

Swiss Society for Microbiology



73rd Annual Meeting and Assembly Workshop "Outreach activities in Switzerland"

Palazzo dei Congressi, Lugano, 28-29 May, 2015

Abstract Booklet and Programme



© 2015 Roche Roche Diagnostics (Schweiz) AG 6343 Rotkreuz Accurate HCV viral load monitoring is essential for current and future HCV treatments: to evaluate virological response, guide treatment duration, and decide on futility. This demands an HCV RNA assay that can correctly distinguish true signals from background noise. See what truly matters with the innovative HCV Dual Probe assay from Roche Molecular Diagnostics.

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Welcome to Lugano

It is a pleasure and a honour for us to welcome you in Lugano for the 73rd Annual Meeting and Assembly of the Swiss Society for Microbiology.

The Palazzo dei Congressi, conveniently located at walking distance from the Main Station and downtown, is the ideal venue to host a meeting that we hope will be enjoyable and highly educational. The Meeting's theme is a very exciting and comprehensive one, aiming at linking human and animal microbiology and epidemiology with environmental and fundamental microbiology.

"Microbiology in the times of Ecohealth" – why this title? While One Health seeks the benefits of a closer cooperation between human and animal health, EcoHealth embraces One Health and extends it to considerations of sustained ecosystem services and health in socio-ecological systems. A broader perspective on human and animal health and ecosystems has a huge potential for many fields of microbiology and we hope this congress will make all participants aware of this important topic.

EcoHealth has the power not only to link research fields together, but it has also far-reaching economic and social consequences. For example, the origin of antimicrobial resistance in human patients can be often, but not always, related to environmental sources. Surveillance systems, however, are most often restricted to single sectors such as public health or the food chain. In this Meeting we shall hear how the Canadian Integrated Programme for Antimicrobial Resistance (CIPARS) has already adopted a fully integrated approach for its surveillance and control. Switzerland has only now taken the first steps towards a closer integration of antimicrobial resistance: thus, there is definitely the need to raise awareness for this topic in our country.

Cross-species and zoonotic transmission of pathogens using integrated human and animal studies must be used to characterise human-animal interfaces, to identify sources of infection and to control zoonoses at the source. The understanding of cross-species transmission can also be used for cross-sector economic analyses, for example in the control of brucellosis. The epidemics of Ebola in West Africa shows that an integrated approach is urgently needed in the field of emerging diseases. Wildlife reservoirs and the consumption of bush meat are at the origin of the Ebola transmission to humans and its control and elimination depend not only on improved hygienic capacity of health care providers but also on a safe cohabitation of wildlife and humans.

Keynote speakers and students will highlight the latest progress in research on various aspects of microorganisms and microbiology such as epidemiology and molecular biology, as well as emphasise their medical and environmental relevance. This will not be limited to EcoHealth, though – a wealth of information will be provided also on general fundamental research on bacteria, viruses and fungi. It is our hope that the meeting will be of interest to international experts and Swiss researchers working on all aspects of fundamental, translational, human and veterinary medical, as well as environmental and applied microbiology.

As you may see from the programme, the Scientific Committee was able to gather outstanding international and national experts for plenary talks and parallel sessions. The oral presentations will be complemented by poster sessions allowing, in particular, young scientists to present their work in an interactive and collegial manner.

We wish you a very enjoyable and highly educational stay in Lugano – please take the time to enjoy not only the congress but also the pre- and post-meeting events, as well as the beautiful surroundings. Welcome in Ticino!

Orlando Petrini

Mauro Tonolla

Presidents of the Annual Assembly 2015

For the organizing committee

Committees

Scientific Committee

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Dr. med. Andrea Zbinden, University of Zurich Medical Microbiology

Prof. Dr. Jakob Zinsstag, Swiss TPH, Basle EcoHealth

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SSM - Workshop - 27 May, 2015

Outreach activities in microbiology

Microbes, the most numerous group of organisms on earth, are also the least well known ones and only scant information and communication is available for them. Antibiotic resistance, pathogens and diseases are the major aspects that come to mind when talking about microbes without knowing what they represent.

General information and vulgarization about medical microbiology are of paramount importance, but microbiology encompasses several other extremely important aspects unknown to the general population and in need to be explained. These include biotechnology, in particular enzymes or microbiological processes, food production and ecological cycles, but also the importance of the microbiota and the fact that human beings host a larger number of microbes than human cells.

Several scientists are involved in communication and vulgarization of the microbial world. The goal of this first Swiss workshop on "Outreach Activities in Microbiology" is to exchange information and ideas and to create a network of researchers acting on information and promoting microbiology to schools and the general public. An important part of this workshop will be dedicated to discussions.

We welcome all researchers, scientists or teachers involved or interested in outreach activities in microbiology to take part in this meeting.

We are looking forward to meeting you.

Mauro Tonolla & Karl Perron

INFORMATION

Venue: Palazzo del Congressi

Piazza Independenza 4 CH-6900 Lugano

www.luganoconventions.com

Room C

Date: 27 May, 2015

Registration: For logistic purposes, however, we encourage interested people to register by

sending an e-mail to mauro.tonolla@supsi.ch.

People interested in participating also in the SSM conference on 28-29 May in Lugano

must register through the following link:

http://www.swissmicrobiology.ch/congress/edit registration.php. Please note that

fees will be charged for these 2 SSM days.

Poster session: A poster session will be organized during this workshop to stimulate exchange and

discussion. All speakers and individuals involved in microbiology outreach activities are welcome to submit a poster highlighting their activities. The posters will be displayed on 28-29 May during the SSM meeting, to provide all congress participants with information on activities related to communication in microbiology. The Organizing Committee will return posters after the meeting to those who will attend the workshop on 27 May 2015 but are not able to stay for the SSM Annual Meeting

(28-29 May 2015).

PROGRAMME Wednesday, 27 May, 2015

Outreach activities in Switzerland

Room C Chairs: Karl Perron, Mauro Tonolla

14:00 Welcome address

14:15 Miwelt: Hands-on science for primary school children in the field

OA 001 of microbial biotechnology Karin Kovar, ZHAW Zürich

14:35 **BiOutils: microbiology for everyone!**

OA O02 Massimo Caine, UNIGE

14:55 Phageback: meeting with a bacteria killer

OA 003 Mathilde Ythier, L'Eprouvette-UNIL

15:15 Getting to know microbes: outreach activities in the Piora Valley

OA 004 Aurélia Weber, UNIGE

15: 35-16-15 Coffee break and poster session

Outreach activities in Ticino

Chairs: Mauro Tonolla, Karl Perron

16:15 Successfully carry out microbiology in the classroom

OA 005 Cristina Fragoso, SUPSI, Ticino

16:35 How to increase the interest in biological and chemical science?

OA O06 Gilda Giudici, L'ideatorio, USI, Ticino

16:55 Pratichiamo le Scienze della Vita. Pratica di laboratorio per studenti liceali

OA 007 Giuseppe Laffranchi, STSBC and Liceo di Bellinzona, Ticino

17:15 General discussion/round table and conclusions

17:30 Apéritif

The organizing committee wishes to thank all sponsors. Their support is essential for the success of this workshop.

















Thursday, 28 May 2015

08:30 Regist	ration and Poster set up			Room
oo.oo registi	ation and i oster set up			
10:10 Welco	me and Introduction	Orlando Petrin	i, Mauro Tonolla	Α
10:15 Plenar	y Session I		to One Health and EcoHealth g, Basle, Switzerland	Α
11:00 – 12:00	Session 1 Mycology	Host defense strategies against Candida albicans Salomé LeibUndgut-Landmann, Zurich, Switzerland Immunogenetics of fungal infections Pierre-Yves Bochud, Lausanne, Switzerland		B1
Parallel Sessions 1 - 2	Session 2 Food Microbiology	The contradicting roles of the Streptococcus bovis complex in humans, animals and food Christoph Jans, Zurich, Switzerland		В3
		,	ons selected from Abstracts	
12:00 – 13:30	Poster Session, Technical Exhibition,	13:00 – 13:30	Medical Microbiology and Mycology Poster presentation	
	Lunch	13:00	Poster Walk, Medical Microbiology and Mycology	
12:30 – 13:00		Minisymposi	um Axonlab AG	B1
		opened a windo	c analysis of a bat influenza viruses «we w and in-flew-enza» nmle, Freiburg, Germany aviruses: Present and Past	
	Session 3 Virology	Ronald Dijkma	n, Berne, Switzerland	B1
		Origin and evolution Metzner,	Zurich, Switzerland	
13:30 – 15:30		Oral presentation	ons selected from Abstracts	
Parallel Sessions	Session 4 General Microbiology	Oral presentations selected from Abstracts		В3
3 - 5	This Session starts	Emerging antibiotic resistance in a world of Patrice Nordmann, Fribourg, Switzerland		
	at 13:20	Resistance in agricultural systems: Aspergillus as an example		
	Session 5 Resistance	Paul E. Verwei	i, Nijmegen, The Netherlands	Α
	nesistance	in Canada	nicrobial Resistance Surveillance Guelph, Canada	
		Resistance to a	• •	
15:30 – 16:30	Poster Viewing, Techn		· · · · · · · · · · · · · · · · · · ·	
15.00 10.00		Symposium I	-	D 4
15:30 – 16:30		Rapid Methods	in Microbiology	B1
16:30 Plenary Session		and domesticat	gens as tracers of human migration, trading ion of animals Berne, Switzerland	Α
		for Brucellosis of	and laboratory research control ng, Basle, Switzerland	
17:15 Annua	I Assembly of the SSM	1		Α
18:50 Meetir	g Point Palazzo dei Co		sport to: , Lugano Paradiso	

Friday, 29 May 2015

			Room	
08:30 Plenar	y Session III	Can we link biodiversity with virus diversity? Sandra Junglen, Bonn, Germany Epidemic Ebola in West Africa – Experiences and lessons from Sierra Leone	Α	
		Tim O'Dempsey, Liverpool, UK		
10:00 – 11:00	Poster Viewing, Tech	nical Exhibition, Coffee		
10:00 – 11:00		Symposium Becton Dickinson BD	B1	
	Session 6 Medical Microbiology	Phage therapy, a novel alternative to treat complex infections Gregory Resch, Lausanne, Switzerland	B1	
11:00 Parallel		Oral presentations selected from Abstracts		
Sessions 6 - 7	Session 7	Moulds in the built environment Jens Frisvad, Lyngby, Denmark		
	Microbiology of the built environment	Microorganisms for safeguarding built heritage Edith Joseph, Neuchâtel, Switzerland	В3	
		Oral presentation selected from Abstracts		
12:00 – 13:00	Poster Viewing, Tech	nical Exhibition, Lunch		
12:00 – 13:00		Symposium Bruker Daltonik GmbH	B1	
	Session 8	Ecology of mosquito immunity and resistance to malaria Jacob Koella, Neuchâtel, Switzerland Vector-borne diseases in Switzerland: new challenges for		
	SVEG	veterinary diagnostics Andrea Vögtlin, Mittelhäusern, Switzerland Tick-borne pathogens in Switzerland	B1	
		Rahel Gäumann, Spiez, Switzerland		
13:00 – 14:30		Oral presentations selected from Abstracts		
Parallel Session 8 – 10	Session 9 Mycology	How fungal root mutualistic endophytes suppress host immunity Alga Zuccaro, Max-Planck-Inst., Marburg, Germany	В3	
		Oral presentations selected from Abstracts		
		Single cell genomics and small-scale metagenomics to investigate microbial evolution Manu Tamminen, Zürich, Switzerland		
	Session 10 General Microbiology	The evolution of spatial self-organization in expanding populations of microorganisms Dave Johnson, Dübendorf, Switzerland	A	
iniciobiology		Using microscopic algae to understand community assembly Patrick Venail, Geneva, Switzerland		
14:30 Poste	r Session, Technical	14:30 – 15:00 General Microbiology and Virology Posters presentation		
Exhib	ition, Lunch	14:30 Poster Walk, General Microbiology and Virology		
15:00 Plenar	y Session IV	The secret life of integrative and conjugative elements, agents of open source evolution Jan Roelof van der Meer, Lausanne, Switzerland	Α	
15:45		SSM Award and Poster Awards Poster Awards sponsored by bioMérieux (Suisse) SA, Genève	Α	

Information

Venue Palazzo dei Congressi

Piazza Indipendenza 4

CH-6900 Lugano

Phone: +41 79 659 26 50 / +41 79 515 07 19

Date 28 – 29 May, 2015

Scientific Secretariat PD Dr. Orlando Petrini

POLE Pharma Consulting Via Al Perato 15C CH-6932 Breganzona Phone: +41 91 941 01 06

Phone: +41 91 941 01 06 Fax: +41 91 941 38 33

e-mail: orlando@poleconsult.com

PD Dr. Mauro Tonolla

University of applied sciences of Southern Switzerland - SUPSI

Department of Environment, Constructions and Design

Laboratory of applied microbiology (LAM)

Microbiology Unit BIVEG, UNIGE

Via Mirasole 22a CH-6500 Bellinzona

Phone: +41 91 814 60 74 Fax: +41 91 814 60 39

e-mail: mauro.tonolla@supsi.ch

Administrative Secretariat cosem / Irene Müller & Martine Moreillon

Sonnenrain 10

CH-3150 Schwarzenburg

Phone: +41 31 731 25 93 Fax: +41 31 731 25 94 e-mail: cosem@bluewin.ch Martine.Moreillon@unil.ch

Congress Secretariat Palazzo dei Congressi

Piazza Indipendenza 4

CH-6900 Lugano

Thursday 28 May 2015 07.30 – 18.00 h Friday 29 May 2015 07.30 – 16.30 h

Registration fees

(incl. Lunches, Coffee breaks) 2 days 1 day tickets

SSM /SVEG / EcoHealth members CHF 150.- CHF 100.-Graduate / Post-graduate students CHF 125.- CHF 70.-Non SSM members CHF 175.- CHF 120.-

Please wear your badge during the whole meeting

FAMH credit points for the participation in the congress you are credited:

½ day 3 points 1 day 6 points Social Programme Apéritif and Banquet: 28 May 2015, 19.30

Ristorante Capo San Martino, Lugano-Paradiso

Banquet, incl. beverages and coffee CHF 110.- (incl. 8% VAT)
Students. doctoral candidates CHF 90.- (incl. 8% VAT)

No vouchers will be on sale at the meeting

Arrival From the train station, take Bus line Nr. 2 direction "Castagnola" until

Bus stop "Palazzo dei Congressi".

Posters / Poster-Awards Authors should be present at their posters at the times indicated in the

programme overview.

Poster set up: Thursday, 28 May 2015, 08.00 -10.00 Poster removal: Friday, 29 May 2015, after 16.45

Unremoved posters will be destroyed.

The best three posters will be awarded with

CHF 2'500, CHF 1'500 and CHF 1'000, respectively, sponsored by

bioMérieux Suisse S.A. Genève



Poster award ceremony

Friday, 29 May 2015, 15.45 - 16.15, Room A

Oral Presentations Only computer presentations are accepted. Please bring your PowerPoint

presentation on a USB memory stick.

Industrial Exhibition There will be an accompanying industrial exhibition. We would like to thank all

exhibitors for their participation. They are the main sponsors of the Annual Assembly of SSM. Please take the opportunity to visit your suppliers!

A list of exhibitors and maps of where to find them are given on

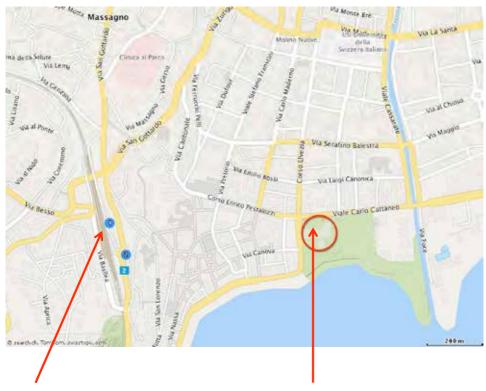
pages 14 - 16.

Additional Information Lugano has many interesting touristic sites. City maps and additional

information are available at the registration desk.

The organizing committee wishes to thank all sponsors and active members. Their support is essential for the success of this meeting.

City Map of Lugano



Train station

Palazzo dei Congressi Piazza Indipendenza 4 6900 Lugano

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BÜHLMANN Laboratories AG CH-4124 Schönenbuch/Basel E-mail: info@buhlmannlabs.ch Phone: +41 61 487 12 12 Fax: +41 61 487 12 34 www.buhlmannlabs.ch

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Blonav

Key journals in Microbiology



Editor-in-Chief: Milton H. Saier Jr. (La Jolla, Calif.)

An excellent forum for basic and applied research

- Publishes original papers from all areas of microbiology and biotechnology
- Reflects the integration of basic and applied microbiology brought about by recent advances in genomics, proteomics and bioinformatics
- Featuring written symposia on selected topics, timely reviews, minireviews and correspondence
- Includes descriptions of novel, microbiologically relevant software



Editor-in-Chief: Jean-Claude Manuguerra (Paris)

The forum for innovative research in all areas of virology

Intervirology welcomes the submission of manuscripts on a wide range of topics in basic and medical virology. The journal's scope encompasses work on the molecular biology of animal viruses, including genome organization and regulation, and the structure and function of viral proteins. The pathogenesis, immunology, diagnosis, epidemiology, prophylaxis and therapy of viral diseases are also considered.



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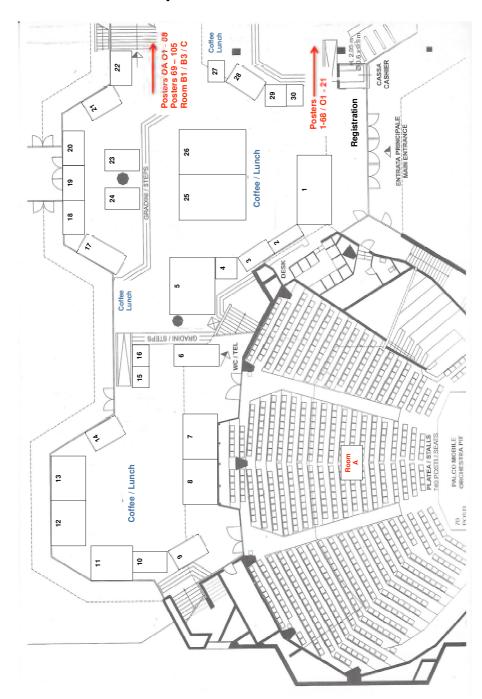
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List of exhibitors

Numerical

1	AXON LAB AG	Baden-Dättwil
2	PROMEGA AG	Dübendorf
3	BÜHLMANN Laboratories AG	Schönenbuch
4	MEINTRUP DWS Laborgeräte GmbH	D-Lähden
5	bioMÉRIEUX (SUISSE) S.A.	Genève
6	CHEMIE BRUNSCHWIG AG	Basel
7	DIASORIN Switzerland AG	Rotkreuz
8	CURETIS AG	D-Holzgerlingen
9	RUWAG Handels AG	Bettlach
10	TEOMED AG	Greifensee
11	ELITECH SÄRL	Estavayer-le-Lac
12	THERMO FISHER SCIENTIFIC	Pratteln
13	BIOLIFE Italiana SRL	I-Milano
14	SYSMEX SUISSE AG	Horgen
15	BIO TEK INSTRUMENTS GmbH	Luzern
16	MED TECH TRADING	Zürich
17	IGZ INSTRUMENTS AG	Zürich
18	SKAN AG	Basel
19	AID	D-Strassberg
20	LABGENE SCIENTIFIC SA	Châtel-St-Denis
21	ABBOTT GmbH & Co. KG	D-Wiesbaden
22	LUBIOSCIENCE GmbH	Luzern
23	QIAGEN	Hombrechtikon
24	EUROIMMUN Schweiz AG	Luzern
25	ROCHE DIAGNOSTICS (Schweiz) AG	Rotkreuz
26	BECTON DICKINSON AG	D-Heidelberg
27	BUCHER BIOTEC AG	Basel
28	BRUKER Daltonik GMBH	D-Bremen
29	PALL CORPORATION	Basel
30	APTEQ AG	Cham

