placenta (n=73); abomasum (n=22)] has been disclosed using Illumina MiSeq sequencing technology. DNA was extracted with the QIAamp Mini Kit (Qiagen), and amplicon sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region was carried out. Data analysis was performed using the software Qiime, in combination with the reference database UNITE, to define operational taxonomic units (OTUs) and to assign each OTU the taxonomic identity at different taxonomic levels. An average of 34 (SD = 7.5) representative OTUs in placenta samples (55.55% Ascomycota, 8.38% Basidiomycota, 6.28% Neocallimastigomycota, 4.10% Zygomycota and 25.69% other - unidentified - unassigned) and an average of 31 (SD = 5.66) representative OTUs in abomasum samples (57.99% Ascomycota, 15.06% Basidiomycota, 0.29% Neocallimastigomycota, 1.83% Zygomycota and 24.82% other - unidentified - unassigned) were found. In both organs the most representative families were Saccharomycetaceae (19.39% in placenta and 12.28% in abomasum) and Mycosphaerellaceae (12.36% in placenta and 24.02% in abomasum). The results revealed some genera which may play a role in abortion including Aspergillus, Fusarium, Penicillium, Mucor, Candida, Cryptococcus and Mortierella. Moreover, Rhodotorula, Scedosporium and Alternaria, three genera that can cause opportunistic infections in animals and humans were found. Comparison of associated mycobiota with indicative histopathological findings is now necessary to determine which fungi families are involved in abortion.

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BACTERIAL-FUNGAL INTERACTIONS AND SOIL FUNCTIONING: WHAT DO WE KNOW ABOUT THE FUNGAL PARTNERS?

S BINDSCHEDLER[1], V HERVE[2], T WUNDERLIN[3], A LOHBERGER[1], I JAMIL[1], G CAILLEAU[2], LY WICK[4], EP VERRECCHIA[2], P JUNIER[1]

[1]Laboratory of Microbiology, University of Neuchâtel, Switzerland., [2]Laboratory of Biogeosciences, IDYST, University of Lausanne, Switzerland., [3]Molecular Ecology, Institute for Sustainability Science, Agroscope Zürich, Switzerland, [4]Department of Microbiology, Hemholtz center for environmental research - UFZ, Leipzig, Germany.

Fungi and bacteria are both essential actors of organic matter turnover and nutrient cycling in soils, a crucial aspect of ecosystem functioning. While their individual roles are well described, the influence of bacterial-fungal interactions (BFI) in soil functioning is still poorly understood. Both organisms have shared the same environment for millions of years resulting in a myriad of interactions from mutualism to antagonism. Describing such interactions in complex systems such as soils is challenging, as their regulation depends on both biotic and abiotic factors. Consequently, both reductionist and systemic approaches are needed in order to get a comprehensive overview of BFI related to ecosystem functioning. First, by using model organisms in Petri dish experiments, we are starting to understand some basics regulatory elements of BFI in nature. In the systemic approach, our model is the oxalate-carbonate pathway (OCP), a natural process in which both fungi and bacteria play a crucial role. The OCP has a major impact on soil functioning by triggering alkalinisation in acidic soils, which positively impacts soil nutrient content. As a result, the OCP is a pertinent model to study the impact of microorganisms on soil functioning. Using soil microcosms studies we were able to highlight that BFI have a positive impact on OCP functioning. Traditional microbial ecology has usually focused more on bacteria than fungi. Therefore, in this presentation we will highlight the role of fungi in BFI. In the future we aim at understanding the complexity of BFI in soils and to decipher the instrumental role of BFI in soil nutrient bioavailability and plant health. Although there are still many aspects to disentangle, our studies highlight the overlooked role of BFI in biogeochemical processes. As a consequence, future studies aiming at unravelling the factors that drive soil functioning should definitely benefit from integrating BFI.

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BIO-INOCULATION OF PLANT GROWTH PROMOTING BACTERIA AS ENDOSPORE: A PROMISING STRATEGY TOWARDS SUSTAINABLE AGRICULTURE

I. JAMIL[1], S. BINDSCHEDLER[1], P. JUNIER[1]

[1]Laboratory of microbiology, University of Neuchâtel, Neuchâtel, Switzerland

The search for sustainable alternatives in agriculture has become a priority. In the mycorrhizosphere, bacteria and fungi are considered as crucial actors of soil functioning, as they are involved in many beneficial activities supporting the whole soil ecosystem. Plant growth promoting rhizobacteria (PGPR) are well- known to enhance plant growth through biocontrol and biofertilization activities. Endospore forming bacteria (EFB) have the ability to form dormant structures called endospores, allowing them to survive under harsh environmental conditions. Some EFB are known PGPR species and thus combining both, their plant-growth promoting activity and their application as spores may represent a potential easy-to-use solution for bio-inoculation. In this study we selected endospore-forming bacteria with plant growth promoting abilities to investigate their effect on plant growth and their interactions with soil fungi. For this, a field experiment using oat (Avena sativa) was set using a mixed inoculant of three mesophilic EFB strains having plant growth-promoting activities. Bio-inoculation was carried out in the form of either vegetative cells or endospores onto the seeds. The results obtained so far suggest that bio-inoculation with EFB has a positive effect on plant growth and fitness. Inoculated seeds were observed using scanning electron microscopy and confirmed that the bacteria inoculated (vegetative cells/endospores) were present on the surface of treated seeds as compared to untreated seeds. Analyses of total bacterial and fungal communities and the survival of bio-inoculated EFB in soil are under process. Since the survival of inoculated bacteria is often a critical issue, the inoculation of bacteria in the form of endospores as a delivery system might represent an effective way to supply bio inoculants in the soil.

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FUNGI TO THE RESCUE OF ARCHAEOLOGICAL IRON

L COMENSOLI[1], P JUNIER[1], E JOSEPH[1]

[1]Laboratory of Microbiology, Institute of Biology University of Neuchâtel, Switzerland , [2]Haute Ecole Arc Conservation-Restauration, Neuchâtel, Switzerland

Iron artefacts are the most recurring metal findings recovered during archaeological excavations. Without any conservation-restoration intervention these artworks are affected by corrosion that leads to structural modifications. The main issue with this metallic substrate is the chlorine content of the corrosion layers, which, reacting with H2O and O2, causes an irreversible deterioration of the objects once excavated. The conservation-restoration methods available for this metal perform poorly concerning efficiency and time and in addition they rely on toxic substances. The project presented here proposes sustainable alternatives exploiting fungi. In order to stabilize the corrosion layer of these objects we propose to convert the compounds present in the corrosion layer into more stable iron minerals and to remove chlorine. For this purpose, the interaction between iron and several halotolerant fungi was studied. The ability of different fungal strains to uptake and translocate chlorine was also studied. Crystal formation with several iron sources was investigated, and a simple method to study fungal iron reduction was developed. The production of fungal exopolysaccharides, and their capacity to chelate iron was evaluated. Spectrophotometric analyses and Scanning Electronic Microscopy were conducted to ascertain fungal iron uptake. After 10 days of incubation in liquid cultures amended with 10 mM of FeCl3, Cladosporium sp., Fusarium oxysporum, Fusarium colmorum and Beauveria bassiana were able to uptake iron and accumulate this metal inside the biomass. Nevertheless, different uptake behaviours were noticed. B. bassiana accumulate mainly Fe(III), while, a mix of Fe(II) and Fe(III) was detected in the biomass of Cladosporium sp., F. oxysporum and F. colmorum. The results and their possible application in the conservation-restoration field will be presented here.

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THE POPULATION STRUCTURE OF HYMENOSCYPHUS FRAXINEUS MITOVIRUS 1 SUGGESTS TWO SEPARATE INTRODUCTIONS OF H. FRAXINEUS INTO EUROPE

C.N. SCHOEBEL[1]

[1]Swiss Federal Institute for Forest, Snow and Landscape Research, WSL, Zuercherstrasse 111, 8903 Birmensdorf, Switzerland

The ascomycete fungus Hymenoscyphus fraxineus (synonym: H. pseudoalbidus; basionym: Chalara fraxinea) is a new invasive pathogen causing severe dieback of ash trees (Fraxinus spp) in Europe. We recently detected a novel mycovirus, Hymenoscyphus fraxineus mitovirus 1 (HfMV1), within this pathogen (1). Here, we explored the prevalence and genetic structure of HfMV1 in order to elucidate the pathways of spread of this devastating pathogen. A total of 1127 H. fraxineus isolates, collected in 16 European countries and Japan from fallen petioles and bark lesions were screened for the presence of this RNA virus. The study includes a large-scale comparison, including all countries, as well as a small-scale comparison between Swiss and Lithuanian populations, including isolates sampled at a very detailed sampling scheme. To date Switzerland is considered to be the epidemic front of this disease, while Lithuania was amongst the countries where as dieback was first recorded in the 1990ies. Our data confirm that HfMV1 is highly prevalent throughout the whole distribution range of H. fraxineus, suggesting an efficient transmission of the virus, probably through sexual spores of its host. Our findings (genetic and phylogenetic analyses of 327 sequences (partial RNA dependent RNA polymerase, 497 bp)) also confirm the occurrence of a single mitovirus species throughout Europe. HfMV1 shows scarce population genetic structure with low nucleotide diversity but a high number of haplotypes, which seem to be subjected to a strong purifying selection. Moreover, we suggest that only two (mitovirus-carrying) fungal isolates have been introduced into Europe, more specifically, our data speaks in favor of two separate introductions of one mitovirus-carrying H. fraxineus isolate each.