Exhibition Site Map



List of exhibitors

Alphabetical

21	ABBOTT GmbH & Co. KG	D-Wiesbaden
19	AID	D-Strassberg
30	APTEQ AG	Cham
1	AXON LAB AG	Baden-Dättwil
26	BECTON DICKINSON AG	D-Heidelberg
15	BIO TEK INSTRUMENTS GmbH	Luzern
13	BIOLIFE Italiana SRL	I-Milano
5	bioMERIEUX (SUISSE) S.A.	Genève
28	BRUKER Daltonik GMBH	D-Bremen
27	BUCHER BIOTEC AG	Basel
3	BÜHLMANN Laboratories AG	Schönenbuch
6	CHEMIE BRUNSCHWIG AG	Basel
8	CURETIS AG	D-Holzgerlingen
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22	LUBIOSCIENCE GmbH	Luzern
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18	SKAN AG	Basel
14	SYSMEX SUISSE AG	Horgen
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12	THERMO FISHER SCIENTIFIC	Pratteln

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	Thursday, 28 May 2015	
08:30 – 10:00	Registration, Poster set-up	
10:00 - 10:15 Room A	Welcome and Introduction	
10:15 – 11:00 Room A 10:15 – 11.00 M01	Plenary Session I – EcoHealth Chair: G. Greub, Lausanne, Switzerland An Introduction to One Health and EcoHealth Jakob Zinsstag, Basle, Switzerland	EC⊖HEALTH
11:00 – 12:00	Parallel Sessions 1 – 2	
Room B1	Session 1 Mycology: Fungal infections facing host respo Chair: D. Sanglard, Lausanne, Switzerland	nse and human genetics
11:00 - 11:30 M03	Host defense strategies against <i>Candida albicans</i> Salomé LeibUndgut-Landmann, Zurich, Switzerland	
11:30 – 12:00 M03	Immunogenetics of fungal infections Pierre-Yves Bochud, Lausanne, Switzerland	
Room B3	Session 2 – Food Microbiology Chair: Leo Meile, Zurich, Switzerland	
11:00 – 11:30 M04	The contradicting roles of the <i>Streptococcus bovis</i> com Christoph Jans, Zurich, Switzerland	plex in humans, animals and food
11.30 – 11.45 O01	Coagulase positive- and methicillin-resistant coagulase concurrent carriage among owners and their pets E. Gómez-Sanz, S. Ceballos, B. Duffy, M. Zarazaga	negative staphylococcal
11.45 – 12.00 002	A new surface protein possibly involved in bovine adap strains originating from humans F. Oechslin, S. McCallin, C. Menzi, P. Moreillon, G. Res	. ,
12:00 - 13:30 13:00 - 13:30 13:00	Poster Session, Technical Exhibition, Lunch Medical Poster presentation Poster Walk, Medical Microbiology and Mycology	

Thursday, 28 May 2015

12:30 – 13:00 Satellite Symposium Axonlab AG



Chair: Reinhard Zbinden, Zurich, Switzerland

O03 Comparison of two rapid commercial molecular tests for detection of the most common

carbapenemases: Xpert® Carba-R and eazyplex® V. Hinic, C. Straub, J. Ziegler, D. Goldenberger, R. Frei

O04 Added value of molecular POCT GeneXpert MTB/RIF versus conventional microscopy in the

evaluation of the risk of Tuberculosis transmission in a low-endemic country

O. Opota, L. Senn, G. Greub, K. Jaton

Parallel Sessions 3 - 5

13.30 - 15.30

13.30 - 13.30	raialiei Sessiolis 3 – 3
Room B1	Session 3 – Virology Chair: Andrea Zbinden, Zurich, Switzerland
13:30 – 14:00 M05	Reverse genetic analysis of a bat influenza viruses «we opened a window and in-flew-enza» Martin Schwemmle, Freiburg, Germany
14:00 - 14:30 M06	Zoonotic Coronaviruses: Present and Past Ronald Dijkman, Berne, Switzerland
14:30 - 15:00 M07	Origin and evolution of HIV Karin Metzner, Zurich, Switzerland
15:00 – 15:15 O05	Coronavirus – host interactions in organotypic human airway cultures H.R. Jonsdottir, R. Dijkman, R. Rodriguez, O.J. Hamming, R. Hartmann, V. Thiel
15:15 – 15:30 O06	Citronellavirus - digging into pithoviridae diversity C. Bertelli, L. Mueller, V. Thomas, T. Pillonel, N. Jacquier, G. Greub

Thursday, 28 May 2015

Room B3	Session 4 – General Microbiology – Original contributions Chair: Patrick Viollier, Geneva, Switzerland
13:30 – 14:00 007	The sweetest trick: o-polysaccharides protect plant-beneficial pseudomonads against antimicrobial peptides during insect infection P. Kupferschmied, T. Chai, P. Flury, T.H.M. Smits, M. Maurhofer, C. Keel
14:00 – 14:30 O08	Killing for DNA: the type VI secretion system of <i>Vibrio cholerae</i> fosters horizontal gene transfer M. Blokesch
14:30 – 14:50 O09	Cell cycle constraints on the excision of the <i>Caulobacter crescentus</i> NA1000 mobile genetic element G. Panis, N. Jaunin, D. Martins, P.H. Viollier
14:50 – 15:10 O10	A microfluidic chip to measure bacterial chemotaxis C. Roggo, R. van der Meer
15:10 – 15:30 O11	What triggers sporulation in <i>Serratia ureilytica</i> str. lr5/4? S. Filippidou, W. Kooli, T. Junier, N. Jeanneret, P. Junier
Room A	Session 5 – Resistance This Session starts at 13:20 Chair: Linda Thöny-Meyer, St. Gallen, Switzerland
13:20 - 13:50 M08	Emerging antibiotic resistance in a world of globalisation Patrice Nordmann, Fribourg, Switzerland
13:50 - 14:20 M09	Resistance in agricultural systems: <i>Aspergillus</i> as an example Paul E. Verweij, Nijmegen, The Netherlands
14:20 – 15:00 M10	Integrated antimicrobial resistance surveillance in Canada Rebecca Irwin, Guelph, Canada Lecturer supported by the Laboratorio EOLAB, CH-6500 Bellinzona
15:00 - 15:30 M11	Resistance to antiviral drugs Sabine Yerly, Geneva, Switzerland

Thursday, 28 May 2015

15:30 – 16:30 Satellite Symposium bioMérieux (Suisse) SA Rapid Methods in Microbiology



Chair: Gérard Praz, Sion, Switzerland

O22 Rapid identification of microorganisms by MALDI-TOF

Mass Spectrometry (MALDI-TOF MS) Mauro Tonolla, Bellinzona, Switzerland

O12 Rapid and simple Shigella and E. coli differentiation by MALDI TOF

using the VITEK® MS platform"

Victoria Girard, bioMérieux, R&D Microbiology, Marcy, France

STAT in Microbiology: Yes, We Can!

"How multiplex PCR can change the care of children with respiratory tract infections"

Thomas Bodmer, Liebefeld, Switzerland

Diagnosis of Emerging Antibiotic Resistance Patrice Nordmann, Fribourg, Switzerland

15:30 – 16:30 Poster Viewing, Technical Exhibition, Coffee

Coffee sponsored by: Pharma Consulting Marion Senn GmbH, Burgdorf



Thursday, 28 May 2015

16:30 - 17:15 Room A	Plenary Session II Chair: Jean-Claude Piffaretti, Massagno, Switzerland
16:30 – 16:50 M12	Bacterial pathogens as tracers of human migration, trading and domestication of animals Joachim Frey, Berne, Switzerland
16:50 – 17:15 M13	Combined field and laboratory research for <i>Brucellosis</i> control Esther Schelling, Basle, Switzerland

Room A

17:15

18:50 Meeting Point Palazzo dei Congressi Transport to Capo San Martino (Tourist Trains)

Annual Assembly of the SSM



19:30 Apéritif and Banquet Ristorante Capo San Martino Lugano-Paradiso



Flowers sponsored by:

MED TECH TRADING Diagnostics and Research

Friday, 29 May 2015

08:30 - 10:00 Room A	Plenary Session III Chair: Volker Thiel, Bern, Switzerland
08:30 - 09:15 M14	Can we link biodiversity with virus diversity? Sandra Junglen, Bonn, Germany
09:15 - 10:00 M15	Epidemic Ebola in West Africa – Experiences and lessons from Sierra Leone Tim O'Dempsey, Liverpool, UK

Lecturer supported by the Swiss Tropical and Public Health Institute



10:00 - 11:00 Poster Viewing, Technical Exhibition, Coffee

10:00 – 11:00 Satellite Symposium BD Room B1



10:00 – 10:30	Timely diagnosis of bacterial enteric pathogens – the next level Nadia Wohlwend, MSc, Bern-Liebefeld, Switzerland
10:30 – 11:00	Respiratory diagnostics Adrian Egli, MD PhD, Basel, Switzerland

11:00 – 12:00	Parallel Sessions 6 - 7
Room B1	Session 6 – Medical Microbiology Chair: Reinhard Zbinden, Zurich, Switzerland
11:00 - 11:20 M16	Phage therapy, a novel alternative to treat complex infections Gregory Resch, Lausanne, Switzerland
11:20 – 11:33 O13	The outer membrane protein pora of <i>Campylobacter</i> as a potential vaccine target S. Kittl, P. Kuhnert
11:34 – 11:47 O14	Identification of drugs targeting <i>Mycobacterium tuberculosis</i> M. Dal Molin, M. Meuli, A. Tschumi, S. Laage-Witt, P. Sander
11:48 – 12:00 O15	Replicon typing of plasmids carrying BLA CTX M 15 in <i>Enterobacteriaceae</i> of human, livestock and environmental origin in Switzerland K. Zurfluh, M. Glier, H. Hächler, R. Stephan

Friday, 29 May 2015

Room B3 Session 7 – Microbiology of the built environment

Chair: Mauro Tonolla, Bellinzona, Swiztzerland

Sponsored by:

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SUPSI

11:00 - 11:30	Moulds in the built environment
M17	Jens Frisvad, Lyngby, Denmark

11:30 – 11:50 Microorganisms for safeguarding built heritage M18 Edith Joseph, Neuchâtel, Switzerland

11:50 – 12:05 Chemical and mycological assessment of indoor air quality inside a new flat. A case study T. Teruzzi, S. De Respinis

12:00 - 13:00 Poster Session, Poster, Technical Exhibition, Lunch

12:00 – 13:00 Satellite Symposium Bruker

P032



Chair: Reinhard Zbinden, Zurich, Switzerland

Using the MALDI Biotyper System in a Clinical Microbiology Lab as a System. Past, present, future

Dr. Martin Risch, labormedizinisches Zentrum Dr Risch, FL-Schaan

Multicenter validation of standardized MALDI-TOF based typing of ESBL-producing Escherichia coli outbreak isolates

M. Oberle, R. Frei, F. Maurer, G. Jost, A. Widmer, B. Sakem, N. Wohlwend, D. Jonas,

C. Ottiger, H. Fankhauser, A. Egli

The MALDI Biotyper System – The Next Steps Towards a Pivotal Tool for Clinical Microbiology Sören Lehmann, Bruker Daltonik, D-Bremen

Friday, 29 May 2015

13:00 – 14:30	Parallel Sessions 8 - 10
Room B1	Session 8 – SVEG Chair: Alexander Mathis, Zurich, Switzerland
13:00 – 13:20 M19	Ecology of mosquito immunity and resistance to malaria Jacob Koella, Neuchâtel, Switzerland
13:20 - 13:40 M20	Vector-borne diseases in Switzerland: new challenges for veterinary diagnostics Andrea Vögtlin, Mittelhäusern, Switzerland
13:40 - 14:00 M21	Tick-borne pathogens in Switzerland Rahel Gäumann, Spiez, Switzerland
14:00 – 14:15 O17	Strain-specific estimates of fitness predict the community of a multiple-strain tick-borne pathogen in the field over one decade M.J. Voordouw, J. Durand, M. Jacquet, L. Gern
14:15 – 14:30	Fifteen years of mosquito surveillance in Canton Ticino E. Flacio
Room B3	Session 9 – Mycology – Fungi coping with different environments Chair: Cristina Fragoso Bellinzona, Switzerland
13:00 - 13:30 M22	How fungal root mutualistic endophytes suppress host immunity Alga Zuccaro, Organismic Interactions, Max-Planck-Institute Marburg, Germany
13:30 – 13:45 O18	Heat shock proteins 90 (hsp90) and 70 (hsp70) in <i>Aspergillus fumigatus</i> virulence and antifungal resistance F. Lamoth
13:45 – 14:00 O19	Transcriptomic profiles of <i>Candida albicans</i> in systemic infection of two host organisms S. Amorim-Vaz, A.T. Coste, S. Pradervand, T.V.D. Tran, M. Pagni, D. Sanglard
14:00 – 14:15 O20	The role of nonhematopoietic cells in protective immunity against <i>C. albicans</i> S. Altmeier, S. LeibundGut-Landmann
14:15 –14:30 O21	Combining microfluidics and RNA sequencing to assess the nematode-inducible defensome of a mushroom in spatio-temporal resolution A. Tayyrov, S.S. Schmieder, C. E. Stanley, S. Bleuler-Martinez, A.J. DeMello, M. Aebi, M. Künzler

Friday, 2	29 May	2015
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Room A	Session 10 – General Microbiology – Evolution in microbial communities Chair: Christof Holliger, Lausanne, Switzerland
13:00 – 13:30 M23	Single cell genomics and small-scale metagenomics to investigate microbial evolution Manu Tamminen, Zürich, Switzerland
13:30 - 14:00 M24	The evolution of spatial self-organization in expanding populations of microorganisms Dave Johnson, Dübendorf, Switzerland
14:00 – 14:30 M25	Using microscopic algae to understand community assembly Patrick Venail, Geneva, Switzerland
14:30 - 15:00 14:30 - 15:00 14:30	Poster Session, Technical Exhibition, Coffee authors non-Medical Posters present Poster Walk, General Microbiology and Virology
15:00 – 15:45 Room A 15:00 – 15.45	Plenary Session IV Chair: Christof Holliger, Lausanne, Switzerland The Secret Life of Integrative and Conjugative Elements, Agents of Open Source Evolution

15:45 - 16:15

SSM Award and Poster Awards

Room A

M26

Short Lectures of the SSM Award winner Poster Award sponsored by: **bioMérieux (Suisse) SA, Genève**

Jan Roelof van der Meer, Lausanne, Switzerland



16:15 Room A Closure

73rd ANNUAL ASSEMBLY OF SSM

Palazzo dei Congressi 28 – 29 May 2015

ABSTRACTS

Workshop Outreach activities in Switzerland Main Speakers Speakers selected from Abstracts

SSM Workshop "Outreach activities in microbiology"

OA 001 MIWELT: HANDS-ON SCIENCE FOR PRIMARY SCHOOL CHILDREN IN THE FIELD OF MICROBIAL BIOTECHNOLOGY
K. Kovar, V. Looser, M. Ottinger, C. Ulli, M. Raabe, J. Dürr

OA 002 BIOUTILS: MICROBIOLOGY FOR EVERYONE!
M. Caine, A. Weber, S. Zuchuat, V. Ducret, P. Linder, K. Perron

OA OO3 PHAGEBACK: MEETING WITH A BACTERIA KILLER
M. Ythier, G. Resch, A. Kaufmann, Y. Que

OA 004 GETTING TO KNOW MICROBES: OUTREACH ACTIVITIES IN THE PIORA VALLEY
A. Weber, M. Caine, C. Fragoso-Corti, R. Bachofen, M. Tonolla, R. Peduzzi, K. Perron, P. Linder

OA 005 SUCCESSFULLY CARRY OUT MICROBIOLOGY IN THE CLASSROOM
C. Fragoso Corti, A. Crenna, A. Weber, M. Caine, D. Sartori, K. Perron, P. Linder,
M. Tonolla

OA 006 HOW TO INCREASE THE INTEREST IN BIOLOGICAL AND CHEMICAL SCIENCE?
G. Giudici, M. Luraschi, R. Rezzonico, G. Pellegri

OA 007 PRATICHIAMO LE SCIENZE DELLA VITA: PRATICA DI LABORATORIO PER STUDENTI LICEALI G. Laffranchi

Main speakers

M01 AN INTRODUCTION TO ONE HEALTH AND ECOHEALTH

J. Zinsstag

MO2 HOST DEFENSE STRATEGIES AGAINST CANDIDA ALBICANS

S. LeibUndgut-Landmann

M03 IMMUNOGENETICS OF FUNGAL INFECTIONS

PY. Bochud

M04 THE CONTRADICTING ROLES OF THE STREPTOCOCCUS BOVIS COMPLEX IN HUMANS.

ANIMALS AND FOOD

C. Jans

M05 REVERSE GENETIC ANALYSIS OF A BAT INFLUENZA VIRUS «WE OPENED A WINDOW AND IN-

FLEW-ENZA» M. Schwemmle

M06 ZOONOTIC CORONAVIRUSES: PRESENT AND PAST

R. Dijkman, HR. Jonsdottir, R. Hartmann, C. Drosten, V. Thiel

M07 ORIGIN AND EVOLUTION OF HIV

K.J. Metzner

M08 EMERGING ANTIBIOTIC RESISTANCE IN A WORLD OF GLOBALIZATION

P. Nordmann

M09 RESISTANCE IN AGRICULTURAL SYSTEMS: ASPERGILLUS AS AN EXAMPLE

P. E. Verweij

M10 INTEGRATED ANTIMICROBIAL RESISTANCE SURVEILLANCE IN CANADA

R. J. Irwin

M11 RESISTANCE TO ANTIVIRAL DRUGS

S. Yerly

M12 BACTERIAL PATHOGENS AS TRACERS OF HUMAN MIGRATION, TRADING AND

DOMESTICATION OF ANIMALS

J. Frey

M13 COMBINED FIELD AND LABORATORY RESEARCH FOR BRUCELLOSIS CONTROL

E. Schelling, A. Dean, Z. Baljinnyam, J. Kasymbekov, Y. Kanouté, L. Falzon,

B. Bonfoh, P. Pilo, J. Zinsstag

M14 CAN WE LINK BIODIVERSITY WITH VIRUS DIVERSITY?