(1) Schoebel, C.N., Zoller, S., Rigling, D., 2014. Detection and genetic characterisation of a novel mycovirus in Hymenoscyphus fraxineus, the causal agent of ash dieback. Infect. Genet. Evol. 28: 78-86.

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LEPTOSPIROSIS AS POSSIBLE CAUSE OF ABORTION IN SMALL RUMINANTS

P SCHNYDRIG[1], S VIDAL[1], V PERRETEN[1], S RODRIGUEZ-CAMPOS[1]

[1]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

Leptospira are flexible, spiral-shaped, Gram-negative spirochaete bacteria that cause leptospirosis in a wide range of mammals, including sheep (Ovis aries) and goats (Capra aegagrus hircus). Leptospirosis is characterized in small ruminants by abortions, neonatal deaths and impaired fertility. The gold standard for the diagnosis of leptospirosis is the microscopic agglutination test (MAT) based on detection of antibodies in serum. Real time PCR is also often used to directly detect leptospiral DNA. Between 1991 and 2015, 485 cases of leptospirosis in cattle were notified, however only one case of leptospirosis was reported in small ruminants. In order to better evaluate the importance of this possible abortive agent in small ruminants, we screened 79 tissue samples [placenta (n=50); abomasum (n=29)] corresponding to 61 cases [sheep (n=31); goat (n=30)] from 2012-2016 for presence of pathogenic Leptospira spp. by real time PCR and 27 related serum samples from ewes by MAT. The MAT was performed with a panel of 9 serovars [Grippotyphosa, Australis, Pomona, Tarassovi, Canicola, Icterohaemorrhagiae, Hardjo, Sejroe, Ballum]. The titers of the serum samples were determined from 1:100 to 1:3200. The bacterial DNA was extracted from abortion material with QIAamp Mini Kit (Qiagen) [samples (n=51)] and with Ultra Clean® Tissue&Cells DNA Isolation Kit (MoBio) [samples (n=28)]. Real time PCR was performed in a 7500 Real Time PCR System (Applied Biosystems), targeting the gene coding for the outer membrane protein LipL32. Out of the 27 sera tested by MAT, 8 sera [sheep (n=6); goat (n=2)] were seropositive for one serovar [Australis (n=2), Pomona (n=2), Hardjo (n=2), Ballum (n=2)] and one serum [sheep (n=1)] was positive for two serovars [Sejroe (n=1), Hardjo (n=1)]. From the 79 tissue samples analyzed by real time PCR, 3 samples [placenta (n=2); abomasum (n=1)] from sheep were positive. One of the PCR positive cases was negative in the MAT. These results indicate that even if leptospirosis plays a minor role in abortion in small ruminants, sporadic cases can occur.

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ANTIBIOGRAMS OF CLINICAL SHIGELLA SPP. FROM 2004 TO 2014: PLASMID-ENCODED RESISTANCE TO CIPROFLOXACIN, 3RD GENERATION CEPHALOSPORINS AND AZITHROMYCIN

H HÄCHLER[1], M NÜESCH-INDERBINEN[1], N HEINI[1], K ZURFLUH[1], D ALTHAUS[1], R STEPHAN[1]

[1]National Centre for Enteropathogenic Bacteria and Listeria, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, 8057 Zurich, Switzerland

We analyzed 344 Shigella sp. strains, isolated between 2004 and 2014 in Switzerland for antimicrobial resistance profiles. Overall, 78.5% of the isolates showed multiple drug resistance (MDR), 10.5% were ciprofloxacin resistant and 2% harbored mph(A), a plasmid-mediated gene that confers reduced susceptibility to azithromycin, a last-resort antimicrobial agent for the treatment of MDR shigellosis.

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ESBL AND CARBAPENEMASES DETECTION DIRECTLY FROM RECTAL SWABS WITH THE RAPID ISOTHERMAL AMPLIFICATION-BASED EAZYPLEX® SUPERBUG CRE ASSAY

A. CHERKAOUI[1], G. RENZI[1], P. FRANCOIS[2], J. SCHRENZEL[1]

1Bacteriology Laboratory, Division of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva, University Hospitals, 4 rue Gabrielle-Perret-Gentil, 1205 Geneva, Switzerland, 2Genomic Research Laboratory, Service of Infectious Diseases, Geneva University Hospitals, 4 rue Gabrielle-Perret-Gentil, 1205 Geneva, Switzerland

Objectives: We evaluated the performance of the eazyplex® SuperBug CRE system for the detection of the most common carbapenemases in addition to CTX-M-type ESBLs directly on rectal swabs collected at Geneva University Hospitals.
Methods: Out of 90 rectal swabs tested, 44 grew ESBL producing Enterobacteriaceae, 5 grew carbapenemase producing Enterobacteriaceae (2 KPC, 2 OXA-48, and 1 NDM-1), and 2 grew ESBL and carbapenemase producing Enterobacteriaceae (2 OXA-48). Phenotypic confirmation of ESBL production on culture isolates was performed with the double-disk synergy test and ESBL + AmpC Screen Kit (ROSCO DIAGNOSTICA). For carbapenemase production, the confirmation was assessed using home-brew real-time PCR assays. The corresponding rectal swabs (eSwabTM, Copan) were tested by eazyplex® SuperBug complete B Assay, according to the manufacturer's instructions.
Results: The eazyplex® SuperBug CRE system correctly detected ESBL-encoding genes in 40/46 ESBL-positive isolates, and in the 36/46 corresponding rectal swabs. The remaining 6 culture isolates and corresponding rectal swabs tested ESBL-negative in eazyplex® were all identified as TEM-116 producers, following Sanger sequencing results. This target is absent from this assay. Absolute concordance (100%) was observed in all isolates with phenotypes compatible with the presence of a carbapenemase, with or without an ESBL, based on susceptibility patterns and phenotypic inhibitory profiles. No false-positive results were observed with the eazyplex® SuperBug complete assays. Determinations performed with the eazyplex® SuperBug CRE system took 20 to 30 min.
Conclusions: This study demonstrated that the eazyplex® SuperBug CRE system represents a promising platform with optimal sensitivity for the routine detection of many of the most prevalent carbapenemases as well as CTX-M-type ESBLs in Enterobacteriaceae directly from rectal swabs with a rapid resolution time. The implementation of this system in routine clinical laboratories provides clinicians with early valuable information for the accurate management of patients with infections caused by ESBL and/or carbapenemase producing Enterobacteriaceae.
other

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AUTOMATED IDENTIFICATION AND QUANTIFICATION OF MICROBIAL GROWTH IN BOTH DEFINED AND CLINICAL SAMPLES

A CROXATTO[1], R MARCELPOIL[2], C ORNY[2], D MOREL[2], G PROD'HOM[1], G GREUB[1]

[1]Institute of Microbiology, University of Lausanne and University Hospital Center (CHUV), Lausanne, Switzerland, [2]BD Kiestra, Drachten, The Netherlands

Automation in microbiology laboratories impacts management, workflow, productivity and quality. Further improvements will be driven by the development of intelligent image analysis algorithms allowing earlier detection of microbial growth, automated detection of sterile samples, automated identification and quantification of bacterial colonies as well as automated reading of AST disk diffusion assays. We investigated the potential benefit of intelligent imaging analysis by developing an algorithm allowing automated identification and quantification of bacterial colonies.
Defined monomicrobial samples and clinical urines were inoculated by the BD Kiestra InoqulA BT module. Images of plates were taken with the ImagA BT digital imaging module (BD Kiestra) and were analyzed with the BD KiestraTM OptisTM software. The algorithms were developed and trained using defined data sets and their performance evaluated on both defined and clinical samples.
The algorithm was challenged with various bacterial species and concentrations exhibiting more than 95% accuracy in microbial growth detection. Moreover, quantification accuracy of 84% and of 98% with a 1 log tolerance was obtained with defined monomicrobial samples. Automated detection and quantification was also applied on clinical urine specimens giving a detection accuracy of 95% and quantification accuracy of 90% and of 100% with a 1 log tolerance from expected values, despite the presence of multiple species in the sample. A non parametric Spearman correlation of 0.99 (0.97 – 0.99, 95% CI) was obtained between visual and automated quantification. Preliminary results of automated identification of bacteria growing on chromogenic agar ranged from 70% to 99.9% identification accuracy depending on the tested bacterial species.
The development of intelligent algorithm represents a driving innovation that will increase laboratory quality and productivity while reducing turn-around-times. Further development and validation with a larger volume of defined and clinical samples should be performed before transferring intelligent imaging analysis into diagnostic laboratories.

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LABEL-FREE OPTICAL BIOSENSOR BASED ON PHOTONIC CRYSTAL SURFACE WAVES REVEALS BINDING KINETICS OF ANTIBODIES TO LIVING BACTERIA ESCHERICHIA COLI DH5A.