A. Kopp, M. Marklewitz, F. Zirkel, K. Hermanns, Ch. Drosten, S. Junglen

M15 EPIDEMIC EBOLA IN WEST AFRICA - EXPERIENCES AND LESSONS FROM SIERRA LEONE

T. O 'Dempsey

M16 PHAGE THERAPY. A NOVEL ALTERNATIVE TO TREAT COMPLEX INFECTIONS

YA. Que, G. Resch

M17 MOULDS IN THE BUILT ENVIRONMENT

J. C. Frisvad

M18 MICROORGANISMS FOR SAFEGUARDING BUILT HERITAGE

E. Joseph, M. Albini, L. Comensoli, W. Kooli, L. Mathys, C. Jacquet, M. Rebord,

Y. Braendle, P. Junier

M19 ECOLOGY OF MOSQUITO IMMUNITY AND RESISTANCE TO MALARIA J. Koella M20 VECTOR-BORNE DISEASES IN SWITZERLAND: NEW CHALLENGES FOR VETERINARY **DIAGNOSTICS** A. Vögtlin M21 TICK-BORNE PATHOGENS IN SWITZERLAND R. Gäumann M22 HOW FUNGAL ROOT MUTUALISTIC ENDOPHYTES SUPPRESS HOST IMMUNITY M23 SINGLE CELL GENOMICS AND SMALL-SCALE METAGENOMICS TO INVESTIGATE MICROBIAL **EVOLUTION** M. Tamminen THE EVOLUTION OF SPATIAL SELF-ORGANIZATION IN EXPANDING POPULATIONS OF M24 **MICROORGANISMS** D. R. Johnson M25 USING MICROSCOPIC ALGAE TO UNDERSTAND COMMUNITY ASSEMBLY P. Venail

THE SECRET LIFE OF INTEGRATIVE AND CONJUGATIVE ELEMENTS, AGENTS OF OPEN

S. Sulser, F. Delavat, A. Vucicevic, V. Sentchilo, J. R. van der Meer

M₂₆

SOURCE EVOLUTION

Speakers selected from abstacts

O01	COAGULASE POSITIVE- AND METHICILLIN-RESISTANT COAGULASE NEGATIVE- STAPHYLOCOCCAL CONCURRENT CARRIAGE OF AMONG OWNERS AND THEIR PETS E. Gómez-Sanz, S. Ceballos, B. Duffy, M. Zarazaga, C. Torres
O02	A NEW SURFACE PROTEIN POSSIBLY INVOLVED IN BOVINE ADAPTATION OF STAPHYLOCOCCUS AUREUS CC8 STRAINS ORIGINATING FROM HUMAN F. Oechslin, S. McCallin, C. Menzi, P. Moreillon, G. Resch
O03	COMPARISON OF TWO RAPID COMMERCIAL MOLECULAR TESTS FOR DETECTION OF THE MOST COMMON CARBAPENEMASES: XPERT® CARBA-R AND EAZYPLEX® SUPERBUG COMPLETE B V. Hinic, C. Straub, J. Ziegler, D. Goldenberger, R. Frei
O04	ADDED VALUE OF THE MOLECULAR POCT GENEXPERT MTB/RIF VERSUS CONVENTIONAL MICROSCOPY IN THE EVALUATION OF THE RISK OF TUBERCULOSIS TRANSMISSION IN A LOW-ENDEMIC COUNTRY O. Opota, L. Senn, G. Greub, K. Jaton
O05	CORONAVIRUS - HOST INTERACTIONS IN ORGANOTYPIC HUMAN AIRWAY CULTURES HR. Jonsdottir, R. Dijkman, R. Rodriguez, OJ. Hamming, R. Hartmann, V. Thiel
O06	CITRONELLAVIRUS - DIGGING INTO PITHOVIRIDAE DIVERSITY C. Bertelli, L. Mueller, V. Thomas, T. Pillonel, N. Jacquier, G. Greub
007	THE SWEETEST TRICK: O-POLYSACCHARIDES PROTECT PLANT-BENEFICIAL PSEUDOMONADS AGAINST ANTIMICROBIAL PEPTIDES DURING INSECT INFECTION P. Kupferschmied, T. Chai, P. Flury, T. H.M. Smits, M. Maurhofer, C. Keel
O08	KILLING FOR DNA: THE TYPE VI SECRETION SYSTEM OF VIBRIO CHOLERAE FOSTERS HORIZONTAL GENE TRANSFER M. Blokesch
O09	CELL CYCLE CONSTRAINTS ON THE EXCISION OF THE CAULOBACTER CRESCENTUS NA1000 MOBILE GENETIC ELEMENT. G. Panis, N. Jaunin, D. Martins, PH. Viollier
O10	A MICROFLUIDIC CHIP TO MEASURE BACTERIAL CHEMOTAXIS C. Roggo, J. R. van der Meer
011	WHAT TRIGGERS SPORULATION IN SERRATIA UREILYTICA STR. LR5/4? S. Filippidou, W. Kooli, T. Junier, N. Jeanneret, P. Junier
012	RAPID AND SIMPLE SHIGELLA AND E. COLI DIFFERENTIATION BY MALDI TOF USING THE VITEK MS PLATFORM M. Arsac, V. Monnin, P. Bourne-Branchu, D. Pincus, H. Dwivedi, G. Devulder, G. Durand, A. van Belkum, V. Girard
013	THE OUTER MEMBRANE PROTEIN PORA OF CAMPYLOBACTER AS A POTENTIAL VACCINE TARGET S. Kittl, P. Kuhnert
O14	IDENTIFICATION OF DRUGS TARGETING MYCOBACTERIUM TUBERCULOSIS M. Dal Molin, M. Meuli, A. Tschumi, S. Laage-Witt, P. Sander
O15	REPLICON TYPING OF PLASMIDS CARRYING BLACTX-M-15 IN ENTEROBACTERIACEAE OF HUMAN, LIVESTOCK AND ENVIRONMENTAL ORIGIN IN SWITZERLAND K. Zurfluh, M. Glier, H. Hächler, R. Stephan
O16	CHEMICAL AND MYCOLOGICAL ASSESSMENT OF INDOOR AIR QUALITY INSIDE A RECENTLY CONSTRUCTED FLAT T. Teruzzi, P. Canonica, S. De Respinis, AP. Caminada, V. Guidi, M. Tonolla

STRAIN TICK-BORNE PATHOGEN IN THE FIELD OVER ONE DECADE
MJ. Voordouw, J. Durand, M. Jacquet, L. Gern

O18 HEAT SHOCK PROTEINS 90 (HSP90) AND 70 (HSP70) IN ASPERGILLUS FUMIGATUS
VIRULENCE AND ANTIFUNGAL RESISTANCE
F. Lamoth

O19 TRANSCRIPTOMIC PROFILES OF CANDIDA ALBICANS IN SYSTEMIC INFECTION OF TWO

STRAIN-SPECIFIC ESTIMATES OF FITNESS PREDICT THE COMMUNITY OF A MULTIPLE-

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- O19 TRANSCRIPTOMIC PROFILES OF CANDIDA ALBICANS IN SYSTEMIC INFECTION OF TWO HOST ORGANISMS
 S. Amorim-Vaz, AT. Coste, S. Pradervand, TVD. Tran, M. Pagni, D. Sanglard
- O20 THE ROLE OF NONHEMATOPOIETIC CELLS IN PROTECTIVE IMMUNITY AGAINST C. ALBICANS S. Altmeier, S. LeibundGut-Landmann
- O21 COMBINING MICROFLUIDICS AND RNA SEQUENCING TO ASSESS THE NEMATODE-INDUCIBLE DEFENSOME OF A MUSHROOM IN SPATIO-TEMPORAL RESOLUTION
 A. Tayyrov, S. S. Schmieder, C. E. Stanley, S. Bleuler-Martinez, A. J. DeMello,
 M. Aebi, M. Künzler
- O22 RAPID IDENTIFICATION OF MICROORGANISMS BY MALDI-TOF MASS SPECTROMETRY (MALDI-TOF MS)
 M. Tonolla

OA 001

miwelt: Hands-on science for primary school children in the field of microbial biotechnology

K. Kovar 1, V. Looser 1, M. Ottinger 1, C. Ulli 1, M. Raabe 2, J. Dürr 3

- 1 School of Life Sciences and Facility Management LSFM, Zurich University of Applied Science
- 2 independent journalist, Leipzig, Germany
- 3 freelance childrens books illustrator, Berlin, Germany

In scope of the miwelt-project (funded by the Agora programme of the SNFS), activities for children from 7 to 11 years of age are being developed to encourage exploration of the non-tangible world of microorganisms, their presence in everyday life, and their use in the science laboratory. For this purpose, the scientists involved, together with artists and journalists, have developed illustrated factual stories, thematic excursions, and laboratory experiments in the field of microbial biotechnology. They have broken down complex information into graspable chunks and redesigned originally sophisticated scientific experiments with the use of conventional articles/items so that they can be reproduced outside a well-equipped laboratory, for example, in typical classrooms or the kitchen at home. The pedagogic concept is based on the use of graphic arts and creative activities to trigger and stimulate children's natural curiosity and bring them the excitement of a voyage of discovery. In this way, science becomes more attractive to children.

Children's fascination stimulates their curiosity! As the microbial world cannot be seen at first glance, its notions are built on images of the real, visible world as children experience it. While taking part in laboratory activities, children can observe, question, experiment, document their research and findings in a lab book, have discussions, and dress in white lab coats, gloves and goggles. They experience, for instance, that a good experimental plan may fail, that cells viewed under the microscope are likely to dance, that microbial culturing may be the new face of pastoral and/or arable farming, and that DNA is something a human, animal or plant cannot lose. The delivery concept is based on hands-on experience and a dialogue, both of which emphasise the significance of underlying principles over factual knowledge. Adult researchers developing and supervising these activities are motivated by the unbiased perceptions of the young researchers, and thus inspired to seek deeper understanding of their own knowledge of science.

OA 002

BiOutils: microbiology for everyone!

M. Caine ¹, A. Weber ¹, S. Zuchuat ¹, V. Ducret ¹, P. Linder ¹, K. Perron ¹

1 BiOutils, University of Geneva, Geneva, Switzerland

Experiment, as a teaching strategy in schools, is well-known for enhancing cognitive development, especially when it comes to scientific concepts. Consistently, in the field of Biology, practical sessions are a major part of teaching curriculums. However, the laboratory equipment which is needed to achieve modern classroom experiments is not always affordable for schools' budgets and furthermore teachers often struggle to keep the pace of modern scientific developments.

To tackle this educational gap, BiOutils was founded in 2007 at the University of Geneva. Created by researchers, BiOutils is an interface that aims to promote and support the study of biology in schools. To do so, the platform offers to teachers of primary, secondary I and secondary II schools protocols, material and know-how to perform modern and engaging experiments in class, with an emphasis on molecular biology and microbiology. 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.

The growing success of BiOutils shows that this kind of structure is highly needed: after only 8 years of existence, BiOutils is now providing support to all secondary schools in Geneva. Furthermore, other activities for schools and for the broad public are also regularly organized, such as the Microbiology Days – an annual event addressing key issues in microbiology – laboratory visits, continuous education for teachers, internships and various didactical projects.

Phageback: meeting with a bacteria killer

M. Ythier 1, G. Resch 2, A. Kaufmann 3, Y. Que 4

- 1 L'Eprouvette from the Science-Society Interface, University of Lausanne
- 2 Department of Fundamental Microbiology, University of Lausanne, Switzerland
- 3 Science-Society Interface, University of Lausanne, Switzerland
- 4 Intensive Care Medicine Department, Lausanne University Hospital, Switzerland

Antibiotics are crucial drugs for the treatment of infectious diseases, but the prevalence of multidrug resistant bacteria has continuously challenged their efficacy. Since the number of new drugs under clinical development is limited and their introduction to the market is disappointingly slow, it is critical to evaluate alternative non-antibiotic-based treatments. Bacteriophages are ubiquitous natural bacterial predators that specifically infect and kill bacterial cells during their life cycle.

Shortly after their discovery in the beginning of the 20th century, phages were successfully used for the treatment of bacterial infections. Phage therapy was the only specific treatment targeting bacteria during the pre-antibiotic era. The development and overwhelming success of antibiotic agents in the mid-century shadowed the utilization of phage therapy in the Western world, while East European countries, such as Georgia, Russia, and Poland, continued to produce and use phage preparations. Because of the worldwide spread of antibiotic resistant bacteria, phage therapy has now become a subject of high and renewed interest in the Western world. Research programs have been specifically funded to re-introduce phage approaches to fight multidrug resistant bacterial infections. An European program recently funded PHAGOBURN, the first multi-centric randomized controlled clinical trial on phage therapy in the modern era. This project will evaluate the efficacy and safety of phage cocktails to treat burn wound infections caused by *Escherichia coli and Pseudomonas aeruginosa*.

In this context, an Agora project funded by FNS has started in order to inform the public about bacteriophages and promote debate about issues of phagetherapy in the current alarming context of antibiotic resistance. Workshops, exhibitions, brochures and a dedicated website with forums are in development.

Getting to know microbes: outreach activities in the Piora Valley

A. Weber 1, M. Caine 1, C. Fragoso-Corti 2, R. Bachofen 3, M. Tonolla 2, R. Peduzzi 4, K. Perron 1, P. Linder 1

- 1 BiOutils, University of Geneva
- 2 Laboratory of applied microbiology, University of applied sciences Southern Switzerland
- 3 University of Zurich
- 4 Fondazione Centro Biologia Alpina, Piora

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 <code>Hamatococcus pluvialis</code> and the bacteria <code>Chromatium okenii</code> 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, panels and downloadable content. 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

Successfully carry out microbiology in the classroom

C. Fragoso Corti 1, A. Crenna 1, A. Weber 2, M. Caine 2, D. Sartori 3, K. Perron 2, P. Linder 2, M. Tonolla 1

- 1 SUPSI, Laboratory of Applied Microbiology
- 2 Bioutils. University of Geneve
- 3 Ufficio dell'insegnamento Medio Superiore, Cantone Ticino

The complexity of biology, which nowadays is divided into many sub-disciplines, combined with the quickness with which laboratory technology evolve, makes it extremely difficult for teachers to be constantly updated. These lead to technical skills limitation amongst scientific teaching staff, and a general reluctance to propose practical work to students in classrooms.

For these reasons, we have built up an interface between LMA (Laboratorio Microbiologia Applicata) and schools of Southern Switzerland (Canton Ticino) in tight collaboration with the administration and the teaching staff. This platform will be developed on the same model as BiOutils (University of Geneva), an interface already existing, that promotes life sciences in schools.

The objective is to provide experimental material and scientific skills to biology teachers of the Ticino region, to help them illustrate their courses and support a modern education in microbiology based on experimental work (in accordance with the current School Program for Natural Sciences).

We work in close contact with the teachers to propose activities that fit their needs. We also develop the experimental set-ups, outcome results and reflection on the activities that the teachers undertake with students.

Within our project, we provide experimental protocols, equipment and support to biology teachers to promote science and microbiology in particular.

How to increase the interest in biological and chemical science?

G. Giudici¹, M. Luraschi^{1,2}, R. Rezzonico¹, G.Pellegri¹

Previous investigations done on science and society revealed that the perception that children have of scientist is often stereotypical: scientists tend to work at night, among cobwebs and candles, or surrounded by computers and calculators. They stand alone, slightly apart from the mass. The scientist for many children is a 'mad' person, not young, often bald, strictly male, often a chemist, wearing odd clothes and working in a windowless space on mysterious things, conducting projects that sometimes help to save the world, and sometimes are harmful to our natural environment. This misrepresented outlook on science is surprisingly unchanged by the time these young people reach secondary school, reflecting this negative impact on their future career choices. This is one of the statements leading to the problem of staff shortages in the MINT sectors (Mathematics, Informatics, Natural and Technical science) in Switzerland and to the shortfall in the number of women scientists. These reflections have to be integrated into the various didactic activities addressed to pupils, with the goal of counteracting this stereotypical view, in particular with the creation of dedicated spaces where children can meet a colored, live and useful science – not a 'crazy', but a normal, customary and also female one. The objective of the didactical projects is not to convince young people that science is the safe and secure path for their lives. However, regardless of the trade or profession they are going to end up working in, we believe that a positive, though critical, eve on science will be infinitely better than an unreal and eccentric caricature.

¹ L'ideatorio, Università della Svizzera italiana

² Atgabbes association

Pratichiamo le Scienze della Vita Pratica di laboratorio per studenti liceali

G. Laffranchi 1,2

Il Lavoro di Maturità (LaM) è una produzione personale che l'allievo realizza e presenta alla fine del ciclo di studi liceali ticinesi. Per realizzare il LaM, lo studente deve svolgere un lavoro di analisi basato sulla raccolta, il confronto e la valutazione di informazioni e di dati. Il lavoro di ricerca si svolge nel secondo semestre della terza liceo e nel primo della quarta liceo. Un LaM in biologia o in chimica presuppone in genere che parte dell'indagine venga svolta in laboratorio utilizzando delle metodologie tipiche della ricerca sperimentale. La Società Ticinese delle Scienze Biomediche e Chimiche in collaborazione con l'Ufficio dell'insegnamento medio-superiore cantonale intende promuovere un'iniziativa che permetta agli studenti liceali impegnati in un LaM di biologia o di chimica di approfondire una specifica metodologia sperimentale durante un periodo di pratica svolto in un laboratorio di un'industria o di un ente attivo nel campo delle Scienze della vita. L'obiettivo più ampio è di promuovere gli studi nel campo delle Scienze della vita, informando nel contempo gli studenti liceali sulle possibilità d'impiego professionale in questo settore in Ticino. Gli enti e le aziende che aderiranno all'iniziativa verranno citate come sostenitori.

¹ Società ticinese scienze biomediche e chimiche

² Liceo di Bellinzona, 6500 Bellinzona

An Introduction to One Health and Ecohealth

- J. Zinsstag 1
- 1 Swiss Tropical and Public Health Institute, PO Box, 4002 Basel

Human and veterinary medicine are commonly perceived as two distinct academic disciplines with their own schools although they are strongly interconnected i.e. by the testing of human drugs in animals and the uptake of therapies from human health for animals. However, there arise issues where both medicines do not communicate and collaborate sufficiently which leads to unnecessary morbidity and mortality. "One Health" postulates that an added value in terms of human and animal health and financial savings can be generated by a closer cooperation of human and animal health. Examples are provided from health services for pastoralists and the control of zoonotic diseases. The linkage of humans and animals involves also their environment and ecosystems. Changes in ecosystems and human and animal populations influence each other mutually and often show that ecosystems are affected by human activity. Evidence is growing that long term planning of public health must consider sustained provision ecosystem services inclusively. In the last 15 years scientists have more and more combined approaches to human and animal health with ecosystem assessments under the term "Ecosystem Health". Such systemic approaches to health and ecosystems have also important social components, bringing together actors in participatory transdisciplinary stakeholder processes to identify locally adapted solutions to complex problems. The origin of antimicrobial resistance, or mercury contamination in Amazon fish, or the origins of emerging diseases like Ebola are just a few examples warranting approaches to health as outcomes of social-ecological systems. There remains an unfinished agenda for "One Health" in many areas. But "One Health" is clearly embedded in the much broader concept of "EcoHealth" (www.ecohealth.net). Both integrated approaches to health should work together as closely as possible to secure sufficient translational impact on new policies to protect the health of humans, animals and the ecosystems in which they live.

Host defense strategies against Candida albicans

- S. LeibUndgut-Landmann 1
- 1 Division of Immunology, Institute of Virology, University of Zürich, Switzerland

Fungal pathogens bear a serious health hazard for individuals with a weakened immune system. Although some fungi, such as *Candida albicans*, are present in the normal microbiota, they can cause severe diseases if host defenses are breached. The continuous rise in fungal infections and the increase in resistance against available antifungal drugs urge the development of novel preventive and therapeutic strategies. For this, a detailed understanding of fungal pathogenicity and natural host defense mechanisms is of great importance. Neutrophils and CD4+ T lymphocytes have long been known to play an indispensable role in promoting protective antifungal immunity. More recently, the cytokine interleukin-17 (IL-17) has emerged as a critical player in regulating antifungal defense, in particular in mucosal tissues and the skin. Importantly, rare genetic defects in the IL-17 pathway is associated with an increased susceptibility to *C. albicans*. Using a mouse model of mucocutaneous candidiasis we have gained novel insights into IL-17-dependent and -independent mechanisms of host protection. Here, I will discuss the current view of host-pathogen interactions that adjust the fine balance between fungal colonization and pathogenic infection.

Immunogenetics of fungal infections

PY. Bochud ¹

1 University hospital and University of Lausanne

Fungal infections such as invasive candidiasis and aspergillosis still represent important causes of morbidity and mortality in specific groups patients, including those undergoing intensive chemotherapy and/or hematopoietic stem cell transplantation for the treatment of onco-hematological diseases, surgical patients with multiple abdominal surgery as well as solid organ transplant recipients. While specific risk factors have been identified, such as patient age, co-morbidities, type and severity of immunosuppression, CMV infection, renal failure and type/recurrence of surgery, it is still difficult to accurately predict which patients will develop these complications. An increasing number of studies are highlighting a role for genetic polymorphisms in susceptibility to such infections. So far, due to numerous limitations, existing data have not supported the use of genetic polymorphisms for individual risk stratification in the clinical practice. Major problems have been the inability to replicate the association, especially among different populations at risk, and/or the lack of a definite role for the reported gene and/or its polymorphism(s) in fungal pathogenesis. However, some relevant polymorphisms are now emerging from well-designed studies, with appropriate replication and/or support from strong biological evidence. Such polymorphisms may play a role in the prediction and management of severe fungal infections in high risk patients in the coming years.

THE CONTRADICTING ROLES OF THE STREPTOCOCCUS BOVIS COMPLEX IN HUMANS, ANIMALS AND FOOD

C. Jans 1

1 Laboratory of Food Biotechnology, Institute of Food, Nutrition and Health, ETH Zurich

The Streptococcus bovis/Streptococcus equinus complex (SBSEC) comprises seven (sub)species arranged in four main phylogenetic branches S. equinus, S. infantarius, S. gallolyticus and S. alactolyticus. Their habitat extends across many mammals including ruminants and humans to marsupials, birds and fermented food products. SBSEC members are often commensal bacteria of the gastrointestinal tract and rumen, able to degrade a multitude of complex carbohydrates from plants and other sources. Additionally, SBSEC members are opportunistic pathogens of humans and animals. Originally described as S. bovis, recent taxonomic advances allow for highly improved disease to species associations. S. gallolyticus subsp. gallolyticus (S. bovis biotype I) is implicated in infective endocarditis and associated with colorectal cancer for which the causality is still unclear. S. gallolyticus subsp. pasteurianus and S. lutetiensis are implicated in biliary and urinary tract infections and meningitis. In contrast, S. gallolyticus subsp. macedonicus (Sgm) and S. infantarius subsp. infantarius (Sii) show little disease associations but a high prevalence and predominance in fermented dairy and plant foods in Europe, Asia, America and sub-Saharan Africa. Especially African strains of Sii harbor adaptations to the dairy environment paralleling the evolution of S. thermophilus in the Western dairy system. Sii and Sgm could provide rare occasions to elucidate the evolution from pathogenic origin to dairy fermentation strains, develop future indigenous fermentation starter cultures and contribute to the preservation of biodiversity and technology. Their classification within the opportunistic pathogenic SBSEC, largely unknown virulence factors and incidents of possible Sii/Sgm human infections demand for accurate safety assessment considering the consumption of over 108 live cells/mL of fermented product. Our project therefore aims to elucidate the epidemiology and pathogenicity mechanisms of Sii and SBSEC in the human-animal-food system to provide targeted interventions, improved medical diagnosis, risk assessment for infection and zoonosis and the delineation of potential food-grade strain lineages.

REVERSE GENETIC ANALYSIS OF A BAT INFLUENZA VIRUS "WE OPENED A WINDOW AND IN-FLEW-ENZA"

M Schwemmle 1

1 University Medical Center Freiburg, Freiburg, Germany

Zoonotic transmission of Influenza A viruses pose a constant threat to the human population. In 2012 the complete sequence of distantly related influenza A virus was discovered in the fruit bats *Sturnira Iilium* (little-yellow shouldered bat) from Central America. One year later, a second bat influenza virus from flat-faced fruit bats (*Artibeus planirostris*) from Peru, designated H18N10, was identified, suggesting a broader distribution and diversity of bat influenza viruses at least in Central and South America.

The recent finding of new influenza A virus-like subtype genomes in bats expands the reservoir of potentially dangerous zoonotic influenza A viruses. Bats are known hosts of several life-threatening pathogens including Ebola virus, Hendra virus, rabies virus, SARS- and MERS-coronavirus. It is then of upmost importance to assess the host spectrum, reassortment capacity and possible therapeutic interventions for bat influenza viruses. Unfortunately, infectious bat influenza virus could be neither isolated from infected bats nor reconstituted by reverse genetic approaches, impeding further characterization of these viruses.

By substituting the bat influenza virus glycoproteins with those of an avian influenza virus, we now describe for the first time the generation of infectious chimeric bat influenza viruses. This allowed us to study their host tropism, sensitivity to antivirals, and ability to adapt to multiple species. While these chimeric bat viruses were not restricted for replication in human respiratory cells and quickly adapted to avian and mouse hosts, they were unable to exchange their viral genomes (reassort) with other conventional influenza A virus strains. The latter significantly reduces the risk of emerging bat influenza viruses in non-bat species, including humans.

Zoonotic Coronaviruses: Present and Past

R. Dijkman 1, HR. Jonsdottir 1, R. Hartmann 2, C. Drosten 3, V. Thiel 1

- 1 Institute of Virology and Immunology IVI, Bern, Switzerland
- 2 University of Aarhus, Aarhus, Denmark
- 3 Institute of Virology, Bonn, Germany

Coronaviruses have received considerable attention since the emergence of the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in China 2002/2003. SARS-CoV infection in people exemplified that coronaviruses can cross the species barrier from an animal reservoir to humans and cause severe and lethal disease in humans. The emergence of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012 showed that zoonotic transmission of coronaviruses is not a rare event. Both viruses have close relatives in bats, and moreover, intermediate hosts, such as raccoon dogs, civet cats (SARS-CoV) and dromedary camels (MERS-CoV) have been proposed to facilitate zoonotic transmission. Interestingly, the long known human coronavirus 229E (HCoV-229E; causing common cold in humans), appears to have a similar history of zoonotic transmission, since close relatives were detected in bats as well as in camelid species. Using well-differentiated human airway epithelia cell cultures as surrogate model for human respiratory tract we assess the zoonotic potential of these closely related zoonotic viruses and defining which adaptations are required for zoonotic transmission. Furthermore, despite the potential large socioeconomic burden, there are no efficacious antiviral treatment options available against these zoonotic coronaviruses. Therefore we also evaluate whether Type III IFNs are interesting therapeutic treatment candidates against these zoonotic coronaviruses. Combined knowledge on the requirements of zoonotic transmission and development of antiviral treatment options paves the way for more effective measures against emerging zoonotic coronavirus.

ORIGIN AND EVOLUTION OF HIV

Karin J. Metzner^{1,2}

Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, 8091 Zurich, Switzerland

² Institute of Medical Virology, University of Zurich, 8091 Zurich, Switzerland

Human immunodeficiency viruses type 1 (HIV-1) and type 2 (HIV-2) are the causes of acquired immunodeficiency syndrome (AIDS). Multiple cross-species transmission led to the infection of humans by simian immunodeficiency viruses (SIV) and at least two of them gave rise to the HIV-1 pandemic and the HIV-2 endemic. Today, more than 40 primate species are known to be naturally infected with the respective SIV. What are the risks to be confronted with HIV-3 in the (near) future? The high heterogeneity of HIV-1 is mainly caused by the error-prone virus replication and the high generation rate of virions. On the population level, the amino acid differences can be 35% and more in certain viral proteins. Several HIV-1 subtypes and recombinant forms are circulating and new viral variants continue to emerge reflecting the ongoing process of HIV-1 evolution. On the individual level, the process of HIV-1 transmission involves a bottleneck, i.e., in the majority of acutely HIV-1 infected individuals the infection is established by one (to few) transmitted/founder virus that rapidly adapts to the environment within the new host and generates a new heterogeneous viral population. The ongoing HIV-1 evolution and the high diversity of HIV-1 have major implications on pathogenesis, drug resistance, and vaccine development.

EMERGING ANTIBIOTIC RESISTANCE IN A WORLD OF GLOBALIZATION

- P Nordmann 1
- 1 Departement of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland

Emerging antibiotic resistance in bacteria is dominated nowadays by difficult-to-treat infections due to multidrug and pandrug resistant gram negatives. Those gram negatives are mostly *Enterobacteriacae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Multidrug resistance is not anymore observed only among nosocomial but also among community-acquired pathogens. Focus has been on \(\mathbb{G}\)-lactam resistance since this class of antibiotics represent the most widely prescribed antibiotics and resistance to \(\mathbb{G}\)-lactams is associated to resistance to the other classes of antibiotics such as aminoplycosides and fluoroguinolones.

Extended-spectrum \(\text{B-lactamases} \) (CTX-M) compromise the efficacity of all \(\text{B-lactams} \) except cephamycins and carbapenems. They are observed in many community-acquired pathogens (ca. 5% in \(Escherichia \) coli in Switzerland and 80% in India \(!) \) and mostly in \(Klebsiella \) pneumoniae as a source of nosocomial outbreaks. The ultimate evolution of antibiotic resistance in gram negatives is the spread of carbapenemases that hydrolyze virtually all \(\text{B-lactams}, including the carbapenems. Three main carbapenemase groups have been reported, KPC, NDM and OXA-48. KPC producers are mostly observed as a source of nosocomial infections in \(K \) pneumoniae in the US, Greece, and Italy. NDM and OXA-48 producers are the source of both nosocomial and community-acquired infections. The most important reservoir of NDM producers is South East Asia while that of OXA-48 is North Africa and the Middle-East. In Switzerland, most of the carbapenemase producers are identified from patients hospitalized abroad. Globalization related to tourism and population transfer is the main source of acquisition of those multidrug resistant bacteria by the Swiss patients. Combating the growing reservoirs of those resistant bacteria in developing countries and their early identification on the Swiss territory is of primarily importance to try to control this pandemic situation. Multidrug resistance in gram negatives may become the most important challenge for modern medicine in a very near future.

RESISTANCE IN AGRICULTURAL SYSTEMS: ASPERGILLUS AS AN EXAMPLE

P. E. Verweij 1

1 Radboud University Medical Centre of Nijmegen, Nijmegen, The Netherlands

The saprophytic mold Aspergillus fumigatus is commonly found in decaying organic matter including compost. The fungus is ubiquitous and is not known to be a phytopathogen. However in humans A. fumigatus causes a spectrum of diseases ranging from allergic syndromes to lethal invasive infections. The arsenal of antifungal agents that can be used to treat patients is limited with the azoles representing the most important class. In the past decade acquired resistance has emerged in clinical A. fumigatus isolates. Although azole resistance may develop during therapy, it has become apparent that selection of azole resistance occurs in the environment. Azoles are widely used outside medicine, including applications for crop protection, material preservation and in veterinary practice. There is increasing evidence that exposure of A. fumigatus in the environment to azoles results in resistance selection. Because similar azole molecules are used for abovementioned applications in the environment compared to human medicine, resistance selection in the environment causes loss of activity of medical triazoles. The environmental route of resistance selection is of major concern as it represents the main route of clinical resistance cases, causing up to 80% of azole-resistant cases. This route is characterized by the propensity to migrate with resistance now being reported in Europe, Middle east, Asia, Africa and most recently Australia. Furthermore, azole resistance significantly complicates patient management. Environmental resistance has as consequence that any patient may present with any azole-resistant aspergillus disease, often without a history of previous azole exposure. Early diagnosis is difficult, especially in culture-negative patients. Furthermore, azole-susceptible and azole-resistant co-infections may occur. The reported case-fatality rate is high in azole-resistant culture-positive invasive aspergillosis, reported to be between 88% and 100%.

At present it is important to understand how azole resistance develops in the environment, as over time new resistance mechanisms have emerged and the use of azoles is threatened unless adequate measures are implemented. Research should focus on factors that cause azole resistance to originate in *A. fumigatus* or factors that facilitate the spread of resistance. This will help to initiate a stewardship program that retains the use of azoles for crop protection and material preservation without compromising the efficacy of medical triazoles.

M₁₀

INTEGRATED ANTIMICROBIAL RESISTANCE SURVEILLANCE IN CANADA

R. J. Irwin 1

1 Public Health Agency of Canada, Guelph, Ontario, Canada

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was developed to enable an understanding of how antimicrobials used in agri-food settings contribute to the emergence and spread of antimicrobial resistance along the food chain to impact human health. Enteric micro-organisms along the farm-to-fork continuum are collected along with antimicrobial use data for humans and animals.

This program has successfully collected samples and data from multiple public health and agricultural jurisdictions by developing strong trust among participants, providing relevant expertise (veterinary public health), employing sound epidemiological and microbiological principles for design and analyses, and maintaining efficiencies throughout the system. Continuous consultation with stakeholders has ensured the sustainability of the system and that information needs are recognized and understood.

One of the greatest challenges for integrated surveillance is analyzing all data together and considering it in a holistic way to provide an overall picture. Surveillance integration occurs across communities and regions, over time, between different sources (host species), microorganisms, drugs (in the cases of multi-drug resistance), and culminates with communication between the science and policy realms. The integration of data elements relies on the translation of discipline-specific data elements (e.g. antimicrobial resistance patterns versus drug use data versus farm management information versus public health risk factor data) into a common language. Ultimately we need to deliver an effective, accurate and contextually sound story from the analysis of these varied data inputs to inform science and policy.

Integrated surveillance programs offer significant benefits to understanding the ecology of complex systems. We have had demonstrable successes in reducing resistance, related to the communication of surveillance findings to private industry stakeholders who implemented voluntary changes and to federal and provincial governments who changed policies.

Resistance to antiviral drugs

S. Yerly 1

1 Laboratory of Virology, Geneva University Hospital

There are at present more than 40 licensed antiviral agents available, targeting a wide range of viruses, including herpesviruses, HIV, HBV, HCV and influenza viruses. Antiviral drug resistance is an increasing concern in immunocompromised patients, where ongoing viral replication and prolonged drug exposure lead to the selection of resistant strains, and remains a concern in HBV or HIV infected patients on long-term therapy. The frequency and the timing of drug resistance development differ according to the type of viruses and antiviral drugs. Factors associated with the risk of drug resistance include both viral (replication mechanism, quasi-species, resistance transmission, fitness) and antiviral drug (potency, concentration in target cells, pharmacokinetic) characteristics.

Today, rapid diagnosis of drug resistance can be today performed by the detection of viral mutations to various drugs. Genotypic tests have a faster turnaround time than phenotype assays and use a common technology of PCR amplification and viral sequencing. However these assays depend on knowledge of the viral mutations causing resistance to specific antiviral drugs.

New treatment strategies including antiviral drug with high barrier to resistance development should be studied. The successful control of antiviral resistance (and the transmission of resistant viruses) is dependent on the ability to detect these resistances in both clinical setting and population surveillance.

BACTERIAL PATHOGENS AS TRACERS OF HUMAN MIGRATION, TRADING AND DOMESTICATION OF ANIMALS

J. Frev 1

1 Institute of Veterinary Bacteriology, University of Bern

Molecular genetic markers such as Single Nucleotide Polymorphisms (SNPs) of bacterial pathogens of domestic animals allow studying the root and spread of epidemics and also tracing of human migration, domestication of animals and trading of animal products.

SNP analysis showed that a cluster of *Mycoplasmas* of ruminants, the '*M. mycoides* cluster' emerged about 10'000 years ago when humans started to domesticate ruminants. The severe pathogen *M. mycoides* subsp. *mycoides* thereof, constituting a very tight entity, evolved about 500 years ago when cattle breeding became intensified in Europe.

The ruminants' pathogen, *Bacillus anthracis*, the agent of anthrax, shows slow evolution *via* a bimodal lifestyle alternating between short replication phases of 20–40 generations during infection of the host, where it causes death, followed by long, dormant phases as spores in the environment. Hence, the genome of *B. anthracis* is highly homogeneous. This feature and the worldwide analysis of strains for canonical SNPs has allowed the development of precise molecular clock models to estimate the age of major diversifications and to trace the global spread of this pathogen. This was promoted by movement of domestic cattle with settlers and by international trade of contaminated animal products, in particular wool. In Europe, four genetic main lineages are found, lineage B.Br.CNEVA that is strongly associated to cattle and seems to be introduced to central Europe from the far-East during the Neolithic revolution followed by dispersal westward and establishment along the Alps. The two other, A.Br.011/009 and A.Br.001/002 (Ames) seem to have been introduced via the silk road to Europe and seem to have spread further by settlements during the Columbian times to Northern America. Finally lineage A.Br.Vollum seems to represent a lineage of goat and sheep strains introduced by wool imports from central Asia, affecting mostly workers in wool factories but also contaminating cattle.

Combined field and laboratory research for brucellosis control

E. Schelling^{1,2}, A. Dean^{1,2}, Z. Baljinnyam^{1,2,3}, J. Kasymbekov^{1,2,4}, Y. Kanouté^{1,2}, L. Falzon⁵, B.Bonfoh⁶, P. Pilo⁷, J. Zinsstag^{1,2}

² University of Basel, Basel, Switzerland

The preferential reservoir host for Brucella abortus is cattle and for B. melitensis, sheep and goats. Only few countries are free of ruminant brucellosis, the most important causative agents of brucellosis in people. Cross-sector economics showing cost-benefits of vaccination or test and slaughter of livestock to society assist Governments' decisions to implement control, but require sufficient epidemiological data. Studies are mainly done in assumed high risk populations and cannot be compared to other human disease burdens at national level. Subsequently, health ministries are hesitant to re-allocate limited resources. In Mongolia nonherders had higher seropositivity than herders since they were less informed on preventive measures. Most of the published research focuses either on human or livestock brucellosis. An ongoing systematic review evaluates correlations between human and livestock brucellosis prevalences, stratified by livestock species and region based on data that is comparable in location, time, level of aggregation, details and quality. Simultaneous human and animal studies identify the source of infection, the level of underreporting or serve as the basis for animal-human transmission models and be substantiated by homologous MLVA patterns of Brucella spp isolates from people and livestock. In Mongolia we investigate if camels can maintain brucellosis infection or are a spill-over host alone. In parallel, the achieved vaccination coverage in ruminants is monitored by veterinarians and doctors to identify needed corrective actions. In West Africa we do not know if B. melitensis is present given the strong past bias on cattle. B. abortus isolates from Togolese cattle showed a large deletion in a gene encoding a putative autotransporter which might be involved in virulence and / or host predilection (and may explain the comparably low human seropositivity of ~1%). Combining epidemiological field studies with laboratory research better clarifies brucellosis transmission dynamics, should lead to regional biobanks with well-characterised Brucella sp. isolates to validate and improve serological and molecular diagnosis as well as elucidate mechanisms of pathogenicity and host affinity for different Brucella species and genotypes.

¹ Swiss Tropical and Public Health Institute, Basel, Switzerland

³ Animal Health Project, Swiss Agency for Development and Cooperation, Ulaanbaatar, Mongolia

⁴ Institute of Biotechnology of the National Academy of Science of the Kyrgyz Republic, Bishkek, Kyrgyzstan

⁵ Veterinary Public Health Institute, Vetsuisse Faculty, Bern, Switzerland

⁶ Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte d'Ivoire

⁷ Institute of Veterinary Bacteriology, Bern, Switzerland

CAN WE LINK BIODIVERSITY WITH VIRUS DIVERSITY?

A. Kopp ¹, M. Marklewitz ¹, F. Zirkel ¹, K. Hermanns ¹, Ch. Drosten ¹, S. Junglen ¹

1 Institute of Virology, University of Bonn Medical Centre, Bonn, German

Tropical rainforests show the highest level of terrestrial biodiversity and may be an important contributor to microbial diversity. Exploitation of these ecosystems and accompanying loss of biodiversity may foster the emergence of novel pathogens. It is widely accepted that the majority of emerging disease outbreaks occur in the tropics. At present, however, we lack both an understanding of pathogen diversity in remote rainforest areas, and reliable detection systems for novel pathogens. Moreover, our knowledge on the impact of biodiversity loss on pathogen diversity, as well as on the biological mechanisms that drive emergence of specific pathogens is limited.

We study mosquito and virus abundance patterns along an anthropogenic disturbance gradients in tropical Africa and the Neotropics. We found an extremely high diversity of previously unknown RNA viruses suggesting that tropical ecosystems may contain a much larger spectrum of viruses than previously expected. Virus diversity and prevalence patterns show a decrease in diversity from natural to modified habitat types. The majority of the viruses seemed to be species specific that differed by habitat. Interestingly, several viruses from different viral families increased in prevalence along the gradient. These data suggest that only few viruses benefit from ecosystem disturbance.

Knowledge on the biological mechanisms behind ecosystem modification and arbovirus emergence could provide innovative approaches for epidemic risk assessment and intervention strategies.

Epidemic Ebola in West Africa - Experiences and Lessons from Sierra Leone

T. O 'Dempsey 1

1 Liverpool School of Tropical Medicine (LSTM)

The 2014-15 Ebola epidemic in West Africa, primarily affecting Guinea, Sierra Leone, and Liberia has exceeded all previous Ebola outbreaks in the number of cases and in the international response.

Sierra Leone has experienced more cases of Ebola virus disease than any other country in history. The epidemic was characterised by an exponential increase in cases that far exceeded available resources for surveillance, laboratory confirmation, safe isolation and clinical management. The impact on the health workforce was particularly devastating. Paralysis of already weak health systems and services resulted in significant 'collateral damage': an excessive morbidity and mortality due to diseases other than Ebola.

Following a concerted National and International response, the epidemic is now coming under control. 'Getting to Zero' remains a national priority, however many challenges exist in eliminating transmission among hard-to-reach communities. Cross-border transmission also continues to pose a threat, particularly along Sierra Leone's northern border with Guinea.

Sierra Leone is now moving into a transition period of early recovery and safe reactivation of health systems and services.

The speaker will reflect on the clinical and ethical challenges faced by health workers over the course of the epidemic, the national and international response, current approaches to getting to a 'resilient zero', and the emergence of Ebola as a global security threat.

Phage therapy, a novel alternative to treat complex infections

YA. Que 1, G. Resch 2

- 1 Department of Adult Critical Care Medicine, CHUV, Lausanne, Switzerland
- 2 Department of Fundamental Microbiology, University of Lausanne, Switzerland

Hard-to-treat bacteria pose a public health problem, particularly in critical care settings, where extremely vulnerable patients are being treated. Multidrug-resistant (MDR) pathogens dramatically increase the adverse impact of infections in Intensive Care Units with an important rise in morbidity, mortality, and healthcare costs.

"ESKAPE" pathogens are among the most problematic bacteria. They challenge efficacy of available antibiotics and rapid spread of resistance threatens medical progress and drifts us into a post-antibiotic era where common infections will become orphans. In parallel, the number of antimicrobials under development remains limited and progression into the clinical market is slow, highlighting the urgency for innovative approaches to control MDR infections.

Bacterial viruses - bacteriophages - represent such an alternative. Soon after their discovery, lytic bacteriophages were used therapeutically. The first success of the so-called "phage therapy" has been reported in 1919 when D'Hérelle cured five hospitalized children from severe dysentery. Between 1920 and 1970, phage preparations were used to treat numerous diseases caused by various bacterial species. Even in Lausanne in 1949 Feihl reported 83% success rate in 77 patients suffering from Staphylococcus aureus infections.