E. ROSTOVA[1], S.K. SEKATSKII[1], G. DIETLER[1]

[1]École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland Laboratoire de physique de la matière vivante

According to reviews on antimicrobial resistance, each year around 700'000 people die of infections caused by drug resistant bacteria. The spread of the antimicrobial resistance caused by a substantial misuse of antibiotics is a growing public health concern worldwide. Prescription drug abuse is likely provoked by unknown drug action mechanisms, which are challenging to discover by available technologies. Therefore, an efficient technique is needed for measurements of drug binding interactions.
Drug binding kinetics on living cells can be measured by a biosensor based on photonic crystal surface waves. In the biosensor, two optical surface waves are supported by a specially designed photonic crystal consisting of dielectric layers with different refractive indices. When the sample attaches on the photonic crystal surface, the refractive index of the medium adjacent to the surface increases, and the change of the excitation conditions of the surface waves is monitored by the camera. Sufficient sensitivity of the biosensor is achieved due to a deeper wave penetration into the sample compared to typical optical biosensors.
Using this biosensor, we studied binding kinetics of mono- and polyclonal antibodies against bacterial lipopolysaccharides to living gram-negative bacteria Escherichia coli DH5α. High sensor sensitivity due to a specially designed photonic crystal permits to observe binding of the antibodies at a concentration of 1.25 µg/ml. The data analysis reveals specific binding with the dissociation constant (KD = 6.2 3.4 nM) for the monoclonal antibodies.
Qualitative kinetic information collected in real time on living cells will certainly accelerate and optimize screening of drug candidates. The photonic crystal-based biosensor can serve as a "control unit" in antibody production for rapid selection of the best isoform based on its kinetic properties. Also cancer cells and various drugs are our next focus. Ultimately, the biosensor can be employed to study binding of drugs together with a viability test, eventually leading to the identification of currently unclear mechanisms of drug action.

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CHARACTERISATION OF SECRETED EFFECTORS AND ACCESSORY PROTEINS OF THE TYPE VI SECRETION SYSTEM IN BURKHOLDERIA CENOCEPACIA

S HEDAYAT[1]

[1]Department of infection, immunity and cardiovascular disease, Medical School, University of Sheffield, Sheffield, United Kingdom (UK)

Burkholderia cenocepacia has received increasing attention in recent years due to its pathogenicity in immunocompromised patients and CF patients.The type VI secretion system (T6SS) as a novel type of protein secretion system is present in Burkholderia cenocepacia and may play a role in its virulence. VgrG (TssI) is one of 13 core components of the T6SS machine but can also act as an effector.Two putative effector proteins with an additional domain at C-terminus with phospholipase A1 activity (PLA) are TssI BCAS0667 and TagD (BCAL1296). Downstream of BCAL1296 and BCAS0667 are genes encoding proteins with ankyrin-repeat domains (BCAL1297 and BCAS0666, respectively) that may serve as immunity proteins for the BCAL1296- and BCAS0667-encoded proteins. One of the aims of this project is to determine whether the putative accessory proteins BCAL1297 and BCAS0666 as putative immunity proteins interact with their corresponding effector proteins with phospholipase activity.In this project, the bacterial adenylate cyclase two-hybrid system (BACTH)and pull-down assay were used to investigate protein-protein interactions between BCAS0667 (evolved TssI) and protein BCAS0666, and between BCAL1296 (evolved TagD) and BCAL1297. The result of the two-hybrid and pull down analysis suggests that there are interactions between the two T6SS PLA effector proteins and the putative PLA accessory proteins. Therefore, the results support the idea that BCAS0666 and BCAL1297 are likely to be accessory proteins with a putative anti-toxin role for effectors BCAS0667 and BCAL1296, respectively.This insight suggests an opportunity for development an anti-bacterial therapeutic agent that targets T6SS effector immunity proteins that may benefit CF patients suffering from B. cenocpacia infections.

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PROTEOME-WIDE DISCOVERY OF ENZYME ACTIVITIES IN ESCHERICHIA COLI USING NONTARGETED METABOLOMICS

DC SÉVIN[1], U SAUER[1]

[1]Institute of Molecular Systems Biology, ETH Zurich

Many yet undiscovered enzymes still limit our understanding of metabolism, and a growing body of literature suggests that many known enzymes might promiscuously catalyze additional reactions. Here, we present a high-throughput metabolomics approach to comprehensively profile proteome-scale protein collections for in vitro enzymatic activity. Overexpressed or purified proteins are incubated in a supplemented metabolome extract containing hundreds of biologically relevant candidate substrates. Accumulating or depleting metabolite ions are then identified using nontargeted metabolomics, and a combination of chemometrics and database approaches is used to assign chemical reactions.
Upon extensive method validation, a screen of 1,275 functionally uncharacterized Escherichia coli proteins revealed 241 potential novel enzymes. Moreover, a subsequent screen of all 1,054 known enzymes of E. coli identified previously unknown reactants for 495 enzymes. Intriguingly, many discovered reactions involved non-canonical or unknown metabolites, indicating that these reactions may be part of novel metabolic pathways. Using constraint-based modeling, we finally predict these reactions to enhance metabolic evolvability of E. coli in environments with flucutating nutrient availability by facilitating the evolution of novel catabolic pathways.
Thus, we establish high-throughput nontargeted in vitro metabolomics as an approach for proteome-scale enzyme discovery and offer a glimpse on the astonishing catalytic versatility of E. coli enzymes.

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CHARACTERIZATION OF CHROMOSOMAL TOXIN-ANTITOXIN SYSTEMS IN CAULOBACTER CRESCENTUS

A FRANDI[1], J COLLIER[1]

[1]Dep. Fundamental Microbiology, University of Lausanne, Bat. Biophore, UNIL_Sorge, Lausanne, Switzerland

Toxin-antitoxin systems (TAs) are small genetic elements composed of a toxin gene and its cognate antitoxin. In general, the toxin is more stable than the antitoxin but the latter is expressed at higher level. If supply of the antitoxin stops, as for instance under special growth conditions, the antitoxin is rapidly cleared out and can no longer counteract the toxin. Although bacterial genomes often contain multiple TAs, to date, at most 9 TAs have been found in C. crescentus and only few of these have been characterized. Despite their functions and regulation remain poorly understood, the wealth of information delivered by the increasing number of high-throughput studies in C. crescentus enabled to chart TAs functions and regulation and might foster the discovery of new ones. Here we report the identification of a DnaA related protein, CC1058, which seems to control the expression of the relBE1 and parDE1 TAs and to affect cell division in C. crescentus. CC1058 gene encodes a small protein with homology to the DNA binding domain of DnaA. Loss of CC1058 is easily attainable with no significant effect on cells morphology and viability. However, microarray experiments and in vivo measurement of relBE1 and parDE1 transcription have shown a significant increase in relBE1 and parDE1 mRNA levels in ΔCC1058 cells compared to wild type C. crescentus. Conversely, relBE1 transcription is significantly increased also when CC1058 is overproduced. Cells overproducing CC1058 are filamentous and rapidly loose viability. Increased levels of CC1058 disturb FtsZ to assembly at the correct mid-cell position, leading to the formation of mini- or multiple constricted cells. Division site placement is linked to chromosome segregation in C. crescentus, hence we checked whether altered levels of CC1058 affect DNA replication/segregation. Multiple or partially segregated origins arise in cells overproducing CC1058 suggesting a putative interaction with DNA replication/segregation. On the basis of these preliminary observations we will embark on a study to characterize the biological function and regulation of CC1058 and its interaction with chromosomal TAs loci in C. crescentus.

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LAUSANNEVIRUS ENCODES A FUNCTIONAL DIHYDROFOLATE REDUCTASE SENSITIVE TO PROGUANIL

LM MUELLER[1], FG GAUYE[1], PMH HAUSER[1], GG GREUB[1]

[1]Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

Lausannevirus belongs to the Marseilleviridae family, within the Nucleocytoplasmic large DNA viruses (NCLDVs). These giant viruses exhibit unique features, including a large genome, ranging from 100kb to 2.5 Mb and coding from 150 to more than 2500 genes, as well as the presence of genes coding for proteins involved in transcription and translation.
Lausannevirus replication and cell lysis have been reported only in the Acanthamoeba genus (A. castellanii and A. polyphaga). Although the pathogenicity of Lausannevirus towards humans has not been assessed yet, our previous seroepidemiological study showed that humans are exposed to this giant virus. A similar study conducted on blood samples of healthy blood donors also revealed human exposure to the “Giant Blood Marseille-like virus” (GBM). Furthermore, Senegalvirus (another member of the Marseilleviridae family) was isolated from human stools. Therefore, Marseilleviridae are part of the human virome and may affect human health.
Interestingly, comparative genomics analyses showed that a Dihydrofolate reductase (DHFR) encoding gene is present within the Lausannevirus genome. This enzyme catalyses the reduction of dihydrofolate in tetrahydrofolate, which is a precursor of different cofactors involved into the synthesis of several essential metabolites, it is for example a key factor in the purines de novo biosynthesis. DHFR is the main pharmacological target of antifolates such as trimethoprim, pyrimethamine and proguanil. Using a complementation system in the model organism Saccharomyces cerevisiae, the deleted S. cerevisiae DHFR was complemented with a plasmid expressing the heterologous DHFR of Lausannevirus, demonstrating the function of the latter. Using this heterologous expression system, we demonstrated the in vitro sensitivity of the Lausannevirus DHFR to proguanil and its resistance to pyrimethamine and trimethoprim. Proguanil may provide a unique and useful treatment if Lausannevirus and/or Marseillevirus prove to be a human pathogen.

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ESTABLISHMENT OF A PSEUDOTYPE PLATFORM FOR HANTAVIRUSES

G. TORRIANI[1], S. ROTHENBERGER[1], H. FRITAH[1], G. ZIMMER[2], N. TISCHLER[3], O. ENGLER[4], S. KUNZ[1]

[1]Institute of Microbiology, University Hospital Center and University of Lausanne, Rue du Bugnon 48, 1011 Lausanne, Switzerland, [2]Institute of Virology and Immunology (IVI), 3147 Mittelhäusern, Switzerland, [3]Molecular Virology Laboratory, Fundación Ciencia & Vida, Santiago, Chile, [4]Spiez Laboratory, 3700 Spiez, Switzerland

Hantaviruses are emerging rodent-borne viruses of the Bunyaviridae family that can be associated with severe human diseases with high mortality. In nature, hantaviruses are hosted by small mammals, mainly rodents, but also insectivores such as shrews, moles and bats, where they cause asymptomatic persistent infections. When transmitted to humans, they can cause two severe clinical syndromes with high case fatality rates: hemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome (HCPS).
A major goal of current hantavirus research and the aim of our studies is to understand the fundamental virology of these important emerging pathogens and to develop novel therapeutics.
A crucial step in the life cycle of hantaviruses is the biosynthesis of the viral envelope glycoprotein that is essential for virus-host cell attachment and entry and represents the major target for neutralizing antibodies. The aim of the present study is to characterize the glycoproteins Gn and Gc of hantaviruses and to use these characterized recombinant glycoproteins to establish a viral pseudotype platform. By the process of pseudotyping, the viral glycoproteins, Gn and Gc, provided in trans can be incorporated into replication-deficient vesicular stomatitis virus (VSV) vectors that contain a reporter gene. Such “VSV pseudotypes” are powerful tools to investigate the role of hantaviruses glycoproteins in host cell attachment and entry, as well as serology diagnostics, in particular the detection of neutralizing antibodies in humans and other species. Moreover, the pseudotyped viruses are unable to complete their replication cycle making them suitable for research and diagnostics under BSL2 conditions.

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EXPANDING THE SPECTRUM OF MICROBIOLOGICAL DIAGNOSTICS BY FILMARRAY MULTIPLEX PCR

RF KOLLER[1], MT BARBANI[1], S ZÜRCHER[1], AU LÜTHI[1], JF STEINLIN-SCHOPFER[1], SL LEIB[1], M GORGIEVSKI-HRISOHO[1]

[1]Institute for Infectious Diseases, University of Bern, Switzerland

Fast and reliable identification of pathogens is important for adequate management of infections. PCR methods are usually the most sensitive for direct pathogen detection, but they require trained and experienced lab technicians and have a turnaround time of multiple hours. Therefore they are not ideal for emergency diagnostics. With newly developed all in one multiplex PCR systems, like the FilmArray by BioFire (a bioMérieux company), a broad spectrum of pathogens can be detected in an easy-to-use, fully automated system, in about 70 min. To optimize our service during weekends and off-peak times, we compared this system to our routinely used real-time PCR methods for the detection of viral pathogens by using the Respiratory Panel (RP) and the newly FDA cleared Meningitis/Encephalitis (ME) Panel for the FilmArray system. These panels allow the detection of 20 respiratory pathogens in nasopharyngeal swabs (NPS) and 14 ME pathogens in cerebrospinal fluids (CSF) respectively. To meet diagnostic needs reported from the clinicians in our hospitals we expanded the RP to testing for respiratory viruses in broncheoalveolar lavages (BAL) specimens, by implementing one additional preparation step. We retrospectively tested 23 NPS, 34 BAL, as well as 30 CSF. Currently all patient samples requiring urgent testing are prospectively included. The retrospectively tested NPS scored 100% sensitivity for viral targets by using the RP Panel. The sensitivity for BAL was as follows: 28 positive and 3 negative samples were correctly identified. One Adenovirus (PCR CT>35), and 2 low-positive Coronaviruses (PCR CT>37) were missed. ME Panel sensitivity is currently being assessed. The FilmArray panels provide a fast and comprehensive addition to currently used diagnostic tests. This multiplex PCR System offers a broad menu of pathogens in a single pouch and an easy to use setting with generally excellent performance, lower sample volume usage and less than 8 minutes hands-on time. The system can provide a useful addition for emergency diagnostics by offering a broad amount of pathogens within 2 hours.

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FATAL DISSEMINATED ADENOVIRUS INFECTION DURING MAINTENANCE CHEMOTHERAPY FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

MT BARBANI[1], P AGYEMAN[2], RA AMMANN[2], K LEIBUNDGUT[2], M GORGIEVSKI-HRISOHO[1]

[1]Institute for Infectious Diseases, University of Bern, Switzerland, [2]Department of Paediatrics, University of Bern, Switzerland

Adenovirus (ADV) infections typically present with respiratory symptoms, gastroenteritis, or conjunctivitis and are usually mild and self-limiting in healthy children and adults. However, severe life-threatening infections with fatal outcome represent a challenge in immunocompromised patients and are more common in children than in adults. Patients who have undergone allogeneic haematopoietic stem cell transplantation (HSCT) represent the group with the highest risk of severe infections. We describe a case of fatal ADV infection in a child undergoing maintenance chemotherapy (CT) for acute lymphoblastic leukaemia (ALL). The 6½ year old boy presented with fever, leucopenia, rhinitis and cough on the day of admission. ADV serotype 4 was isolated in stool and different respiratory samples. Viral load in plasma peaked at 11.9 milliard copies/mL. He developed disseminated disease with hepatitis, pneumonia and multi-organ failure and died despite intensive care support and cidofovir therapy started on day 8 after admission. This was the second case of fatal ADV infection in a child undergoing maintenance CT for ALL in our Division of Paediatric Haematology/Oncology during a period of 10 years. The first case was published in 2008(1). To our knowledge only four other reports of disseminated ADV infections in children undergoing intensification or maintenance CT for ALL have been published. Only one child survived. He developed ADV diarrhoea and intermitted loose stools during 6 months before disseminated infection. The knowledge of previous ADV infection allowed prompt suspicion, diagnosis, and initiation of cidofovir therapy. Disseminated ADV infections in children receiving CT for ALL without HSCT are rare but often have a lethal outcome. Algorithms for diagnosis and treatment of ADV infections in this patient population are lacking. Moreover, whole genome analysis of ADV and studies on the host could help in future to better understand why certain children receiving CT for ALL have disseminated ADV infections while the majority have self-limiting infections.

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THE PESTIVIRAL IFN ANTAGONIST E(RNS) CLEAVES DSRNA AS NICKING ENDORIBONUCLEASE

C. LUSSI[1], M. SCHWEIZER[1]

[1]Institute of Virology and Immunology (IVI), Federal Food Safety and Veterinary Office and Vetsuisse Faculty University of Bern, Switzerland

Bovine virus diarrhea virus (BVDV), a pestivirus in the Flaviviridae family, causes persistent infection (PI) in cattle by entering the fetus early in gestation prior to development of adaptive immunity. In addition, the inhibition of interferon (IFN) type I synthesis plays an important role in escaping innate immunity and is a prerequisite for the birth of PI calves. Thus, pestiviruses express two IFN antagonists: the non-structural protein N(pro) that inhibits IFN induction in infected cells by degradation of IRF-3, and the soluble glycoprotein E(rns), which cleaves extracellular dsRNA prior to activation of non-infected cells.
E(rns), which forms covalently-linked homodimers, belongs to the T2 family of ribonucleases that preferably degrade ssRNA. Accordingly, the crystallographic structure of the catalytic domain of E(rns)did not provide evidence for dsRNA to fit into the active site. Thus, it may well be envisaged that E(rns) independently cleaves both strands of dsRNA, which would be in accordance with the fact that ssRNA is degraded by this viral RNase more efficiently than dsRNA. Here we show that wild-type, but not an RNase-inactive mutant form of E(rns), was able to degrade RNA in a RNA/DNA hybrid with the DNA strand being resistant to degradation. Together with the fact that monomeric E(rns)is similarly able to cleave dsRNA, these results support our model that E(rns)might be a ´nicking endoribonuclease' degrading ssRNA within double-stranded substrates. This efficiently prevents the activation of patter recognition receptors (PRRs) and helps to maintain a state of innate immunotolerance in PI animals.