Discovery and massive production of penicillin prompted disappearance of phage therapy in the Occident despite proven efficacy. However, it did not totally disappear. Georgia, Russia, and Poland continue nowadays to produce therapeutic phage preparations and thousands of patients are still successfully treated with only very few side effects reported.

In response to the constant increase of bacterial resistance to antibiotics, phage therapy currently encounters a high renew interest in the Western World. In this context, the EU recently granted Phagoburn, the first multi-centric phase I/II clinical trial aimed at evaluating phage therapy in burn patients infected with *Escherichia coli* or *Pseudomonas aeruginosa* according to the current ethical and regulatory standards.

MOULDS IN THE BUILT ENVIRONMENT

J. C. Frisvad 1

1 Danmarks Tekniske Universitet, Lingby, Denmark

Many molds can be found in the built environment. Some of these molds are clearly associated to indoor surfaces, furniture etc. and can actually grow on such material and cause disease with the known sick building syndrome diagnostics. However when examining indoor air or dust from houses it is common to find other kind of molds that do usually not thrive on any wall or other indoor material. These include food-borne fungi, epiphytic fungi and soil fungi. Among the food-borne fungi found are *Penicillum allii* (from garlic), *P. glabrum* (from onions), *P. italicum* and *P. digitatum* (from citrus fruits), *P. expansum* (from rotting apples), *P. camemberti*, *P. commune* and *P. roqueforti* (from cheeses) and *P. nalgiovense* from mold-salami. These do not grow on inddor material. Furthermore soil fungi such as *P. janczewskii* and *P. canescens* indicate that soild has been sampled rather that true indoor-associated fungi. However, the real indoor air fungi are first of all *Phoma* and *Cladosporium* spp (in bathrooms), *Aspergillus reber* and other members of *Aspergillus* section *Versicolores*, *P. rubens* and *P. chrysogenum* and several others. Species in important genera of indoor molds have been thoroughly revised recently and examples will be given on which changes have been made and how to identify all these fungi.

MICROORGANISMS FOR SAFEGUARDING BUILT HERITAGE

E. Joseph^{1,2}, M. Albini¹, L. Comensoli¹, W. Kooli¹, L. Mathys¹, C. Jacquet¹, M. Rebord¹, Y. Braendle¹, P. Junier¹

While often considered as harmful for built heritage, microorganisms can also be used for its safeguarding. Indeed, there is a growing interest for the development of biological technologies that are environmental friendly (close to ambient temperature and pressure, neutral pH) and do not require the use of toxic materials. A real progress could be expected in terms of durability, effectiveness and toxicity. Over the last decades, the development of biological methods and materials became a significant alternative for the preservation of ancient built heritage. In particular, microbial mechanisms are exploited aiming to consolidate, clean, stabilize or even protect surfaces of cultural items. This contribution is intended as a review of the current biotechnological applications in the field of cultural heritage. Different examples related to the preservation of stone, metal and wood artefacts will be presented.

¹ Laboratory of Microbiology, Institute of Biology, University of Neuchâtel, Switzerland

² Haute Ecole Arc Conservation-Restauration, Neuchâtel, Switzerland

ECOLOGY OF MOSQUITO IMMUNITY AND RESISTANCE TO MALARIA

J. Koella 1

1 University of Neuchâtel, Switzerland

Insect immunity is a complex system. With simplifying ideas we have made considerable progress in understanding it. One simplification is to think of it as a problem of how to move from (host) genetic information to the phenotype (i.e. resistance), with immune mechanisms supplying the pathway. This approach one is concerned with finding the genetic basis and the mechanisms underlying resistance. An alternative one, sometimes called ecological immunity, is to consider the immune response as a black (or at least grey) box, and to focus on the ecological interactions between host, parasite and environment and and on the trade-offs between resistance and other important traits. Using the example of malaria-mosquito interactions, I will give examples for the two approaches and will provide links between them where possible. I will conclude by suggesting that adequately addressing the complexity of insect immunity will require a novel approach that integrates several different levels – interactions among genes, among traits, between host and parasite, and between individuals and the environment.

Vector-borne diseases in Switzerland: new challenges for veterinary diagnostics

A. Vöatlin 1

₁ FDHA, FSVO, IVI, Diagnostics, Sensemattstrasse 293, Mittelhäusern

Arboviruses represent a group of viruses, which are transmitted by arthropods and originate from different virus families. Infections with these pathogens can lead to diseases in various species and therefore represent a major threat to animals and humans. The significance of these infections is demonstrated by the increasing number of scientific publications, the presence of this issue on websites from various animal and public health organisations such as Cdc, Ecdc or OIE and most important by the elevated number of reported disease outbreaks. Many factors including the existence of susceptible hosts, the presence of the vector as well as environmental conditions play a major role for the development of diseases due to arboviral infections.

Recent outbreaks of vector-borne diseases in Switzerland concerning the field of veterinary medicine included infections with the Bluetongue virus and the Schmallenberg virus.

What are the challenges for diagnostics with regard to such emerging diseases? What did we learn from these former outbreaks and how do we prepare us for future outbreaks?

TICK-BORNE PATHOGENS IN SWITZERLAND

R. Gäumann 1

1 Division of Biology, SPIEZ LABORATORY, Spiez

¹Division of Biology, SPIEZ LABORATORY, Spiez

²National Reference Centre for tick-transmitted diseases

Ticks and tick-transmitted diseases are increasingly recognized as an important public health issue by experts as well as the general public. The predominant tick species in Switzerland, transmitting the vast majority of pathogens, is *Ixodes ricinus*. The most significant tick-transmitted diseases in our country are *Borrelia burgdorferi sensu lato* and the European subtype tick-borne encephalitis virus (TBEV). An estimated 7'000 – 12'000 cases of lyme borreliosis and 100 – 170 cases of tick-borne encephalitis occur each year. From local and national tick surveillance studies, the tick carrier rates with these pathogens are known to be about 0.5 % for TBEV (in so-called natural foci), and up to 50 % for *B. burgdorferi s.l.* In addition to these well-established agents, more unfamiliar or emerging pathogens such as *Anaplasma phagocytophilum*, *Rickettsia helvetica*, *Rickettsia monacensis*, *Candidatus* Neoehrlichia mikurensis, *Borrelia miyamotoi*, *Francisella tularensis* or members of the genus *Babesia* are increasingly detected in ticks. This presentation will give a brief overview on established and emerging tick-borne pathogens in Switzerland.

HOW FUNGAL ROOT MUTUALISTIC ENDOPHYTES SUPPRESS HOST IMMUNITY

Alga Zuccaro

Organismic Interactions, Max Planck Institute Marburg, Marburg, Germany and University of Cologne, Botanical Institute, Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany.

Root endophytism and mycorrhizal associations are complex, derived traits in fungi that shape plant physiology. Sebacinales (Hymenomycetes, Basidiomycetes) display highly diverse interactions with plants. While basal lineages are root endophytes loosely colonizing root cells and/or have saprotrophic abilities, derived clades are biotrophs forming mycorrhizal associations, sometimes uncultivable. Sebacinales thus document the transition from saprotrophy to endophytism and to mycorrhizal nutrition within one family.

Genomic traits associated with this transition are keys to the understanding of fungal root symbioses and will be discussed together with recent advances in effector research in the sebacinoid root endophyte Piriformospora indica. A special focus will be given on functional insights into the molecular mechanisms of symbioses with the monocot host plant barley and the dicot model plant Arabidopsis thaliana.

SINGLE CELL GENOMICS AND SMALL-SCALE METAGENOMICS TO INVESTIGATE MICROBIAL EVOLUTION

M. Tamminen 1

1 ETHZ, Zürich, Switzerland

Single cell genomics has in recent years led to important discoveries in multiple areas of biology. For instance, in environmental microbiology single cell genomics provides a cultivation-free platform that complements higher-throughput but lower-resolution metagenomic sequencing. Current protocols used to prepare individual microbial genomes for sequencing involve DNA amplification and sequencing library preparation, both of which are infrastructure-intensive, laborious and expensive procedures. Thus, the amount of single cell genome sequences that can reasonably be produced from a sample is in the order of hundreds of cells, after which sequencing costs and manual labor requirements make the effort unfeasible. I discuss here new approaches that aim to increase the throughput of single cell genomics by orders of magnitude while considerably reducing the costs per genome. This involves techniques that carry out the amplification and library preparation reactions inside emulsion compartments, combining ideas from molecular biology, microfluidics and combinatorial chemistry. These techniques are equally applicable to single cells as well as microscopic cell communities and aim to combine the resolution of single cell genomics with the throughput of metagenomics. The increased resolution is providing novel insights into microbial evolution, horizontal gene transfer, spatial structuring of microbial communities and host-microbe interactions.

THE EVOLUTION OF SPATIAL SELF-ORGANIZATION IN EXPANDING POPULATIONS OF MICROORGANISMS

D. R. Johnson 1

1 Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland

The fate of an individual cell rarely depends on its genotype and abiotic environment alone, but instead also depends on its spatial positioning with respect to other cells. Spatial positioning determines whether different cells interact with each other (e.g., they may have positive or negative effects on each other), and how these interactions lead to emergent community behaviors. As cells expand their range from an initial colonization event, random drift has a segregating effect that increases the spatial distance between different cell types. Range expansions should thus disfavor the evolution and maintenance of interactions. Here we investigate how interdependencies between different cell types can mitigate these effects and promote the evolution of spatial self-organizing patterns that maintain close spatial proximity between different cell types.

To address our objective, we constructed a synthetic microbial ecosystem consisting of two cell types that cross-feed a metabolic intermediate. One cell type consumes a parent substrate into an intermediate, and another cell type then consumes the intermediate. We manipulated the interaction between the two cell types by changing the reactivity of the intermediate. The cell types form a commensalistic interaction when the intermediate is non-toxic and form a mutualistic interaction when the intermediate is toxic. We could therefore experimentally manipulate the strength of interdependence between the two cell types and measure the consequences on evolving patterns of spatial self-organization.

We found that the expanding populations rapidly and repeatedly evolved spatial self-organization that decreased the distance between the two cell types, thus overcoming the segregating effects of neutral drift. We present experimental results and mathematical simulations that point towards specific underlying processes that cause these patterns. We also argue that our results may be generalizable, and are therefore of potentially broad interest for explaining patterns of spatial self-organization in the natural environment.

Using microscopic algae to understand community assembly

P. Venail 1

1 Institut F.-A. Forel, Université de Genève

The competition-relatedness hypothesis (CRH) predicts that the strength of competition is the strongest among closely related organisms and decreases as these become less related. This hypothesis is based on the assumption that common ancestry causes close relatives to share biological traits that lead to greater ecological similarity. Although intuitively appealing, the extent to which phylogeny can predict competition and co-occurrence among species has only recently been rigorously tested, with mixed results. When studies have failed to support the CRH, critics have pointed out at least three limitations: (i) the use of data poor phylogenies that provide inaccurate estimates of relatedness, (ii) the use of inappropriate statistical models that fail to detect relationships between relatedness and ecological interactions, and (iii) overly simplified laboratory conditions that fail to allow eco-evolutionary relationships to emerge. We addressed these limitations and find they do not explain why evolutionary relatedness fails to predict the strength of ecological interactions or probabilities of coexistence among freshwater green algae. First, we construct a new data-rich, transcriptome-based phylogeny of common freshwater green algae that are commonly cultured and used for laboratory experiments. Using this new phylogeny, we re-analyze ecological data from three laboratory experiments. After accounting for the possibility of nonlinearities and heterogeneity of variances across levels of relatedness, we find no relationship between phylogenetic distance and ecological traits. In addition, we show that communities of North American green algae are randomly composed with respect to their evolutionary relationships in 99% of 1077 lakes spanning the continental United States. Together, these analyses result in one of the most comprehensive case studies of how evolutionary history influences species interactions and community assembly in both natural and experimental systems. Our results challenge the generality of the CRH and suggest it may be time to re-evaluate the validity and assumptions of this hypothesis.

The Secret Life of Integrative and Conjugative Elements, Agents of Open Source Evolution

S. Sulser ¹, F. Delavat ¹, A. Vucicevic ¹, V. Sentchilo ¹, J.R. van der Meer ¹

1 Department of Fundamental Microbiology, University of Lausanne, 1015-Lausanne

Recent genome sequencing projects have dramatically illustrated the extent to which bacterial genomes are infested with horizontally transferred DNA (HT-DNA). Based on their assumed modes of transfer and key genetic elements, various types of HT-DNA have been recognized. The latest coherent new group "on the block" being Integrative and Conjugative Elements or, in short, ICE. ICE integrate into the host genome at one or more specific sites, where they can remain largely silent and are replicated concurrent with the replication cycle of the host. At low frequencies, between 10e-7 and 10e-2 per cell (depending on the system), ICE can excise through site-specific recombination, forming an "excised" circular ICE molecule that can be conjugated to a new recipient cell. In the recipient cell, the ICE will again integrate. Sequence comparisons of ICE have illustrated their large capacity as agents of open source evolution, carrying within their boundaries a changing repertoire of genes with potential adaptive benefit. Distinct ICE families exist, which have spread to overlapping but also different host species.

So what is so secret about the life of ICE? Recent efforts in our group have started to uncover the highly intricate regulatory mechanisms that control the switch between the integrated and excised state of the ICE. In line with the low observed frequencies of ICE transfer, we could show that actually only few cells in a population become "transfer competent", a (primitive) developmental process that turns the host cell into a dedicated optimal ICE transfer machine. Transfer competence is a bistable process for individual cells, from which, once initiated, they cannot escape; but which has been selected in an evolutionary and ecological balance between minimizing host damage and optimizing ICE transfer.

Literature:

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001

COAGULASE POSITIVE- AND METHICILLIN-RESISTANT COAGULASE NEGATIVE- STAPHYLOCOCCAL CONCURRENT CARRIAGE OF AMONG OWNERS AND THEIR PETS

E. Gómez-Sanz 1, S. Ceballos 2, B. Duffy 3, M. Zarazaga 2, C. Torres 2

- 1 Zurich University of Applied Sciences,, Wädenswil, Switzerland
- 2 University of La Rioja, Logroño, Spain
- 3 Zurich University of Applied Sciences, Wädenswil, Switzerland

Staphylococcus aureus (SA) and Staphylococcus pseudintermedius (SP) are nasal colonizers and opportunistic pathogens of humans and dogs, respectively. Interspecies transmission (IT) of SA/SP between owners and in-contact pets has been described. Concomitant carriage of SA/SP and methicillin-resistant coagulase-negative staphylococci (MRCoNS) might represent a risk for acquisition of antimicrobial resistance determinants.

In a former study, all nasal coagulase-positive staphylococci (CoPS) recovered from 133 individuals (67 owners, 66 pets –dogs, cats) at one sampling (T0) were studied (Gómez-Sanz et al., 36:83–94, 2013 CIMID). Here, MRCoNS from T0 were recovered and characterized. Co-carriage of MRCoNS and the CoPS (36 SA, 18 SP) present was investigated to address (i) whether owner-pet co-inhabitance may favour MRCoNS and SA/SP co-carriage and (ii) any possible transference of the β -lactams resistance determinant (mecA) between both bacterial groups.

Thirty-one MRCoNS were recovered (28.4% humans, 18.2% dogs). A total of 56.7% of owners and 45.5% of pets were positive for MRCoNS and/or CoPS. MRCoNS carriage as single species was similar in owners and pets (11.9% and 12.1%). However, coexistence of MRCoNS and SA was more common among owners (13.4% versus 3.0%). Both populations predominantly carried only CoPS (>50% of positive individuals) followed by (i) co-carriage of MRCoNS+CoPS in owners (29.0%) and (ii) carriage of MRCoNS only in pets (26.7%).

Two individuals carried more than one concomitant MRCoNS and/or CoPS, which were involved in cases of direct IT. Likewise, most individuals positive for more than one bacterial species originated from households with cases of IT. Two owners and their co-inhabitant pet carried methicillin-resistant- SA, CoNS and SP, respectively, but different genetic backgrounds enclosing the *mecA* gene were observed.

Concurrent carriage of MRCoNS and SA/SP seems common. Owners seem to be more prone to carry MRCoNS in concomitance with SA. Co-inhabitance with pets seems to increase the possibility to co-carry nasal staphylococci. Transmission potential of β-lactams resistance from MRCoNS to CoPS appears low.

O02

A NEW SURFACE PROTEIN POSSIBLY INVOLVED IN BOVINE ADAPTATION OF STAPHYLOCOCCUS AUREUS CC8 STRAINS ORIGINATING FROM HUMAN

F. Oechslin 1, S. McCallin 1, C. Menzi 1, P. Moreillon 1, G. Resch 1

1 Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland

Staphylococcus aureus is a human and animal opportunistic pathogen that can produce a variety of diseases. In humans, the major reservoir is represented by healthy carriers, who account for up to 30% of the population. Carriage has also been reported in numerous animal species. However, while S. aureus is quite ubiquitous in terms of host species, different animals tend to harbor different lineages. Several studies suggest that critical modulators of this apparent host specificity may be mobile genetic elements, gene decay, or adaptive evolution of surface proteins.

We recently described bovine-adapted *S. aureus* strains of the Clonal Complex 8 (CC8) that were highly suspected to originate from humans. We further compared the genetic makeup of collections of human and bovine CC8 strains using a comparative, whole-genome sequencing approach in order to identify new factors that could correlate with a human to bovine jump. One of these factors is potentially a new staphylococcal cassette chromosome (SCC), which was present in 38/39 of unrelated bovine CC8 isolates. Interestingly, this SCC element, which was never found in human CC8 isolates, harbors a gene encoding a previously unknown LPXTG-protein (referred to as LPXTG-bov).

The surface-attached nature of LPXTG-bov was confirmed by cloning and expression in *Lactococcus lactis*, in which it was detected both by surface trypsin shaving followed by proteomic detection and by ELISA. Lactococci expressing LPXTG-bov also demonstrated a striking star-like colony morphology that was lost after the loss of the gene. Finally, PCR-amplification of LPXTG-bov genes from several unrelated bovine CC8 isolates showed substantial inter-isolate sequence length variation related to variation in internal repeats and reminiscent of sequence length variations in the spa and spa-clfB genes.

We propose that the new LPXTG-bov is not only specific for the bovine host, but that it was established long ago enough to undergo micro-evolutionary changes, either in response to functional needs or by stochastic genetic rearrangements.

O03

Comparison of two rapid commercial molecular tests for detection of the most common carbapenemases: Xpert® Carba-R and eazyplex® SuperBug complete B

V. Hinic ¹, C. Straub ¹, J. Ziegler ¹, D. Goldenberger ¹, R. Frei ¹

₁ Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland

Objectives: Rapid and reliable detection of carbapenemases is an imperative for clinical microbiology laboratories, but is often challenging. Xpert® Carba-R, developed for GeneXpert® platform (Cepheid, USA), detects KPC, NDM, VIM, IMP-1 and OXA-48 in gram-negative rods directly from rectal swabs. eazyplex® SuperBug complete B test (Amplex Biosystems, Germany) developed for Genie® II (Optigene, UK) instrument is based on isothermal amplification technique. It detects KPC, NDM, VIM, OXA-48, OXA-181, OXA-23 and OXA-40 directly from rectal swabs and bacterial cultures. We evaluated these two rapid commercial molecular tests on culture isolates.

Materials and Methods: The protocol for Xpert® was adapted for testing bacterial isolates. The eazyplex® test was performed according to the manufacturer's instructions. 45 well characterized carbapenemase-producing clinical strains from our strain collection were tested: 38 *Enterobacteriaceae* (10 KPC, 10 NDM, 7 VIM, 8 OXA-48 "sensu stricto", 1 OXA-181, 2 non-carbapenemase-producing strains were analyzed including ESBL and AmpC.

Results: Both assays detected all KPC, NDM, VIM and OXA-48 "sensu stricto" in *Enterobacteriaceae* and all VIMand NDM-producing *P. aeruginosa*. Xpert® did not detect two OXA-181 (OXA-48-like) strains, which on the other hand were detected by eazyplex®. One *P. aeruginosa* IMP-18-producing strain was found negative by both assays. The hands-on time for both assays was 2-3 min. The turnaround time (TAT) for Xpert® and eazyplex® was 60 and 9-16 min, respectively.