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MicroRNA-17 IS A CRITICAL HOST FACTOR FOR PESTIVIRUS REPLICATION

M. LINIGER[1], T.K.H. SCHEEL[2], J.M. LUNA[3], G. AURAY[1], M. GERBER[1], I. KELLER[4], R. BRUGGMANN[4], N. RUGGLI[1], C.M. RICE[3]

[1]Department of Virology, Institute of Virology and Immunology IVI, Mittelhäusern, Switzerland, [2]Copenhagen Hepatitis C Program, Department of Infectious Disease and Clinical Research Centre, Copenhagen University Hospital, Hvidovre, Denmark, [3]Laboratory of Virology and Infectious Disease, Center for the Study of Hepatitis C, The Rockefeller University, New York, NY, USA, [4]Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland

Crosslinking immunoprecipitation of Argonaute proteins combined with high throughput sequencing was used in the context of viral infections to search for microRNA (miRNA) interactions with viral genomic RNA. This revealed specific miR-17 binding at the 3' non-translated region of bovine viral diarrhoea virus (BVDV). The miR-17 binding site of BVDV is conserved in the related pestivirus classical swine fever virus (CSFV). Therefore, we studied the functional relevance of miR-17 binding by CSFV. To this end, CSFV with a defective miR-17 seed site was generated (CSFV-p3p4). Replication of the parent and mutant CSFV were analysed in the presence of locked antisense oligonucleotides (tinyLNA17) for miR-17 inhibition and of artificial miR-17p3p4 for seed site complementation, respectively. TinyLNA17 repressed the propagation of CSFV, suggesting that CSFV depends on miR-17 for efficient replication. In addition, replication of the CSFV-p3p4 mutant was strongly impaired. Complementation with artificial miR-17p3p4 restored the growth characteristics of CSFV-p3p4 completely. Luciferase-based miR-17 reporter assays suggested a virus-mediated sequestration of miR-17. Accordingly, differential transcriptome analysis of CSFV versus mock-infected cells showed functional de-repression of cellular miR-17 target mRNAs, thereby altering the host transcriptome. Taken together, these data demonstrate that miR-17 is a critical host factor for replication of CSFV.

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ISOLATION AND CHARACTERIZATION OF DISINFECTION-RESISTANT ENTEROVIRUSES

S MEISTER[1], M KLINGER[1], T KOHN[1]

[1]ENAC, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

Human enteric viruses are a leading cause of waterborne disease outbreak worldwide. Their environmental persistence and resistance to disinfection treatments are two main factors that can influence the dissemination of these viruses, thus increasing the risk of person-to-person transmission. In this study, we isolated and characterized the resistance pattern for several environmental viruses, with the long-term goal of understand the molecular mechanisms underlying resistance and persistence. To obtain environmental isolates, we implemented a method allowing an efficient isolation and concentration of enteric viruses from Lausanne and Minneapolis wastewater treatment plant influents. Single strain viruses were isolated by BGMK cells plaque assay and further characterized by sequencing. In this manner, five Coxsackieviruses B5 (CVB5) and one Coxsackievirus B4 were isolated for further experiments. Several disinfection treatments were used to test the inactivation kinetics of our isolates: UV-C light, Simulated sunlight, Free-chlorine, Chlorine dioxide, and Heat. Their respective inactivation rate constants were measured and compared to CVB5 Faulkner and Echovirus 11 laboratory strains as well as MS2 bacteriophage, a frequently used surrogate. Interestingly MS2 exhibited the most extreme disinfection behaviour, with a low susceptibility for UV-C and sunlight and a high susceptibility for free-chlorine and chlorine dioxide. In contrast, CVB5 isolates shared a more homogenous susceptibility to all disinfectants, though the lab strain was typically less resistant. In addition, a pronounced resistance pattern to chemical disinfectants could be observed in two of our isolates (L3 and L18). This pattern was also observed in heat inactivation experiments, with L3 exhibiting the strongest thermal resistance. In conclusion, we can highlight different resistant patterns even among highly related viruses, possibly due to the high mutation rate of these RNA viruses. In future work, the sequencing of the whole virus genome could help us to understand the molecular mechanisms involved in this resistance, as well as the disinfection cross-resistance observed among our isolates.

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DEVELOPMENT OF A VIRUS NEUTRALIZATION TEST FOR LYSSAVIRUSES USING PROPAGATION-INCOMPETENT VESICULAR STOMATITIS VIRUS PSEUDOTYPES

S. MOESCHLER[1], B. KRAEMER[2], G. ZIMMER[1]

[1]Institute of Virology and Immunology (IVI), University of Bern, CH-3147 Mittelhäusern, Switzerland, [2]Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel, Paul-Ehrlich-Institut, 63225 Langen, Germany

Rabies virus (RABV) is a zoonotic virus of the genus Lyssavirus that causes fatality rates of nearly 100% in unvaccinated individuals. Dog-mediated human rabies is responsible for at least 55 000 human deaths annually, mostly affecting rural poor communities in Asia and Africa. Rabies infection is 100% preventable and mass dog-vaccination campaigns are implemented in order to eliminate the disease at its source. The quantification of virus neutralizing antibodies (VNA) against RABV is important to assess the efficacy of anti-rabies vaccines and should be coupled to mass vaccination. The conventional methods for the titration of rabies VNA rely on propagation-competent RABV and require enhanced biosafety measures that can hinder their implementation in poor areas. Moreover, these tests are time-consuming, expensive and limited to the detection of VNA directed against the classical RABV (genotype 1). Here we developed a pseudotype-based neutralization test that makes use of Vesicular Stomatitis Virus (VSV) replicons lacking the VSV glycoprotein. VSV replicons are trans-complemented with RABV glycoproteins on helper cell lines in a process called pseudotyping. The use of propagation- incompetent VSV pseudotypes alleviates the enhanced biosafety measures required for the conventional neutralization setups. Furthermore, the readout is facilitated by the expression of reporter genes (GFP, luciferase) in the VSV vector. The pseudotype-based neutralization test turned out to be at least as sensitive as the conventional tests and results strongly correlated (r=0.9). Finally, this novel method is highly versatile as pseudotyping with various lyssavirus glycoproteins extended its use to serological studies on other lyssavirus genotypes.

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GLOBAL GENE EXPRESSION ANALYSIS OF ADENO-ASSOCIATED VIRUS 2 (AAV2)-INFECTED CELLS

S.O. SUTTER[1], K. TOBLER[1], M. ACKERMANN[1], C. FRAEFEL[1]

[1]Institute of Virology, University of Zurich, Zurich, Switzerland

parvovirus with a single stranded DNA genome of 4.6 kb, encoding two clusters of genes, rep and cap. In absence of a helper virus, AAV can integrate its genome at a specific site, termed adeno-associated virus preintegration site (AAVS1) on human chromosome 19. In the presence of a helper virus, such as herpes simplex virus type 1 (HSV-1), adenovirus (Ad) or human papilloma virus (HPV), AAV can enter a lytic replication cycle with the production of progeny virus particles. To investigate the global effect of AAV2 infection on a host cell, a global transcriptome analysis by next generation sequencing (NGS) of wild type (wt) AAV2-infected or mock-infected normal human fibroblasts (NHF) was performed. Networking and enrichment analysis of the transcriptome (1930 genes with $p < 0.01$ and number of reads > 40) revealed several affected biological processes such as chromatin organization, intracellular transport, regulation of the cell cycle, cytoskeleton and organelle organization. To further evaluate the enrichment analysis of the transcriptome, heat maps of the top 50 differentially regulated genes between wtAAV2 and mock-infected cells were created. Further reduction of the NGS data revealed a list of 872 genes, which were either up- or down-regulated at least 1.5-fold in wtAAV2 versus mock-infected cells. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis of the 872 genes revealed that the top most affected pathways include cell cycle regulation and p53 signaling.
To confirm the NGS data, 10 genes were selected based on their differential expression profile (down-regulated, close to zero, or up-regulated) in wtAAV2-infected and mock-infected cells and the fold difference in target gene expression was assessed by quantitative RT-PCR (RT-qPCR) resulting in a multiple correlation coefficiency (R2) of 0.897. Moreover, the F-statistics revealed a significant relationship between the variables and the linear regression model (p-value = 0.0003543).
Next, the differential expression of the selected genes will be assessed in mock-infected NHF cells and cells infected with double-stranded self-complementary (sc) AAV2 vectors, rep-negative single stranded AAV2 vectors or inactivated wtAAV2, in order to identify the AAV components responsible for the interference with cellular target genes and the cell cycle progression.

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IDENTIFICATION OF KEY RESIDUES IN CANINE DISTEMPER VIRUS ATTACHMENT PROTEIN-STALK DOMAIN REGULATING HOST CELL ENTRY

M. HERREN[1], F. BRINGOLF[1], M. WYSS[1], P. PLATTET[1]

[1]Division of Neurological Sciences, Department of Clinical Research and Veterinary Public Health (DCRVPH),Vetsuisse Faculty, University of Bern, Bern, Switzerland

The fusion machinery of Morbillivirus (i.e. canine distemper [CDV], measles [MeV]), consisting of tightly interacting attachment (H) and fusion (F) protein, is crucial for host cell invasion. The H-ectodomain is composed of an F-activating stalk region and a cuboidal receptor-binding head domain, which are separated by a short “connector” segment. The current model of F-activation assumes that the H-heads move away from the stalk after receptor engagement; a motion enabled by putative structural flexibility of the connectors. Subsequently, H-stalk rearranges and activates F, which in turn undergoes drastic conformational changes ultimately leading to membrane merging. We aim to unravel the role of the connector microdomain of the Morbillivirus attachment protein H (residues 142-170) in fusion activation. The role of specific amino acids in the H-connector domain is examined by site-directed mutagenesis. The mutants’ surface expression, fusion promoting activity, interaction with F and receptor binding capability are investigated in receptor negative and positive cells using established methods. We found that the isoleucine residue at position 146 can be mutated into various amino acids while keeping wildtype-like surface expression. Interestingly, the fusion promotion of those H-I146 mutants was modulated when substituted into hydrophobic amino acids, while their bioactivity was severely impaired when exchanged into non-conserved polar/charged amino acids. Our preliminary data suggests that, while irrelevant for transport-competence, hydrophobicity of residue I146 of the H-connector is crucial for F-triggering. Whether the H-I146 mutants impacted fusion-promotion by locking H in a “pre-receptor-binding” conformation or induced premature conformational change is under current investigations.

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OPTIMIZING GENOTYPIC DETERMINATION OF THE HIV-1 TROPISM

M. ZINGG[1], A. HAAS[1], N. LABHARDT[2], T. KLIMKAIT[1]

[1]Molecular Virology, Department Biomedicine – Petersplatz, University of Basel, Switzerland, [2]Swiss Tropical Public Health Institute, Basel

Background:
Knowledge of the host cell tropism of HIV is critical for initiating a therapy with a coreceptor antagonist and may be of importance for judging the clinical course. Genotypic methods are today available that are mainly based on the variable loop 3 (V3) in the viral env gene, either on the plain sequence (Geno2Pheno) or by analyzing duplex properties (XTrack). Since the V3 loop is flanked by sequences with a higher degree of conservation, amplification typically utilizes these regions for PCR amplification. Thereby commonly used primes may reach slightly into the region, and this may potentially introduce a bias, as the primer-provided nucleotides cannot be taken into account for interpretation.

Methods:
To overcome this bias we now designed shorter primers that, through 2'-modifications, are able to retain comparable binding affinity despite the shortening. These primers now do not reach into the V3 loop and allow full representation of patient-derived V3 sequences. The amplified sequences were in parallel analyzed by duplex-tracking (XTrack) and using the genotypic Geno2Pheno (G2P) assay, which is based on tropism prediction using the amino acid sequence of the V3 loop. In silico G2P analysis of V3 sequences differing only in the affected primer region was also performed with consensus sequences from various subtypes.
Replicative phenotyping were used as gold standard in order to clarify contradictory results.

Results:
A comparison of sets of sequences from subtype AE and –C viruses revealed that for most of them there is no principal difference (CXCR4- vs. CCR5-tropism), and no significant differences in the False Positive Rates (FPR) between the two V3 sequence lengths for XTrack or the G2P assays. Surprisingly even the V3-sequence of consensus HIV-2 does not yield a principally different interpretation. Complete omission of the respective flanking sequences renders interpretation difficult – as suggested by the conserved nature of this region among virus isolates.

Conclusion:
This study illustrates the importance to consider the entire sequence of the V3 loop for tropism determination. On the other side our data clearly show the limited contribution from mutations and variation in the V3-flank to a given viral tropism.
Of note, the biggest changes in the FPR and thereby shift towards a tropism change was seen for isolates of the –AE subtype CRF_01.
Larger data sets are still needed to gain a better validity for clinical utility of our findings.

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THE VIROME OF SWISS WATERBUFFALOES

J. LECHMANN[1], C. BACHOFEN[1], M. ACKERMANN[1]

[1]Institute of Virology, Vetsuisse Faculty, University of Zurich, Switzerland

In the last two decades the population of waterbuffaloes in Switzerland steadily increased and meanwhile it is counting over 1200 animals distributed over 75 farms. While being the most important all-purpose life-stock animals in Asia, buffaloes in Switzerland are mainly kept for beef and dairy production, for example mozzarella cheese. The import and the increasing number of exotic animals in Switzerland goes along with an increased risk of introduction and distribution of novel pathogenic agents such as viruses, bacteria and protozoa. Transmission of these newly introduced agents to our native animals and also to humans may cause severe illness. On the other hand, water buffaloes may succumb to illness when getting in contact with native pathogens that are relatively harmless for native species. In order to obtain more information on the virome of Swiss water buffaloes, i.e. the spectrum of viruses present, blood samples of 48 water buffaloes and of contact animals (26 small ruminants) from three Swiss farms were analysed by a wide range of routine diagnostic means as well as by next generation sequencing (NGS). In summary, we found single waterbuffaloes positive in ELISA or PCR for viruses of bovine origin (mainly herpes- and pestiviruses), but there was no indication for virus transmission from or to small ruminants. While virome analysis by NGS supported these findings, it additionally showed that the blood samples of more than half of the animals from one single farm were positive for a small circular DNA virus of the Gemycircularvirus family. Members of this recently detected virus family have mainly been found in environmental samples but have also been described in bovine serum and human cerebrospinal liquid. In our case, a statistically significant association to sequences of bacterial origin points to environmental contamination during the blood sampling process on this farm. While the actual place of replication of this newly detected virus remains to be determined, its finding highlights the importance to critically challenge NGS data.

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INNATE SENSING OF MIRNA MIMETICS PROVIDES BROAD RANGE ANTI-VIRAL EFFECTS

A. KANNAN[1], M. SUOMALAINEN[1], M. AMSLER[1], H.V. TRINH[3], A. FRANCESCHINI[1], C. VON MERING[1], S. HEMMI[1], U.F. GREBER[1]

[1]Institute of Molecular Life Sciences, University of Zurich, Switzerland, [3]present address: US Military HIV Research Program, WRAIR and Henry M. Jackson Foundation for the Advancement of Military Medicine, MD, USA

Viruses elicit unprecedented complexities when they interact with their host cells. This involves changes in signalling, gene expressions, metabolites and lipids. Together these changes can drive the infected cell towards the state of a viral factory. For example adenovirus infected cancer cells produce thousands of progeny viruses in the nucleus, and release the virions in a poorly understood process of oncolysis. Adenoviruses are non-enveloped DNA viruses infecting many different vertebrates, including humans. They cause upper respiratory infections in children, and recurring infections in adults. For immune-compromised individuals, adenoviruses are a major risk factor, and can lead to severe health problems and fatalities. To better understand systems aspects of adenovirus infection, we carried out microRNA profiling with mirBase18 upon HAdV-C5 (species C) infection of human lung adenocarcinoma A549 cells. Cellular microRNAs are important regulators of gene expression in both normal and pathological processes. They are short ~22 nucleotide RNA sequences and bind to complementary sequences in the 3' UTR of target mRNAs which can result in mRNA silencing. Using micro-array profiling and quantitative reverse transcription polymerase chain reaction (qRT-PCR), we found downregulated host miRNAs in HAdV-C5 infected cells, and also in HAdV-B3 infected cells. We show that chemically synthesized miRNA mimetics of the downregulated host microRNAs reduced HAdV-C5 infections of transformed and non-transformed cells. They also reduced Influenza A virus, Vesicular Stomatitis virus, and Semliki Forest virus infections of cultured cells. Transfected miRNA mimetics led to transcriptional activation of a range of interferon inducible genes. In silico analyses using canonical and noncanonical interaction models with potential target mRNAs revealed potential candidate target genes for expression modulation. Further studies on the mode of anti-viral action of the microRNA mimetics revealed the activation of dsRNA sensors and downstream innate immune responses, independent of canonical seed sequences recognizing target mRNAs. This study reveals new properties of host and viral regulatory networks and underlying mechanisms by which microRNA mimetics antagonize viral infections.

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ASSESSMENT OF THE ANTIVIRAL ACTIVITY OF MXA AGAINST INFLUENZA A VIRUS

F. STEINER[1], P. NIGG[2], L. MURER[1], M. CRAMERI[1], E. MORITZ[1], J. PAVLOVIC[1]

[1]Institute of Medical Virology, University of Zurich, Zurich, Switzerland, [2]Friedrich Miescher Institute for Biomedical Research, University of Basel, Basel, Switzerland, [3]Institute of Virology, University of Freiburg, Freiburg, Germany

Mx proteins belong to the family of dynamin-like, large GTPases and are primarily active against negative-stranded RNA viruses, including influenza A (IAV). Human MxA is able to form higher order oligomeric structures, however, the mode of action of MxA remains to be fully elucidated. Increasing evidence suggests that MxA requires auxiliary cellular factors for its antiviral activity. Recently, we have shown that MxA interacts with the DEAD-box helicase UAP56, which is an essential factor for efficient replication of IAV. There is increasing evidence that NP represents the viral target of MxA. For instance, the resistance phenotype of human IAV segregates with a discrete cluster of surface-exposed amino acids in the NP. We showed that the dimeric form of MxA is able to form stable complexes with viral NP as well as UAP56 suggesting that the dimeric form of MxA plays a central role in antiviral function. In line with these findings, co-immunoprecipitation (Co-IP) experiments revealed that NPs of several MxA-sensitive or resistant strains exhibit different binding affinities to dimeric MxA. Furthermore, we observed that binding of MxA to UAP56 is greatly enhanced in the presence of NP.
Currently we characterize the interactions of MxA, NP and UAP56 by Co-IP and a tripartite split-GFP system. In particular, we investigate the effect of (i) GTPase activity mutants of MxA, (ii) ATPase and RNA binding activity mutants of UAP56 as well as (iii) NP mutants with altered MxA sensitivity. Furthermore, we assess the influence of these mutations on MxA antiviral activity.