Conclusions: Both assays were very reliable for rapid detection of the carbapenemases that are covered by the assay. While Xpert® did not detect all carbapenemases of the evolving OXA-48 family (such as OXA-181) and other members of the IMP family except IMP-1, eazyplex® does not cover any IMP-carbapenemases. While the TAT of both tests is very short, results of eazyplex® were available already after 9-16 min due to the rapid isothermal amplification technology.

004

ADDED VALUE OF THE MOLECULAR POCT GENEXPERT MTB/RIF VERSUS CONVENTIONAL MICROSCOPY IN THE EVALUATION OF THE RISK OF TUBERCULOSIS TRANSMISSION IN A LOW-ENDEMIC COUNTRY

O. Opota 1, L. Senn 2, G. Greub 3, K. Jaton 1

- 1 Institute of Microbiology, University Hospital, Lausanne, Switzerland
- 2 Preventive Medicine and Infect. Diseases, University Hospital, Lausanne, Switzerland
- 3 Microbiology and Infectious Diseases Service, University Hospital, Lausanne, Switzerland

Airborne precautions are required for patients suspected for tuberculosis. The isolation is maintained until 3 serially collected acid-fast bacilli (AFB) sputum smears are negative, which has a great impact on patient flow management in the hospital. The molecular POCT GeneXpert MTB/RIF designed for the rapid detection of *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin is more sensitive than microscopy and gives a semi-quantitative result within 2 hours. We evaluated the possibility to replace the result of the AFB in the first sputum smear by the GeneXpert for the evaluation of the transmission risk of MTB.

The performance of the GeneXpert was compared with the results of AFB microscopy examination for respiratory specimens (n=200) collected from May 2010 to March 2014 in our 1,000-bed tertiary-care university hospital negative or positive for MTB (culture as gold-standard).

Compared with AFB microscopy, sensitivity of the GeneXpert was 100% (42/42 positives results) and negative predictive value was 100% (134/134 negative results). Twenty-four negative results by AFB microscopy were GeneXpert positive, suggesting a higher performance of GeneXpert. 100% (24/24) of the samples positive with the GeneXpert at a medium or high quantity were AFB positive, when 54% (14/26) of the samples positive with the GeneXpert at a low quantity were found AFB positive and 25% (4/16) of the samples positive with the GeneXpert at a very low quantity were found AFB positive.

Thus, the GeneXpert MTB/Rif could make the decision of specific isolation care more accurate and more rapid than AFB sputum examination with major impact on preventing tuberculosis transmission and on patient flow management in the hospital as: 1) negative GeneXpert result corresponds to a low risk of transmission and 2) positive GeneXpert result at medium or high quantity corresponds to a high risk of transmission. For patients with positive GeneXpert at low or very-low quantity an evaluation of the clinical chart would help to decide whether airborne isolation is needed.

O05

CORONAVIRUS - HOST INTERACTIONS IN ORGANOTYPIC HUMAN AIRWAY CULTURES

HR. Jonsdottir ¹, R. Dijkman ¹, R. Rodriguez ², OJ. Hamming ³, R. Hartmann ³, V. Thiel ¹

- 1 Institute of Virology and Immunology, Bern, Switzerland
- 2 Department of Pathology, Kantonsspital St.Gallen, St.Gallen, Switzerland
- 3 Centre for Structural Biology, Dept. of Molecular Biology and Genetics, Univ. of Aarhus

Human airway epithelium represents the entry port of respiratory viruses. Advances in isolation and cultivation of primary human bronchial cells have enabled comprehensive studies of these viruses in air-liquid-interface cultures (HAEs) that morphologically resemble the conducting airways in vivo. After differentiation these organotypic cultures contain different cell types such as basal, ciliated and goblet cells. We have shown HAEs to be a unique platform to study virus-host interactions in natural target cells. There we have focused on the human coronaviruses (HCoVs), using the commonly circulating HCoV-229E. This virus effectively infects HAEs and can persist for weeks. No pronounced inflammatory cytokine or interferon response is observed in the system following HCoV-229E infection although HAEs generally respond to both type I and III interferon, indicated by increased expression of various interferon stimulated genes (i.e. MX1, 2'-5'OAS) and various RNA sensors (i.e MDA5, TLR3) post-induction. HCoV-229E replication can also be effectively reduced by interferon treatment. To shed further light on the molecular interactions during HCoV infection the HAEs should be made amenable to genetic modification to allow for shRNA-mediated knock-down of specific host genes. To accomplish this, an efficient method of primary cell transduction has been developed and undifferentiated bronchial cells were readily transduced in suspension with high efficacy. Transgene expression remains stable during differentiation and cellular composition of transgenic cultures remains intact. Since primary cells have a finite life-span, we increased their proliferation capacity, while preserving their differentiation potential, by inhibiting Rho-associated protein kinase. This will enable us to sort transduced primary bronchial cells to near homogeneity to generate fully transgenic HAEs. Genetically modified HAEs will facilitate detailed studies of host innate immune responses to HCoV infection. Furthermore, this system can be adapted to animal airway cultures to study host-specific responses to viral infection and to elucidate basic parameters of zoonotic transmission.

O06

CITRONELLAVIRUS - DIGGING INTO PITHOVIRIDAE DIVERSITY

- C. Bertelli* 1, L. Mueller* 1, V. Thomas 2, T. Pillonel 1, N. Jacquier 1, G. Greub 1
- 1 Institute of Microbiology, University Hospital Centre and University of Lausanne, Lausanne
- 2 Enterome Bioscience, Paris, France

Amoeba-infecting viruses raised the interest of scientists due to their uncommon genome size, up to 2.8 Mb, and their very large particles, around 1 µm, thus causing them to be retained in bacterial filters (0.22 µm) widely used to recover viruses. To date, amoeba-infecting viruses representative of 4 families have been isolated and sequenced. The presence of proteins for transcription and translation challenged the viral dogma of a complete host cell dependence for translation. Viruses belonging to the families Mimiviridae and Marseilleviridae present an icosahedral capsid. Very recently, the discovery of Pandoravirus, the largest known giant viruses, and of the 30'000 year-old Pithovirus revealed new viral particle morphologies presenting amphora-like shapes. Here we report the discovery and the characterization of a new virus, isolated from a Seine river (France) water sample, that we named Citronellavirus. This newly identified microorganism exhibits an AT-rich genome of 575'161 bp and an amphora-shaped particle of about 0.80 µm long. Among the numerous host cells tested, the viral replication only occurred in A. castellanii leading to host cell lysis within 24 hours. Such replication, taking place in the cytoplasm, is reminiscent of Mimiviridae. Citronellavirus and Pithovirus share a similar amphora-shaped particle with an apical plug and are phylogenetically closely related, both belonging to a new family of Pithoviridae. However, Citronellavirus genome is essentially devoid of the numerous repeats harboured by Pithovirus. The genetic relatedness of Pithovirus and Citronellavirus denote the widespread distribution of this family of viruses, not only in ancient samples but also in actual water environments.

^{*}Equally contributed to this work

O07

THE SWEETEST TRICK: O-POLYSACCHARIDES PROTECT PLANT-BENEFICIAL PSEUDOMONADS AGAINST ANTIMICROBIAL PEPTIDES DURING INSECT INFECTION

P. Kupferschmied ¹, T. Chai ¹, P. Flury ², T.H.M. Smits ³, M. Maurhofer ², C. Keel ¹

- 1 Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland
- ² Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland
- 3 Institute for Natural Resources Sciences, ZHAW, Wädenswil, Switzerland

Some plant-beneficial Pseudomonas species display insect pathogenicity in addition to their ability of protecting roots from phytopathogenic fungi. Although the bacterial surface is the site where direct microbe-host interactions take place, its characteristics have barely been studied in these pseudomonads. Lipopolysaccharide (LPS) is the major constituent of the outer membrane of Gram-negative bacteria and generally consists of lipid A, core oligosaccharide and O-antigenic polysaccharide (O-antigen). The O-antigen is the exposed part of LPS and was shown to contribute to virulence in pathogenic bacteria and to symbiosis of rhizobia with legumes. We explored the genetic basis of O-antigen biosynthesis in recently sequenced, insect-pathogenic pseudomonads by a combined bioinformatic and genetic approach. Gene clusters that were identified as putative O-polysaccharide biosynthesis loci in these microorganisms were individually mutated in two model strains and their involvement in LPS decoration was assessed by LPS extraction and detection. Insecticidal pseudomonads were found to produce at least two forms of O-antigen simultaneously. Mutant strains lacking these sugar chains were significantly attenuated for virulence in a Galleria mellonella infection model. Cationic antimicrobial peptides play a central role in the immune system of insects. We found that many insect-pathogenic Pseudomonas strains were highly resistant to a model antimicrobial peptide while non-insecticidal strains were susceptible. The potential of causing disease in insects might thus be correlated with the resistance to antimicrobial peptides in plant-beneficial pseudomonads. In Pseudomonas protegens CHA0, high resistance to these antimicrobial compounds was dependent on the biosynthesis of a short O-polysaccharide in addition to modification of lipid A with aminoarabinose. This study provided the necessary basis for future research on the role, regulation and evolution of O-antigens in plant-beneficial pseudomonads with insect-pathogenic properties and represents the first step in the investigation of how cell surface constituents modulate host interactions in these bacteria.

KILLING FOR DNA: THE TYPE VI SECRETION SYSTEM OF VIBRIO CHOLERAE FOSTERS HORIZONTAL GENE TRANSFER

M. Blokesch 1

1 School of Life Sciences, Swiss Federal Institute of Technology Lausanne, Switzerland

Vibrio cholerae, the causative agent of cholera, is considered an important model organism for studying infectious diseases and for elucidating virulence regulation. In the latter context, the involvement of quorum sensing (QS) and the type VI secretion system (T6SS) have been extensively studied even though the T6SS is been considered to be "silent" in pandemic V. cholerae strains tested under laboratory conditions. Much less is known about the bacterium's lifestyle in its natural environment, where it often associates with chitinous surfaces of small crustaceans. Upon growth on chitin, V. cholerae enters the state of natural competence for transformation [1], which enables the bacterium to take up free DNA from the environment. We initially investigated the regulatory network driving natural competence and transformation [2,3] and more recently also the mechanistic aspects of the DNA uptake machinery [4,5].

Here, we show that the chitin and QS-dependent competence regulon of pandemic *V. cholerae* strains includes the T6SS-encoding gene cluster and that the T6SS contributes to enhanced horizontal gene transfer by means of natural transformation. Moreover, we used live cell imaging to visualize the competence-induced and T6SS-mediated killing of prey cells and the subsequent uptake of their DNA by the competent predator cell. Our results indicate that the competence-mediated induction of the T6SS enhances horizontal gene transfer by deliberate killing of neighboring non-immune cells and absorption up their DNA [6].

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O09

Cell cycle constraints on the excision of the Caulobacter crescentus NA1000 mobile genetic element

G. Panis 1, N. Jaunin 1, D. Martins 1, PH. Viollier 1

1 Faculty of Medicine UniGe, Geneva, Switzerland

Caulobacter crescentus NA1000 houses a unique Mobile Genetic Element (MGE) on his chromosome. This MGE is integrated at the Ser tRNA locus and allows to physically separate non-capsulated swarmer and capsulated stalked cells by density gradient (i.e. synchronizability). Indeed, many of the genes contained within this MGE have predicted functions in capsular carbohydrate metabolism and biosynthesis.

Integration and excision MGE are mediated by regulated Site-Specific Recombination (SSR) catalyzed by tyrosine or serine recombinases (also called integrases). Despite the essentially catalytic role of the integrase in both integrative and excisive recombination reactions, a second accessory protein called Recombination Directionality Factor (RDF) is required to direct the reaction towards excision and to prevent re-integration of the MGE. During this work, we showed in vivo that the integrase (Int) protein encoded on the MGE is the dedicated recombinase used for both integrative and excisive reactions. We also demonstrated that overproduction of the encoded RDF protein allowed the excision of the MGE from the bacterial chromosome. However, and following this excision test. it was impossible to obtain a viable strain that had lost this MGE. We demonstrated that this MGE was maintained in C. crescentus thanks to the presence of a functional Toxin-Antitoxin (TA) system encoded on it. Concerning the SSR mechanism of this MGE, we mapped the Int and RDF binding sites on both attL and attR regions. The number and organization of identified recombination sites suggest that the architecture of the NA1000 MGE intasome is related to the intasome architecture of the P4 bacteriophage family. Regarding the int gene regulation, we demonstrated that the Antitoxin binding sites, which overlap its own promoter and the int promoter region, maintained a basal level of int gene transcription all along the cell cycle. Integrase protein levels are limited in the cell by ClpXP, a proteolysis system active in the stalked cell compartment. Thus both level of controls of Int, transcriptional and post-translational, restrict Int to the Sw cell stage of the C. crescentus cell cycle. In agreement, we demonstrated that after Mitomycin C treatment (inducer of the SOS response and many MGEs) this MGE could only be excised in the Sw cells.

A microfluidic chip to measure bacterial chemotaxis

C. Roggo 1, J. R. van der Meer 1

1 Department of Fundamental Microbiology, University of Lausanne, Switzerland

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. In order to measure chemotaxis quantitatively at the minute scale, we used microfluidic chips.

Here we pursue the design of microfluidic chips in which a gradient of attractant can be generated which enables measurement of bacterial chemotaxis. The principle of the design is based on filter channels with a height of 700 nm, which allow the diffusion of small chemical molecules but prevent the passage of the bacterial cells. The chips are composed of an inner channel, where cells are introduced, connected via the filters to two parallel side channels, in which attractant solution and buffer are flowed in order to produce a gradient. The attractant molecules diffuse between the source and the sink channel, which creates a stable gradient perpendicular to the flow in the inner channel

Motile cells detect the gradient and swim toward the highest concentration of the attractant, which leads to an accumulation of cells on one side of the channel. The chemotaxis response of fluorescent bacteria is observed by microscopy and the distribution of the cells across the channel is determined.

We show that chemical gradients can be produced in a microfluidic design consisting of three parallel channels and that *Escherichia coli* cells introduced in the middle channel experience chemo-attraction toward different molecules and concentrations. Moreover, this flow-based microfluidic chips offers the possibility of continuous measurements with flow of different samples and reuse of cells.

WHAT TRIGGERS SPORULATION IN SERRATIA UREILYTICA STR. LR5/4?

S. Filippidou ¹, W. Kooli ¹, T. Junier ¹, N. Jeanneret ¹, P. Junier ¹

1 Laboratory of Microbiology, University of Neuchatel, Switzerland

Spore or spore-like structures are only found in four bacterial phyla: Actinobacteria, Cyanobacteria, Proteobacteria and Firmicutes. These structures provide resistance to adverse conditions. The ability to form spores is not, however, a widely spread characteristic and it is restricted to only some orders within those phyla. For example, amongst Proteobacteria, solely 5-proteobacteria can produce spore-like fruiting bodies, or so we knew. A novel y-proteobacterium, Serratia ureilytica str. Lr5/4, was found to produce spores that not only resemble structurally to those produced by endospore-forming Firmicutes, but also provide heat-resistance.

Sporulation is supposed to be triggered, in most cases, by nutrient starvation. Upon the discovery of a new sporulating bacterium, our aim was to investigate what triggers sporulation, whether its spores are resistant to various extreme conditions and finally, what the genetic imprints of the sporulation triggering factors and the resistance are.

In order to address these questions, *S. ureilytica* Lr5/4 vegetative cells were exposed to nutrient starvation, heat and freezing shocks, high salinity, desiccation and UV radiation. In the same environmental conditions, spores were tested for their resistance. These tests were performed in comparison to *Bacillus* sp. for which sporulation triggers and spore resistance are well studied. Moreover, sequencing and annotation of its full genome was performed in order to describe the featured genes devoted to sporulation and resistance. Comparative genomics were used to investigate similar as well as differentiating genomic features of sporulation between *S. ureilytica* Lr5/4 and all sporulating phyla.

The present study demonstrates a novel mechanism for the formation of the described spores of *S. ureilytica* Lr5/4. Moreover, it provides insights in under which environmental conditions survival strategies are expressed.

RAPID AND SIMPLE SHIGELLA AND E. COLI DIFFERENTIATION BY MALDI TOF USING THE VITEK MS PLATFORM

M. Arsac¹, V. Monnin², P. Bourne-Branchu², D. Pincus³, H. Dwivedi³, G. Devulder³, G. Durand², A. van Belkum², V. Girard²

- Biomathematics department, bioMérieux, Marcy and R&D microbiology, bioMérieux,
- La Balme les Grottes, France
- ² R&D microbiology, bioMérieux Inc, Hazelwood, US

Shigella species and E.coli are very closely related and their differentiation is needed from a clinical and veterinary perspective. Shigella species are always considered pathogenic; E.coli can be either pathogenic or part of the commensal flora. Shigella spp and E.coli are to date difficult to distinguish using MALDI-TOF MS. Biochemical and serological methods are conventionally used and their differentiation remains a diagnostic challenge. The objective of this study was to set up a simple MALDI TOF MS method that could be implemented routinely and would allow distinguishing these closely related species.

106 well characterized strains of *Shigella* and *E. coli* including the pathogenic serovar 0157 where used to acquire 400 MALDI TOF MS spectra using a simple extraction procedure. After processing of the spectra, a predictive identification model was built and discriminative peaks were identified. Data reduction was performed using multi-dimensional scaling (MDS).

Cross-validation and data exploration using MDS showed that 100% of *E. coli* 0157 strains were correctly identified at the serogroup level. Non-0157 *E. coli* and the four *Shigella* species (S. *boydi*, *S. dysenteriae*, *S. flexneri and S. sonnei*) were identified to the species level in 82%, 89%, 90%, 100% and 95% of the cases, respectively. Several discriminative peaks allow the species differentiation. The validation of the prediction model on an external dataset of 62 Shiga-toxin producing *E. coli* (STEC) strains from different serogroups (excluding 0157) showed that 100% of the strains could be identified to the species level. However, identification at the serogroup level was not possible.

Closely related *Shigella* spp and *E. coli* can be distinguished at the species level using MALDI TOF MS. It was not possible to distinguish serogroups, with 0157 being the only exception. This finding could be of great importance in the management of outbreaks and in epidemiological and surveillance studies.

The outer membrane protein PorA of Campylobacter as a potential vaccine target

S. Kittl 1, P. Kuhnert 1

1 Institute of Vet. Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Campylobacter is the major zoonotic pathogen in industrialized countries, with chicken constituting the main reservoir host. Campylobacteriosis is characterized by mild to severe gastroenteritis and can entail severe complications such as Guillain-Barré syndrome, reactive arthritis and irritable bowel syndrome. Even though many potential virulence factors have been proposed, the pathogenesis of the disease is not yet clear. Likewise, no vaccine for either chickens or humans has yet become commercially available.

The major outer membrane protein (PorA) constitutes a possible vaccine target and may be involved in adhesion to host cells. The surface exposed loop regions are highly variable, indicating positive immune selection and infected humans have been shown to produce antibodies against PorA. However, the effect of such antibodies remains unclear. While in one study antibodies induced by the recombinant protein did not bind intact bacteria, in another study oral vaccination with a recombinant version of the protein provided some protection in a mouse model.

To further investigate PorA as a potential vaccine target we produced recombinant PorA using the gene sequence from a clinical isolate of *Campylobacter coli* to generate rabbit antiserum. We then determined the effect of the antiserum on a chicken strain as well as a human isolate with identical PorA sequences. A higher binding of antiserum compared to the preserum in a dot blot with intact bacteria could be observed for both strains. This was however not the case for *Campylobacter jejuni* strains expressing other variants of PorA.

A higher complement dependent bactericidal effect of the antiserum compared to the preserum could be demonstrated for both *Campylobacter coli* strains indicating that the induced antibodies might be protective. Thus far our results indicate that PorA could be a possible vaccine target, however if recombinant protein is used alleles from different strains would probably have to be combined.

Identification of drugs targeting Mycobacterium tuberculosis

M. Dal Molin 1, M. Meuli 1, A. Tschumi 2, S. Laage-Witt 2, P. Sander 1

- 1 Instutute for Medical Microbiology, University of Zurich, Gloriastrasse 32, 8006 Zurich
- 2 Hoffmann-La Roche AG, Grenzacherstrasse 124, 4070 Basel

Mycobacterium tuberculosis, the causative agent of Tuberculosis (TB), is still one of the major concerns in human health care. Annually, 1.5 million people die of TB and 9 million new cases are registered worldwide. Especially Sub-Saharan Africa and South-Eastern Asia are highly affected by TB. Increasing resistance towards the administered first-line (Rifampicin and Isoniazid) and second-line (e.g., Fluoroquinolones and Aminoglycosides) treatments requires development of novel efficient drugs also targeting highly resistant M. tuberculosis (also referred as MDR-TB and XDR-TB). At current stage 3.7% and 0.33% of all new cases are MDR-TB or XDR-TB, respectively. In our study, we performed a Mycobacterium bovis BCG GFP-based whole cell high-throughput drug screening assay against a comprehensive library containing ~400'000 chemical compounds. The live vaccine M. bovis BCG is hereby used as model organism due to its 99% sequence identity compared with M. tuberculosis. The goal is to identify novel potent chemical compounds targeting essential pathways in M. tuberculosis which ideally i) decrease the current six month treatment duration and ii) are effective against MDR-TB and XDR-TB. The initial screening of the ~400'000 compounds resulted in 6356 Hits. These are chemical compounds showing inhibiting activity (>85%) at a defined single concentration. In a secondary set up, Hit compounds of the initial screening were tested for their potency in a Minimal Inhibitory Concentration (MIC) assay using M. bovis BCG-GFP.

REPLICON TYPING OF PLASMIDS CARRYING BLACTX-M-15 IN ENTEROBACTERIACEAE OF HUMAN, LIVESTOCK AND ENVIRONMENTAL ORIGIN IN SWITZERLAND

K. Zurfluh * 1, M. Glier 1, H. Hächler 1, R. Stephan 1

1 University of Zürich, Inst. Food Safety, Zürich, Switzerland

One of the currently most important antibiotic resistance mechanisms in $\it Enterobacteriaceae$ is based on the production of ESBL enzymes that inactivate β -lactam antibiotics including cephalosporins and monobactams by hydrolysing their β -lactam ring.

In humans, the most prevalent ESBL enzyme type is encoded by *blac*TX-M-15. CTX-M-15 producing enterobacterial strains were also frequently isolated from environmental samples like surface water. Plasmids, which can be grouped in different plasmid incompatibility types, play a key role in the horizontal spread of these multidrug resistance genes.

The aim of this study was to investigate the diversity of plasmids that carry *bla_{CTX-M-15}* genes among *Enterobacteriaceae* isolated at the human, livestock and environment interface.

In total, 81 blactx.m-15-harbouring isolates collected between 2009 and 2014 were tested for its ability to transfer blactx.m-15 by conjugation. blactx.m-15 harbouring plasmids were further typed. Transfer of a single blactx.m-15 harbouring plasmid was observed in 31 (38.3%) of the isolates. The most frequent replicon types detected among these plasmids are IncF-type plasmids (n=12) (mostly multi replicon plasmids with a combination of following replicon: IncFII, IncFIIA and IncFIB), followed by IncI1 (n=3), IncK (n=3) and IncR (n=1). A noticeable number of plasmids (n=8) could not be assigned to one of the tested replicon types.

CHEMICAL AND MYCOLOGICAL ASSESSMENT OF INDOOR AIR QUALITY INSIDE A RECENTLY CONSTRUCTED FLAT

- T. Teruzzi ¹, P. Canonica ¹, S. De Respinis ², AP. Caminada ², V. Guidi ², M. Tonolla ²
- 1 Institute for materials and constructions, SUPSI, Canobbio, Switzerland
- 2 Laboratory of applied microbiology, SUPSI, Bellinzona, Switzerland

The owners of a recently constructed flat (2014) in Ticino (Switzerland) asked our research units to assess the indoor air quality, to possibly discover the causal agents of occupants' adverse health effects, such as eye irritation symptoms, dizziness and fatigue and, especially for one member of the family, increased frequency of asthma attacks. The assessment focused on the presence of chemical and biological contaminants. The search for bioaerosol was motivated by a high level of perceived dampness.

Indoor air was analyzed for the presence of volatile organic compounds (VOC) and filamentous fungi. For the chemical characterization of indoor air, the volatile organic compounds were sampled by means of activated charcoal tubes and DNPH cartridges. The sampling media were analyzed gas chromatographically with flame ionization detector. The results show a total VOC concentration as high as 1'800 mg/m³. Anomalous concentrations of some pollutants belonging to the groups of aliphatic hydrocarbons, ketones and esters are observed.

For the quantification and identification of indoor fungi, the indoor air was aspirated with a SAS air sampler and directly inoculated on solid culture media. After culturing on different culture media, the growing fungi resulted to be as high as 57 CFU/m³ in the bedroom and 94 CFU/m³ in the living room. Most of the grown molds were sub-cultured and analyzed by Matrix-Assisted Laser Desorption/lonization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Some representatives were subsequently analyzed by beta-tubulin partial gene sequencing and identified at the species level. Most of the strains belonged to genera *Aspergillus* (Section Versicolores), *Penicillium* and *Cladosporium*. Work is ongoing for their identification at the species level.

The overall results provide evidence of the existence of a possible causal relationship between the poor quality of indoor air and the adverse health effects reported by the occupants.

Strain-specific estimates of fitness predict the community of a multiple-strain tick-borne pathogen in the field over one decade

MJ. Voordouw 1, J. Durand 1, M. Jacquet 1, L. Gern 1

1 University of Neuchâtel, Neuchâtel, Switzerland

Immunodominant pathogen antigens play a key role in structuring the populations of multi-strain pathogens. Strong immune selection against these antigens will cause the pathogen strains to organize themselves into a set of unique serotypes that minimizes cross-reactive acquired immunity. These sets of antigenically distinct strains can remain stable over long periods of time and the frequency of each strain depends on its intrinsic fitness. The tick-borne pathogen, *Borrelia afzelii*, causes Lyme disease in humans. The polymorphic ospC gene of B. afzelii codes for the immunodominant outer surface protein C and is a useful strain-specific marker. We used next generation sequencing to characterize the community of B. afzelii ospC strains in a local population of ticks over a period of 11 years. We also used experimental infections of mice to estimate the intrinsic fitness of six B. afzelii ospC strains in the lab. We found that the frequency distribution of the B. afzelii ospC strains was stable over the duration of the study. Our laboratory estimates of strain fitness explained over 65% of the variation in the strain-specific frequencies in the field. The pattern of genetic variation at the ospC locus suggested there was strong selection against intermediately divergent ospC alleles. Our results are consistent with theoretical models on how acquired immunity structures multi-strain pathogen populations.

Heat Shock Proteins 90 (Hsp90) and 70 (Hsp70) in Aspergillus fumigatus virulence and antifungal resistance

F. Lamoth 1

1 Institute of Microbiology, Lausanne University Hospital, Switzerland

Invasive aspergillosis (IA) is a major cause of infectious death among patients with hematological malignancies or other immunosuppressive conditions. The pathogenesis of IA relies on a complex balance between microbial and host factors. At the pathogen level, environmental conditions triggering morphological changes (germination of conidia and hyphal extension), thermal adaptation and compensatory responses to the cell wall/membrane stress induced by antifungal drugs are all important determinants contributing to invasion and progression of the disease.

The heat shock proteins Hsp90 and Hsp70 are essential molecular chaperones controlling the folding and activation of a major part of the proteome in eukaryotes. They act as sensors of environmental stress triggering adaptive mechanisms via activation of multiple intracellular pathways, such as the calcineurin pathway.

In fungi, Hsp90 was shown to have a key role in basal and acquired resistance to the most important antifungal classes (azoles, echinocandins and polyenes). Hsp70 is involved in the transfer of client proteins to Hsp90 via Hop/Sti1 (StiA in *A. fumigatus*) for their subsequent activation.

The objective of this presentation is to discuss the role and mechanisms of the Hsp90-Hsp70 machinery in virulence, stress adaptation and antifungal resistance of *Aspergillus fumigatus*. Hsp90 is crucial for all steps of *A. fumigatus* morphogenesis (conidiation, germination and hyphal elongation). I have recently shown how Hsp90 triggers basal resistance to caspofungin and the paradoxical effect of this drug (i.e. resistance at high concentrations) via a short promoter region. Acetylation of Hsp90, rather than phosphorylation, governs Hsp90 function in this pathway. Deciphering the functional and physical interactions of the Hsp70-StiA-Hsp90 complex also revealed distinct roles of the C-terminal domain of Hsp70 and the co-chaperone StiA in response to heat and caspofungin stress. Targeting this complex at different levels (Hsp90 or Hsp70 inhibitors, lysine deacetylase inhibitors) represents a promising novel antifungal strategy against invasive aspergillosis.

Transcriptomic profiles of Candida albicans in systemic infection of two host organisms

- S. Amorim-Vaz ¹, AT. Coste ¹, S. Pradervand ², TVD. Tran ³, M. Pagni ³, D. Sanglard ¹
- 1 Institute of Microbiology, University of Lausanne, Switzerland
- ² Genomic Technologies Facility, University of Lausanne, Switzerland
- 3 Vital-IT Group, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland

Candida albicans is the most prevalent fungal pathogen and mortality rates of systemic infections in immunocompromised patients are around 50%. The available antifungal therapies need to be complemented by new drugs directed at new targets. To identify potential targets in C. albicans, a deeper understanding of the mechanisms that allow its adaptation to the different environments in the infected host is needed, for which transcriptional regulation is crucial. Microarray studies have been carried out in vitro, but although informative, they are limited in revealing the full complexity of transcriptional regulation that takes place during infection. In vivo analyses are confronted with the low proportion of fungal biomass in organs recovered from infected organisms as compared to host material. Here, we developed a novel strategy for high-resolution analysis of C. albicans transcriptome directly from early and late stages of systemic infection in two different host models, mouse (kidneys) and G. mellonella. We applied a procedure to enrich RNAseq libraries in fungal transcripts by more than 50-fold, using biotinylated probes to capture C. albicans sequences, and prove that this approach does not bias the resulting RNA populations. Our analysis revealed that groups of genes involved in stress response, adhesion and iron acquisition were, as expected, highly induced during infection in the two models. We also observed upregulation of biofilm-related genes in these models. Many genes with still unknown function were differentially regulated as well, and their study is now of great interest. Additionally, our analysis included dynamics of infection by comparing the fungal response at distinct infection time points in the two host models. Besides, model-specific genes were identified. In conclusion, RNA enrichment from host tissues allows a yet still unprecedented resolution of the C. albicans transcriptome, allowing the detection of over 6000 genes, and may be applied to other microbial pathogens.

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THE ROLE OF NONHEMATOPOIETIC CELLS IN PROTECTIVE IMMUNITY AGAINST C. ALBICANS

S. Altmeier 1, S. LeibundGut-Landmann 1

1 ETH Zürich, Zürich, Switzerland

The pathogenic fungus *Candida albicans* is a major risk factor for immunosuppressed individuals such as those suffering from AIDS. During infection of the mucosa and the skin, *C. albicans* initially interacts with the epithelium, which not only serves as a physical barrier, but is also involved in the activation of inflammation and immunity. As such, the sensing of *C. albicans* by epithelial cells results in the recruitment of neutrophils to the site of infection, which is essential for fungal control. Neutrophil mobilization from the bone marrow is mediated by granulocyte colony stimulating factor (G□CSF), which is produced locally at the site of infection and can be detected at high levels in the serum. Using a mouse model of oropharyngeal candidiasis we found, that, interestingly, endothelial cells in addition to epithelial cells serve as an important cellular source of G-CSF in the infected tissue *in vivo*. A direct crosstalk between the two cell types via soluble factors results in the induction of endothelium-derived G-CSF. The data obtained from this study will advance our current understanding of the intimate interplay between different cells of the nonhematopoietic compartment that coordinate the induction of protective immunity against *C. albicans* in the oral mucosa.

Combining microfluidics and RNA sequencing to assess the nematode-inducible defensome of a mushroom in spatio-temporal resolution

A. Tavvrov 1, S. S. Schmieder 1, C. E. Stanley 2, S. Bleuler-Martinez 1, A. J. DeMello 2, M. Aebi 1, M. Künzler 1

- 1 Institute of Microbiology, Department of Biology, ETH Zürich, Zürich, Switzerland
- 2 Institute for Chemical and Bioengineering, ETH Zürich, Zürich, Switzerland

Preliminary studies on the antagonistic interaction between multicellular fungi and fungivorous nematodes have shown that induction of fungal defense genes is limited to the part of the mycelium that is in a direct contact with the worm (S. S. Schmieder, unpublished). Hence, for the identification of the complete set of genes of a fungus that is differentially expressed upon challenge with such an antagonist, it is crucial to use an experimental setup that maximizes the zone of direct interaction between the mycelium and the worm and allows retrieval of the fungus from this zone. In this respect, conventional co-cultivation methods on agar plates fail. Here, we present a novel microfluidics platform that allows confrontation between the fungus and the nematode in a confined area and retrieval of the organisms from this area for analysis. We tested the effectiveness of the technique by conducting a genome-wide analysis of transcription (RNA sequencing) of the model mushroom Coprinopsis cinerea challenged by the fungivorous nematode Aphelenchus avenae. RNA was extracted from the induced part of the vegetative C. cinerea mycelium at three time points (4, 8 and 20 h) in the presence and absence of the fungivorous nematode. Libraries from all samples with three biological replicates were sequenced on the Illumina HiSeq 2500 platform. Initial results show that approximately 1200 genes were significantly differentially expressed (FDR ≤0.05, FC ≥4) upon challenging with the nematode. In addition to the previously well-known effector proteins such as lectins (Bleuler-Martinez et al., 2011), the list of upregulated genes includes many genes encoding potential defense effector proteins. In conclusion, the application of the microfluidics device allowed us to extract and analyze samples from highly induced sections of mycelia.

Rapid identification of microorganisms by MALDI-TOF Mass Spectrometry (MALDI-TOF MS)

M Tonolla1,2

MALDI-TOF MS is a technique that is increasingly used for the identification of microorganisms. Its versatility and speed have rapidly conquered several fields related to the characterization of bacteria and fungi. Widely recognized advantages of MALDI TOF MS are its speed, accuracy, sensitivity, specificity, high throughput, low operational cost, minimal sample processing, and ease of operation. These are substantial advantages when compared to biochemical and genotype-based methods. This technology takes advantage of the fact that whole cells can be analyzed after a simple extraction procedure without any further preparation steps. The resulting mass spectra display peak patterns that have been shown to be good markers in a mass range of 2–20 kDa. MALDI-TOF MS now plays an important role in medical and veterinary diagnostics, as well as in water, air and food quality monitoring, forensic investigations, and environmental microbiology. Unfortunately, speed of analysis is offset by the need to grow cells in culture media before identification. In clinical microbiology diagnostics efforts have thus been made toward MALDI-TOF MS protocols for the direct detection and identification of pathogens causing sepsis and urinary tract infections. Direct identification, intraspecific typing, detection of antibiotic resistance by MALDI-TOF represent major fields of investigation that would increase considerably the already high potential of this technology.

¹ Laboratory of Applied Microbiology, University of Applied Sciences and Arts of Southern Switzerland (SUPSI), Bellinzona, Switzerland

² Microbiology Unit, Plant Biology Dpt., University of Geneva, Switzerland

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Identification of Neisseria meningitidis serological groups A, B, C, W135, Y, X by multiplex PCR in the nasopharynx of students in Kashan- Iran during 2011-2012

A. Khorshidi ¹, M. Valipor ¹, A. Pirozmand ¹, H. Akbari ¹, M. Mirzaee ¹

1 Kashan University of Medical Sciences, Kashan, Iran

<u>Background:</u> *Neisseria meningitidis* (*N. meningitidis*) is a pathogen that colonizes the nasopharynx without any clinical manifestations. Among the 13 different serological groups, only the serogroups A, B, C, W135, Y, X play a major role in disease development. This study aimed to identify these serological groups of *N. meningitidis* in the nasopharynx students in Kashan schools using a multiplex PCR method.

<u>Materials and Methods:</u> This cross-sectional study was conducted on 1289 students in Kashan Iran during 2011-2012. Samples were collected from the students' nasopharynx using sterile swabs and cultured on a selective medium. Strains were identified by biochemical tests. The serological groups were determined using the multiplex PCR method.

Results: One-hundred and fifteen (8.9%) out of 1289 students were N.meningitidis carriers; 75 (65.2%) were male and 40 (34.8%) female. There was a significant difference in the rate of carriers between genders (P=0.032). The highest rate of carriers (12.3%) was in the 15 to 19-year age group. There was a significant relationship between the rate of carriers and the number of family members (P<0.001). In this study, only the serological groups B (8 cases) and C (107 cases) were detected.

<u>Conclusion:</u> Since the serological group C is involved in the outbreak and there is no vaccine currently available for the serological group B to prevent the infection, detection of these serological groups can be important.

PERFORMANCE OF THE EUROBLOTONE - EUROLINE WESTERN-BLOT SYSTEM (EUROIMMUN) COMPARED TO A HOME-MADE WESTERN-BLOT PROCEDURE FOR THE CONFIRMATORY SEROLOGICAL DIAGNOSTICS OF LYME BORRELIOSIS

M. Cheseaux 1. A. Dumoulin 1

1 Hôpital du Valais - Institut Central

The diagnostics of the Lyme Borreliosis mainly relies on the detection of IgM and IgG antibodies directed against *Borrelia burgdorferi* sensu lato. Due to specificity issues, positive screening tests should be confirmed by western-blot or other methods allowing detection of antibodies against specific antigens. Our laboratory has a long experience in production and routine use of in-house western-blot for this confirmatory diagnostics. However, this method is time-intensive, requires a high-level of standardization for the production of the membrane strips and can only be partially automatized.

We evaluated the EuroblotOne system (EBO, Euroimmun, Germany) that allows fully automated sample processing, digitalization and analysis of western-blots, thereby reducing the turn-around-time from 8h to 3h approximately. The performance of the EBO system and the *Borrelia* western-blot EuroLine WB (ELWB) was compared to our in-house western-blot procedure, by testing 74 well-characterized archived samples covering different stages of the disease.

Preliminary results indicated a good sensitivity of the EBO-ELWB system (IgG 93%; IgM 85%). However, specificity was unsatisfactory (IgG 50%; IgM 51%). Modification of the interpretation algorithm to use criteria closer to the in-house procedure (increased number of specific bands required for positivity; increased positivity-threshold for individual bands) allowed a significant improvement of sensitivity (IgG 93%; IgM 93%) and specificity (IgG 81; IgM 89%). Further analysis on samples from routine diagnostics will be performed in order to validate the modified algorithm.

EVALUATION OF TWO SCREENING TESTS FOR SYPHILIS SEROLOGY

R. Lienhard 1, S. Mauvais 2, M-L. Tritten 1, H.H. Siegrist 1, A. Sarraj 2

- 1 ADMED Microbiologie, La Chaux-de-Fonds, Switzerland 2 SRNJTS Transfusion Center, La Chaux-de-Fonds, Switzerland
- Aim: To compare IgG EIA test results of two instruments to screen for syphilis infection and determine their sensitivity and specificity.

Methods: Two IgG anti-Treponema pallidum assays were used as screening test: Liaison Treponema Screen (Diasorin) and Elecsys® Syphilis on Cobas 6000 (Roche). A collection of 84 sera from patients presenting syphilis at all stages including treatment follow-up was used to determine sensitivity. Specificity was tested with 67 selected clinical sera on both systems. More extensive testing was conducted using clinical samples on Liaison and blood donor plasma on Cobas. Confirmation was obtained with agglutination assays as RPR (bioMérieux), TPPA (Fujirebio) and discrepant results confirmed by IgG and IgM immunoblots (Sekisui, Virotec).

Results and discussion: Sensitivity was 100% for both assays. Specificity was comparable with 92.5% and 91% for Liaison and Cobas, respectively, only 2 sera gave false positive results on both assays. None of the 9 false positive sera was confirmed on immunoblots. Testing 432 routine clinical samples on Liaison and 555 blood donor plasma on Cobas, we obtained very good specificity of 99.5% and 100%, respectively. The comparison of quantitative results gave an unsatisfactory correlation coefficient (R2 <0.75). However, qualitative comparison resulted in a very good Kappa index of 0.92. Comparing TPPA agglutination titers with EIA values showed poor correlation confirming that these assays cannot be used for follow-up after treatment of syphilis.

Conclusion: The IgG EIA assays on Liaison and Cobas are suitable for syphilis screening and allow for easy and rapid determination. However, all reactive results must be confirmed by titer determination on treponemic TPPA/TPHA and non treponemic RPR/VDRL assays. Immunoblots may be useful to confirm specificity (IgG) or early infection stages (IgM).