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EFFECT OF HUMAN BURN WOUND EXUDATE ON PSEUDOMONAS AERUGINOSA VIRULENCE

M. R. GONZALEZ[1], B. FLEUCHOT[1], L. L. LAUCIELLO[1], P. JAFARI[2], L. A. APPLEGATE[2], W. RAFFOUL[2], Y.-A. QUE[3], K. PERRON[1]

[1]Microbiology Unit, Dept. of Botany and Plant Biology, University of Geneva., [2]Plastic, Reconstructive & Hand Surgery, Unit of Regenerative Therapy, UNIL/CHUV Lausanne, [3]Service of Adult Intensive Care Medicine & Burns, CHUV Lausanne

BACKGROUND: Burn wound sepsis are currently the main cause of morbidity and mortality after severe burn injury. Bacterial pathogens such as Pseudomonas aeruginosa, Staphylococcus aureus and Acinetobacter baumanii impair patient recovery and lead to fatal issue. The specialized medical care required after burn injury faces major challenges to prevent bacterial development on the burn wounds. The Biological Biodegradable and anti-Bacterial Burn-wound Bandages (B5) platform, granted by Swiss TransMed, was created in order to improve the current biological dressing used to treat burn wound and to better understand the bacterial pathogenesis in a wound environment.

AIMS: Our specific research topics are i) the pathogenicity characterization of the major burn wound pathogen P. aeruginosa while growing in burn wound exudate and ii) the analysis of burn wound exudate composition in order to establish of an artificial burn wound medium.

RESULTS: We investigated the effect of burn wound exudates (BWE) on the virulence of those pathogens. BWE were collected within 7 days after burn trauma from 5 burn patients. We first monitored their effect on pathogen growth. In contrast to A. baumanii or S. aureus, P. aeruginosa was the only pathogen able to grow within these human fluids. Expression of typical virulence factors such as pyocyanin and pyoverdine were even enhanced compared to standard laboratory medium. An RNAseq analysis on P. aeruginosa growing in burn wound exudate was performed and is currently analyzed. A detailed chemical composition analysis of BWE was done, which enabled us to determine the major components of BWE and underline the metabolic modifications induced by burn trauma. These data are essential for the development of an artificial medium mimicking the burn wound environment and the establishment of an in vitro system to analyze the initial steps of burn wound infections.

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COMPARING EARLY AND LATE VIRAL GENE EXPRESSION OF 13 HUMAN POLYOMAVIRUSES

E. AJUH[1], T. BETHGE, H.H. HIRSCH

[1]Transplantation & Clinical Virology, Department of Biomedicine-Haus Petersplatz, University of Basel, CH-4009 Basel, Switzerland

Introduction: The regulation of the early viral gene region (EVGR) and late viral gene region (LVGR) of human polyomavirus (HPyV) infections has been best studied for BKPyV causing nephropathy (PyVAN) and hemorrhagic cystitis in transplant patients, and JCPyV causing progressive multitfocal leukoencephalopathy (PML). For BKPyV and JCPyV, EVGR and LVGR expression is coordinated by the non-coding control region (NCCR). In patients with PyVAN and PML, however, the NCCR of BKPyV and JCPyV have been found to be rearranged, and constitutively increase EVGR expression and accelerate viral replication in cell culture. Little information is available for any of the 11 novel HPyVs. Moreover, none of the 11 HPyV has been effectively propagated in cell culture. Too close this gap, we compared NCCR-driven EVGR and LVGR expression of the novel HPyVs in different cell culture systems.
Method: NCCRs of all 13 HPyVs were cloned into a bidirectional reporter vector (pRG13D12) bearing the RFP and GFP as markers of EVGR and LVGR, respectively. Expression was assayed in HEK293, HEK293T, and HEK293TT and CV-1 and, Cos-7 to estimate the potential effect of SV40 large T-antigen (LTag). Expression was also monitored in selected cell lines reflecting different human organs using fluorescence microscopy and flow cytometry analysis.
Results: Our results show that a hierarchy of EVGR expression, whereby by MCPyV and HPyV12 were the strongest in 6 of the 7 cell lines. KIPyV EVGR was strong in 3 of the 7 cell lines transfected. HPyV 6, 7 and 9 displayed lowest EVGR expression in most of the cell lines. LVGR expression was generally stronger than EVGR for all archetypes HPyV NCCRs. BKPyV, JCPyV and TSPyV showed strongest LVGR expression in most cell lines. Examining natural variants with „rearranged“ or point mutant HPyV NCCRs from patients, we found that HPyV7-PITT1, HPyV9-UF1 and MCPyV-MCVw156 displayed slightly higher EVGR and LVGR activity than the corresponding “archetype“. HEK293T, HEK293TT, and COS-7 constitutively expressing SV40 LTag increased the NCCR activity of most HPyVs suggesting that the reporter results were modulated as expected in the viral life cycle.
Conclusion: A hierarchy of HPyV NCCR activity could be established. Natural HPyV NCCR variants from patients with HPyV disease showed higher activity suggesting that NCCR are important pathogenicity determinants. LTag expression modulates NCCR activity as expected in the viral life cycle. Together, the results suggest that factors of cell differentiation and activation including small molecules or cytokine should permit identifying suitable cell culture conditions for the novel HPyV, and identify pathogenetic cofactors, and potential inhibitors.

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EXPERIMENTAL INFECTION OF HORSES WITH NONPRIMATE HEPACIVIRUS MEDIATES IMMUNE PROTECTION AGAINST RE-INFECTION

S. PFAENDER[1], S. WALTER[1], D. TODT[1], E. GRABSKI[2], T. GATHER[3], S. MÖLLER[3], R.J.P. BROWN[1], A. POSTEL[4], K. HAHN[5], C. PUFF[5], V. PFANKUCHE[5], F. HANSMANN[5], P. BECHER[4], U. KALINKE[2], W. BAUMGÄRTNER[5], K. FEIGE[3], T. PIETSCHMANN[1], J-M.V. CAVALLERI[3], E.STEINMANN[1]

[1]Institute for Experimental Virology, TWINCORE Centre for Experimental and Clinical Infection Research, Hannover, Germany, [2]Institute for Experimental Infection Research, TWINCORE Centre for Experimental and Clinical Infection Research, Hannover, [3]Clinic for Horses, University of Veterinary Medicine Hannover, Bünteweg 9, 30559 Hannover
[4]Institute of Virology, University of Veterinary Medicine Hannover, [5]Department of Pathology, University of Veterinary Medicine Hannover, Bünteweg 17, 30559 Hannover

Hepatitis C virus (HCV) has a restricted host species tropism, naturally infecting only humans, although chimpanzees are susceptible to experimental infection. A robust immunocompetent animal model is still lacking, hampering mechanistic analysis of virus pathogenesis, immune control and prophylactic vaccine development. The closest homolog of HCV discovered to date is the nonprimate hepacivirus (NPHV) which shares similar features with HCV and thus could represent a useful model to study hepacivirus infections in their natural hosts. In this study, we aimed to dissect equine immune responses after experimental NPHV infection. In addition, we conducted challenge experiments to investigate immune protection against secondary NPHV infections. To this end, three horses were intravenously injected with NPHV containing plasma. Fluorescent activated cell sorting (FACS)-based assays were established to monitor immune cell frequencies and activation status. Cytokines were measured on mRNA level by the development of quantitative real-time polymerase chain reactions and on protein level by a bead-based multiplex assay. To study whether infected horses are protected against a secondary infection, horses were re-challenged with the same as well as with a distinct NPHV inoculum. All infected horses became viremic after one or two weeks and viremia could be detected for several weeks followed by a delayed seroconversion and viral clearance. Histopathological examinations were conducted revealing moderate, periportal infiltrations of lymphocytes with some horses displaying signs of hepatitis. Immune responses differed between all horses; however, no distinct immune pattern could be observed. Importantly, after a primary NPHV infection horses were protected against re-challenge with the identical as well as a distinct isolate. In conclusion, horses were successfully infected with NPHV containing plasma. Techniques were established to monitor the underlying immune responses and to dissect mechanisms contributing to viral clearance and immune protection. Detailed understanding of hepacivirus infections in their natural hosts could facilitate the development of new therapeutic strategies including protective vaccines against HCV.

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BIOPROCESS OPTIMIZATION FOR THE PRODUCTION OF POLY(3-HYDROXYALKANOATES) FROM SYNGAS WITH RHODOSPIRILLUM RUBRUM

M. ROMANINO[2], S. FOLLONIER[1], S. KARMANN[3], M. ZINN[1]

[1]Institute of Life Technologies, HES-SO Valais Wallis, Sion, Switzerland, [2]2 Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Italy, [3]Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

As concerns about the environmental impact of petrol-based plastics and their fate in the environment are growing, there is a need to develop sustainable alternatives. Poly(3-hydroxyalkanoates) (PHA) have emerged as promising bio-based polyester candidates due to their unique features in terms of biodegradability and biocompatibility.
We are investigating how to improve the cost-efficiency and sustainability of PHA production with a bioprocess using syngas, the gas mixture obtained from the pyrolysis of organic wastes and made consisting mainly of CO, CO2, H2 and N2. This bioprocess relies on Rhodospirillum rubrum, a purple nonsulfur photosynthetic bacterium able to both metabolize CO and synthesize PHA intracellularly. It is composed of three phases: 1) heterotrophic, aerobic growth on fructose, 2) adaptation to anaerobic growth with fructose as substrate, and 3) PHA production from syngas and acetate.
A cutting-edge process analytical technology platform was set up to monitor both the bioprocess and cell physiology. It involved measurements of dissolved oxygen and redox potential (in-line), exhaust gas concentrations with a mass spectrometer (on-line), cell concentration and PHA content by flow-cytometry (at-line), as well as optical density, dissolved CO, cell dry weight, PHA content and composition by gas chromatography, and substrate concentrations by high pressure liquid chromatography (off-line).
With this approach it was possible to produce 1.6 g L-1 of PHA (PHA content = 18.5 w%) while consuming 14.3 moles of CO and generating 3.7 moles of H2 as valuable byproduct. The cells exhibited a μmax of 0.12 h-1 during the aerobic phase on fructose and grew linearly up to 8.5 g L-1 CDW during the syngas phase as a result of CO mass transfer limitation. The specific uptake rate of fructose was 0.2 g g-1 h-1 during both the aerobic and the anaerobic phase, and the one of acetate during the syngas phase was 0.03 g g-1 h-1. This study clearly showed the potential of the followed strategy while pinpointing which parameters need to be optimized for further improving process productivity (e.g. CO mass transfer and fructose/acetate concentrations).

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SYNTHETIC TWO COMPONENT BIOREPORTER IN FUNCTIONAL SYSTEMS

V MAFFENBEIER[1], J ROELOF VAN DER MEER[1]

1University of Lausanne, Department of Fundamental Microbiology

Two-component signaling systems interact with environmental stimuli upon which they trigger a cytoplasmic response. Their modular structure allows easy exchange of domains. The first functional chimeric transmembrane receptor (called TrzI) was constructed by Baumgartner et al. in 1994 by fusing the ligand binding domain of the chemoreceptor Trg to the kinase domain of the osmosensor EnvZ. Several other chimeras have been reported since but their functionality has not always been established. In order to be able to exploit the large natural diversity of bacterial chemoreceptors in simplified synthetic biosensor constructions, this thesis focuses on (I) understanding the requirements for the fusion site enabling chimera functionality, (II) predicting ligand-binding in chimeras, and (III) constructing functional biosensors with new specificities.

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CHANGING THE SPECIFICITY OF ESCHERICHIA COLI PERIPLASMIC BINDING PROTEIN RBSB FROM RIBOSE TOWARDS 1,3-CYCLOHEXANEDIOL AND CYCLOHEXANOL

DIOGO TAVARES[1], JAN ROELOF VAN DER MEER[1]

[1]UNIL, DMF

Biosensors have proven to be a powerful and cheap tool for analytics of specific target molecules or conditions (e.g., toxicity). Bioreporter bacteria are genetically engineered to produce a measurable signal (reporter protein) in direct response to a specific chemical or physical agent present in their environment. The design of bioreporter bacteria typically starts with known sensory/regulatory proteins, but it has been proposed that computational prediction of substrate binding interactions in a well-known receptor protein could lead to design of mutant proteins with novel target recognition specificities. Here a mutant library based on the ribose binding protein of Escherichia coli (RbsB) was produced with the goal to find mutations which would permit binding of ribose-similar molecules such as 1,3–cyclohexanediol and cyclohexanol. Rosetta simulation was used to predict critical amino acid residues and potential mutations in the RbsB binding pocket which might permit binding of the two new target molecules. The result was a set of 6 million mutants containing one of 5 possible substitutions at each of 9 amino acid positions. The library was introduced into an E. coli expression strain, which carries a hybrid signaling pathway through which GFP is produced upon binding of the target molecule by the (mutant) RbsB. For better reproducibility, cells were grown as individual microcolonies in alginate beads which were screened by fluorescence activated cell sorting (FACS) for gain-of-function GFP expression in presence of 1,3-cyclohexanediol. Alternate rounds of screening and separation were performed to enrich potential responsive mutants but to eliminate constitutive GFP producers. Since FACS only allows screening of end-points of GFP expression in microcolonies, but not the induction itself, the enriched library is currently being screened by high-throughput microscopy in order to recover potential inducible mutants by 1,3-cyclohexanediol.

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MIWELT: DISCOVER THE HIDDEN WORLD OF MICROBES

M RAABE[2] , V LOOSER[1] , M OTTINGER[1] , K HECHT[1] , M STRAUMANN[1] , C. ULLI[1] , J DÜRR[3] , AND K KOVAR[1] [1] SCHOOL OF LIFE SCIENCES AND FACILITY MANAGEMENT LSFM, ZURICH UNIVERSITY OF APPLIED SCIENCES ZHAW, WÄDENSWIL, SWITZERLAND [2] INDEPENDENT JOURNALIST, LEIPZIG, GERMANY [3] FREELANCE CHILDREN'S BOOKS ILLUSTRATOR, BERLIN, GERMANY SCHREIB@MIWELT.NET

The miwelt project – funded by the Agora instrument of the Swiss National Science Foundation – uses an innovative didactic approach, a combination of arts and science, to familiarise children from 7 to 11 years with concepts in microbial biotechnology. Using everyday experiences packaged as short stories, their curiosity will be stimulated. Thematic excursions provide insights into microbial habitats, and experiments performed in real laboratories explore microbial behaviour. This concept continues to develop in teaching aids and lab-books illustrating experiments that can be repeated at home or at school with little equipment. The people in miwelt open a dialogue that encourages scientists to explain, using simple terms and illustrations, why their work on microbial systems is important and how it becomes the scientific foundation for applications in industrial manufacturing. Although primarily childcentred, the concept is also an asset to adults who lack a strong scientific background.

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BIOUTILS: MICROBIOLOGY FOR EVERYONE!
Education / Outreach

M CAINE[1], A WEBER[1], S ZUCHUAT[1], V DUCRET[1], P LINDER[2], K PERRON[1]

[1]Microbiology Unit, Department of Plant Biology 30, University of Geneva, Quai Ernest-Ansermet Geneva 4 CH 1211, Switzerland, [2]Department of Microbiology and Molecular Medicine, Medical Faculty, University of Geneva, 1 Rue Michel Servet, Geneva 4 CH 1211, Switzerland

Created by researchers, BiOutils is an interface that aims to promote and support the study of biology in schools. The platform provides schools with protocols, material and know-how to perform modern and engaging experiments in class, with activities ranging from ecology to modern molecular biology.
Its innovative edge, compared to other structures that offer educational activities in the field of science, is the fact that the platform is fully integrated in a university research group. Therefore, experimental methods and material provided are always up-to-date with the latest technological and scientific advances.

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GETTING TO KNOW MICROBES: OUTREACH ACTIVITIES IN THE PIORA VALLEY

A. WEBER[1] , M. CAINE[1] , C. FRAGOSO-CORTI[2,3], A. SPINELLI4 , R. BACHOFEN5 , G. PELLEGRI6 , D. SARTORI[7] , M. TONOLLA[1,2,3], R. PEDUZZI[1,3,] K. PERRON[1] , P. LINDER[1]

1 BiOutils, University of Geneva 2 Laboratory of applied microbiology, University of applied sciences Southern Switzerland, Bellinzona 3 Fondazione Centro Biologia Alpina, Piora 4 Pura, Ticino 5 University of Zurich 6 Ideatorio, USI, Università della Svizzera italiana 7 Ufficio dell'insegnamento medio superiore, Cantone Ticino

The largest part of the planet's living material consists of bacteria and other microorganisms that play a vital role in many biological processes. In spite of their huge importance and beneficial effects, microorganisms are largely unknown and mostly negatively perceived. Microbiology has many industrial, medical and environmental applications and it is necessary that society gets an accurate knowledge of microbes and their influence. In this context, outreach activities that address the public are crucial, to give a better understanding of this hidden world. The aim of the present project is to raise awareness about the importance of microorganisms in preserving biological and ecological systems, by offering to the public and schoolchildren in particular the opportunity to see microbes and learn about their metabolism, ecology and utility. To this purpose, a "microbiological path" will be developed in the exceptional environment of Val Piora in Ticino, where several colonies and specimens can be observed directly in nature. It will show that microorganisms are present in the environment and can be seen, in some cases, without any special equipment. For example, the algae Haematococcus pluvialis and the bacteria Chromatium okenii form very distinct red coloration in the water. To guide the visitors along the didactic path, informative material will be available, such as a descriptive leaflet and panels. Visits and activities with local experts will also be organized for schools, to provide complementary education about microbes. This Agora project is supported by the FNS

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