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DYSREGULATION OF THE LUNG MICROBIOTA IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

M. Mika 1, L. Morf 2, S. Beyeler 2, W. Qi 3, C. von Garnier 2, M. Hilty 1

- 1 Institute for Infectious Diseases, University of Bern, Bern, Switzerland
- 2 Department of Clinical Research, University of Bern, Bern, Switzerland
- 3 Functional Genomics Center, Swiss Federal Institute of Technology Zurich, Switzerland

We hypothesize that a dysregulated microbiota drives chronic lung diseases, such as Chronic Obstructive Pulmonary Disease (COPD). Here, we investigate the microbiota from upper to lower airways in patients with different COPD severity degrees.

Brushes from the upper and lower airways, as well as bronchoalveolar lavages (BALs) were obtained by bronchoscopy from non-COPD (n=8), mild (COPD1; n=5), and moderate (COPD2; n=4) COPD patients. Patients had no previous antibiotic treatment. Microbiota analysis was performed by amplification of the bacterial 16S rRNA gene and subsequent 454 pyrosequencing. Sequences were analyzed based on 97% sequencing identity.

Within-sample diversity, measured by the Shannon Diversity Index (SDI), was decreased in the upper and lower airways samples of COPD2 patients compared to non-COPD patients. This trend was significant for samples of the main bronchus (*P*=0.02). In contrast, COPD1 patients did not show a significant decreased SDI in the upper and/or lower airways samples. *Streptococcaceae*, *Moraxellaceae*, and *Prevotellaceae* were the most abundant bacterial families found in non-COPD, COPD1, and COPD2 patients. COPD2 patients harbored a significantly increased mean relative abundance of Streptococcaceae in the main bronchus (30.5%), upper (32.0%), and middle lobar (44.1%) bronchi samples as compared to non-COPD patients (11.0%, 12.0%, and 5.6%, respectively. Multiple t-test; *P*=0.005, 0.03, 0.001, respectively). COPD2 patients showed a trend of decreased abundance of *Prevotellaceae* and *Acidaminococcaceae* as compared to non-COPD patients, indicating microbial dysregulation in more severe degrees of COPD.

COPD2 patients showed lower sample diversity and increased abundance of *Streptococcaceae*, indicating an outgrowth of this bacterial family. Thus, regional changes in microbial composition detected in main and lobar bronchi may be associated with disease progression in COPD.

Development of an artificial burn wound exudate medium to study the mechanisms of burn wound infection

B. Fleuchot ¹, M. R. Gonzalez ¹, Y. A. Que ², P. Jafari ², L. A. Appelgate ², W. Raffoul ², K. Perron ¹

- ¹ Microbiology Unit, Dept. of Botany and Plant Biology, University of Geneva, Geneva
- 2 Functional Unit of Burn Research, Lausanne University Hospital, Lausanne, Switzerland

BACKGROUND: Severe burns are very devastating forms of trauma, which require immediate and specialized medical cares. Despite recent treatment advances that reduced fatal outcomes, the vast majority of all deaths are still related to sepsis induced by burn wound infections. Prevention of bacterial development on burn wounds is therefore a major medical challenge. Biological dressings based on collagen matrices are currently used in treatment at the CHUV in Lausanne to protect the wound and stimulate healing. In order to improve the antibacterial properties of those bandages, the study of bacterial development in burn wound exudates is of prime interest.

AIMS: The aims of this project are i) to study the growth and physiology of *P. aeruginosa*, the major burn wound pathogen, in burn wound exudates, ii) to determine the precise composition of burn wound exudates and iii) to develop an artificial burn wound exudate medium.

RESULTS: Physico-chemical analyses of 15 burn wound exudates collected from 5 patients were performed. Their composition showed a very similar concentration regarding the trace elements, the protein and the amino acids contents. All exudates displayed a basic pH with a mean value of 8.9. Interestingly, the exudates compositions suggest that release of liquid by the wound contributes to a strong loss in amino acid but not in lipids. Inoculation of *P. aeruginosa* in these exudates showed various growth profiles and virulence factors production. These variations were dependent both on patient treatments and on the chemical composition of the exudates.

According to the precise chemical composition of burn wound exudates, an artificial medium was formulated. This medium will greatly improve the study of burn wound infections and provide new tools to understand the biology of wound-associated pathogens. Future investigations using these approaches will help in the development of efficient antibacterial strategies.

Predictive value of procalcitonin: Non-specific in Mycoplasma pneumoniae infections.

O. Ottiger 1, M. Oberle 1, L. Bernasconi 1, H. Fankhauser 1

1 Institute of Laboratory Medicine, Cantonal Hospital Aarau

Introduction: The effectiveness and safety of procalcitonin (PCT)-guided antibiotic therapy in lower respiratory tract infections had been studied. According to the clinical outcome, it was noted that the duration of antibiotic treatment could be safely reduced (Albrich et al. Arch Intern Med. 2012, 14;172,715-22). The presence of bacteria and viruses, respectively, in the respiratory samples had not been checked in that patient collective.

In the present study, we examined the level of PCT in a patient collective with bacterial respiratory infection, to support the above mentioned concept.

Method: Respiratory samples between October 2014 and February 2015, where PCT in serum samples were requested, were examined. These samples had been tested either by conventional culture or by a molecular multiplex PCR (FilmArray, Biofire, Biomerieux).

Results: The data from our collective showed a positivity rate of relevant bacteria in a third of patients; among them were patients with *Mycoplasma pneumonia* infections. Patients with respiratory infections with *Streptococcus pneumonia* and *Haemophilus influenzae* showed an increased PCT level. However, PCT was not increase in most cases of infections with *Mycoplasma pneumonia*.

Conclusion: The presence of classical bacteria species correlates with an increased level of PCT. However, the concept of PCT-guided antibiotic therapy may be hampered in infections with intracellular bacterial species, such as *Mycoplasma pneumonia*, and therefore, antibiotic treatment must nonetheless be considered according to the clinical situation.

EVALUATION OF TWO COMMERCIAL DIAGNOSTIC KITS FOR THE DETECTION OF PARASITIC PROTOZOA IN NATIVE AND SAF PRESERVED STOOLS

M.L. Tritten-Arber 1, C. Parel 1, R. Lienhard 1, H.H. Siegrist 1

1 ADMED Microbiologie, La Chaux-de-Fonds, Switzerland

Objectives: To test the performance of two commercial diagnostic kits for the molecular detection of intestinal protozoa in native stool specimens, evaluate the effect of SAF on PCR, assess the performance of our routine procedures and evaluate the interest of introducing PCR based diagnostic tools in our routine.

Material and methods: Three hundred and one pairs of native and SAF preserved stool specimens for which a parasitological examination was requested were tested with two commercial real-time PCR diagnostic tests: RIDA® GENE Parasitic Stool Panel II and RIDA®GENE Dientamoeba fragilis (R Biopharm) after an easyMAG (BioMérieux) nucleic acid extraction. These two kits are intended for the detection of four intestinal protozoa: Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum and Dientamoeba fragilis. All specimens were also examined with our routine procedures and with a commercial ELISA test for the detection of Cryptosporidium parvum (RIDASCREEN® Cryptosporidium, R-Biopharm).

Results: We obtained (native and SAF) PCR and routine results for 294 samples. Overall, 60 samples tested positive. *Giardia lamblia* (25) and *Dientamoeba fragilis* (30) were most frequently detected. Among those, 8 *Giardia* and 26 *Dientamoeba* were detected by PCR only. Five *Cryptosporidium* were found. Only 1 true *Entamoeba histolytica* was diagnosed. As suspected, SAF had an important inhibitory effect on PCR leading to a loss of sensitivity.

Conclusions: The two diagnostic kits proved to be more sensitive than microscopic examination or ELISA testing particularly for *Dientamoeba fragilis* and *Giardia lamblia*. These tests are easy to perform, can be batch-processed and deliver same day results. They could be used as screening tests. However, it would require careful selection of stools suspected to contain other protozoa or helminths according to clinical and anamnestic indications, in order to perform additional enrichments and microscopic examinations. Unfortunately our routine easyMAG extraction is not sufficient to counteract the inhibitory effect of SAF.

THE UTILITY OF MALDI-TOF IN THE RAPID IDENTIFICATION OF POSITIVE BLOOD CULTURES

R. Lienhard ¹, M. Hassan ¹, L. Bertaiola Monnerat ², C. Juvet ¹, M. Allaz ², M-L. Tritten ¹, H. H. Siegrist ¹

1 ADMED Microbiologie, La Chaux-de-Fonds, Switzerland 2 Laboratoire de Microbiologie, Hôpital de Fribourg, Switzerland

Purpose: To validate an easy and quick method for the rapid identification of bacteria in blood cultures enabling adaptive treatment of patients with bacteraemia.

Method: Blood cultures bottles obtained were from BACTEC (BT), Becton Dickinson and BacT/ALERT (BA), bioMérieux, systems. Blood samples were from 3 different regions: Canton Berne Jura (JBE) using BA, canton Fribourg (HFR) and canton Neuchâtel (NE) using BT. From positive vials broth was collected in a gel separator tube Vacutainer SSTII (BD) and centrifuged to isolate bacteria. Washed pellets were used for identification on MALDI-TOF Microflex using Biotyper software (Bruker). The method was evaluated in two hospital microbiology labs, HFR and our lab (ADMED). Results were compared with the final identifications.

Results and discussions: During a period of 10 months, 989 bottles of positive blood cultures were processed; 653 NE, 238 JBE and 98 FR. Most of those (75.9%) were collected in BT bottles as only JBE used the BA system. Scores >1.6 were accepted as identification as they never failed to correspond to the final result. Globally, 72.2% of all strains in the positive blood cultures could be identified directly. Of all Gram negative bacilli (GNB) and Gram positive cocci (GPC) 88.1% and60.3% could be identified, respectively. A significant difference was observed on GNB and GCP identification comparing BT versus BA: 90.2% and 71.3% versus 79.8% and 29.9%, respectively, showing a poor success rate with BA vials. Slightly better results were obtained from anaerobe versus aerobe vials for BT and BA: 80.8% vs 77.6% and 48.2% vs 42.5%, respectively.

Conclusion: This easy and rapid method to obtain pellets enables a very efficient identification of the aetiology in bacteraemic patient. Pellets are also available to further determine antibiotic susceptibility testing or even rapid detection of resistance.

Development and application of a dual-targeted real-time PCR for detection of Streptococcus pneumoniae

- S. Selvanayakam ¹, C. Straub ¹, K. Furrer ², R. Frei ¹, D. Goldenberger ¹
- 1 Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland
- ² Clinic of Thoracic Surgery, University Hospital Basel, Basel, Switzerland

Objectives: Streptococcus pneumoniae is an important human pathogen and represents a major cause of community-acquired pneumonia, meningitis, sinusitis, and otitis media. Due to its autolytic activity or prior antibiotic administration, the culture of S. pneumoniae often fails. In addition, the close phylogenetic relationship to oher species of the S. mitis group such as S. oralis, S. mitis and S. pseudopneumoniae makes a correct identification of S. pneumoniae challenging. Specific and rapid real-time PCR amplification of S. pneumoniae DNA may improve reliable identification from culture isolates and direct detection in clinical specimens.

Materials and Methods: We developed a dual-targeted real-time PCR for detection of part of the autolysin (*lytA*) and capsular polysaccharid (*cpsA*) gene of *S. pneumoniae*. For test evaluation we determined linear regression of standard curves, sensitivity and specificity of the assay using reference strains and clinical isolates. The test then was applied to prospectively collected clinical specimens from patients with thoracic empyema.

Results: A total of 89 culture isolates including *S. pneumoniae* (n=39), *S. mitis/oralis* (n=32), *S. pseudopneumoniae* (n=11), streptococci other than *Streptococcus mitis* group isolates (n=11), and non-streptococcal bacteria (n=6) were analyzed. The specificity of *lytA* and *cpsA* was 98.9% and 95.5%, respectively. The analytical sensitivity was 23 fg of purified DNA for both targets, corresponding to 10.5 copies of *S. pneumonia* per PCR. The linear regression of standard curves indicated very high correlations (R²=0.949 for *lytA*, R²=0.997 for *cpsA*). Furthermore, our PCR assay was applied to 217 pleural specimens (166 biopsies, 51 pleural fluids) from 54 patients with thoracic empyema. 30 (13.8%) samples from 8 patients were PCR-positive. In the corresponding cultures of all these specimens *S. pneumoniae* could not be detected.

Conclusion: This new dual-targeted PCR may be very useful for identification of atypical isolates from the *S. mitis* group in routine diagnostic work. It also showed to be a powerful assay in culture-negative samples from suspected

S. pneumoniae infection. In addition, the real-time PCR format may allow quantitative detection.

DIRECT IDENTIFICATION OF BACTERIA FROM POSITIVE BLOOD CULTURE BOTTLES BY MATRIX-ASSISTED LASER DESORBTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

B. Schulthess 1, P. Demolli 1, C. Meijerink 1, M. Hombach 1

1 Institute of Medical Microbiology, University of Zurich, Zurich

Sepsis is a life-threatening complication of infection with high morbidity and mortality. For accurate antibiotic treatment of patients an early diagnosis of sepsis and its underlying cause is crucial. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has the potential to speed up the diagnostic of causative microorganisms. In this study, we evaluated the same day identification of microorganisms directly from positive BacT/ALERT blood culture bottles (bioMérieux) using the SepsiTyper Kit and the MALDI Biotyper System of Bruker Daltonics. In parallel, conventional identification methods were performed on all clinical samples. Of the 174 positive, resin-containing blood cultures analyzed, 155 were monomicrobial, and of these contained 48% Gram-positive organisms, 48% Gram-negative organisms, and 4% yeasts. Using a score cutoff of 2.0, 59% of the organisms were correctly identified to the species level. The identification rate of Gram-negative organisms (79%) was found to be significantly greater than that of Gram-positive organisms (35%). Reducing the species score cutoff increased the identification rate to up to 85% and 57% for Gram-negative and Gram-positive bacteria, respectively. No misidentification was observed. Charcoal-containing bottles had substantially lower identification rates compared to resin-containing bottles. The analysis of mixed cultures resulted in identification of only one of the organisms. This study showed that the identification of bacteria directly from positive blood culture bottles using the SepsiTyper Kit and MALDI-TOF MS is highly reliable, and that identification rates can be improved by adapting score cutoffs.

SEEGENE ANYPLEX(TM) II ASSAYS ARE COMPATIBLE WITH THE FULLY AUTOMATED BD MAX(TM) PLATFORM

D. M. Gezer ¹, B. Nadesalingam ¹, M. Hug ¹, M. Altwegg ¹

1 Bioanalytica, Lucerne, Switzerland

AnyplexTM II diagnostic reagents (Seegene) are based on specially designed primers (Dual Priming Oligonucleotides, DPOTM) in combination with a novel multiplex amplification/detection technology (Tagging Oligonucleotide Cleavage and Extension, TOCETM) which allows semi-quantitation through repeated melting curve analysis. This technology has been designed specifically for use with a CFX96TM thermocycler (BioRad) and the corresponding software. In this study we wanted to determine whether the AnyplexTM II reagents are compatible with the BD MAXTM (Becton Dickinson) platform which is a walk-away instrument that allows fully automated analysis by combining nucleic acid extraction, PCR set-up and amplification/detection. As the BD MAXTM software does not allow repeated melting curves, the intention was to use cycle threshold (Ct) and melting point (Tm) values for semi-quantitation and identification, respectively, with the restriction that only one Ct value is measured in each channel for two different targets This might cause quantitation problems in the case of double infections; however, the areas under the respective melting curves would allow to estimate the relative amounts of the two targets.

In our preliminary study with the AnyplexTM II STI-5 assay (detection/identification of *Trichomonas vaginalis*, *Mycoplasma genitalium*, *M. hominis*, *Ureaplasma urealyticum*, *U. parvum*) on the BD MAXTM we were able to show that:

- 1. All targets can be reliably separated by Tm;
- 2. Ct values for M. hominis and U. urealyticum/parvum correlate with semi-quantitative culture results;
- 3. Denaturation time can be reduced from 30sec to 10sec without significantly affecting Ct values or signal intensities:
- 4. Deacreasing annealing and extension time results in higher Ct values and lower signal intensities whereas longer annealing and extension has no positive effect.

We conclude that Seegene AnyplexTM II STI-5 reagents are compatible with fully automated BD MAXTManalysis . A clinical validation study has now been started.

EVALUATION OF FOUR DIFFERENT RAPID STOOL ANTIGEN TESTS FOR THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION

S. Pranghofer 1, L. Schneider 1

1 Bioanalytica AG, Luzern, Switzerland

Background: For the diagnosis of *Helicobacter pylori* infections several non-invasive tests based on the detection of *H. pylori* antigens in stool samples are commercially available. The aim of this study was to compare four rapid one-step immunochromatographic stool antigen tests (Rapid Hp StAR (Oxoid), H. pylori Card (Certest Biotec), Pylori-Strip (Coris), SD H. pylori Ag (Alere)) with an enzyme immunoassay (Amplified IDEIA Hp StAR (Oxoid)) for the diagnosis of *H. pylori* infections in patients with dyspeptic symptoms.

Methods: A total of 240 patients (5-85 years old) were included in the study. 160 patients were *H. pylori* infected defined by HpSA-EIA and 80 patients showed negative results. Stool samples were analysed with the IDEIA Hp StAR upon arrival and then stored at -80°C before being analysed by the four rapid antigen tests.

Results: In comparison the Amplified IDEIA Hp StAR test, the *H. pylori* Card showed an excellent performance with an overall sensitivity of 95.95% and specificity of 98.91%. Rapid Hp StAR showed the same sensitivity but a lower specificity (97.83%) and lower cost efficiency. Pylori-Strip and SD H, pylori Ag showed poor sensitivities of 66.89% and 70.27%, respectively. Their specificity was 100% and 97.22%, respectively.

Conclusion: The *H. pylori* Card was the most accurate rapid one-step immunochromatographic stool antigen test for diagnosing H.pylori infections in dyspeptic patients and it can be used as an alternative to the time consuming stool antigen enzyme immunoassay.

RAPID IDENTIFICATION OF MICROORGANISMS DIRECTLY FROM POSITIVE BLOOD CULTURES USING MALDI-TOF MS AND VACUTAINER SSTII ADVANCE TUBE

G. Jost 1, N. Liassine 1

1 Dianalabs, Geneva, Switzerland

Objectives: Detection of microorganisms in blood specimens using MALDI-TOF is widely used in clinical laboratories. However, identification of bacteria directly from blood cultures is more demanding. The aim of this study is to evaluate the performance of a simplified method for direct identification of bacteria in positive blood cultures by the combined use of MALDI-TOF and Vacutainer SSTII Advance tube.

Methods: 44 positive blood cultures (BacT/ALERT 3D, bioMérieux) from 26 different patients were investigated. For the testing protocol, 5 mL of blood was injected into Vacutainer SSTII Advance tube with separator gel (Becton Dickinson) and centrifuged at 2500 rpm for 10 minutes. For direct identification, MALDI-TOF plate was prepared in triplicate with 1 µl of the pellet followed by protein extraction with formic acid 70%. In addition, five drops of the pellet were inoculated on blood and chocolate agar incubated at 35°C with CO₂. Additional identifications were performed in triplicate by MALDI-TOF after 2 and 6 hours of incubation. A score value cut-off of > 2 or > 1.4 with four successive proposals of the same species were applied for identification at the species level. Standard MALDI-TOF identification on the overnight culture was used as the gold standard.

Results: Sensitivity was higher for Gram-negative rods (GNR) than for Gram-positive cocci (GPC). Bacteria correctly identified at the species level directly from blood cultures were all Enterobacteriaceae (11/11), Enterococcus faecium (3/3) and Listeria monocytogenes (1/1). Concerning staphylococci, only 1/4 of S. aureus, 2/4 of S. epidermidis and 1/5 of S. hominis were correctly identified directly from blood cultures; they were all correctly identified after 6 hours of incubation. No incorrect identification was observed with the score value cut-off applied.

Conclusions: The current work highlights the reliability and rapidity of identifying isolates directly from blood cultures, particularly for GNR, by a simplified method with minimum manipulations and additive costs.

TO BE OR NOT TO BE - CULTIVATION OF GONOCOCCI AFTER THEIR DETECTION BY MOLECULAR METHODS

L. Berlinger 1, S. Pranghofer 1, M. Altwegg 1

1 Bioanalytica AG, Lucerne, Switzerland

Sexually transmitted infections are on the rise worldwide. For *Neisseria gonorrhoeae*, one of the important implicated organisms, a worrisome increase of resistance not only to quinolones but also to cephalosporins has been observed. Diagnosis of gonorrhoea in Switzerland is mainly based on molecular methods which, however, do not provide any information about the antibiotic susceptibility of these organisms. This resulted in new guidelines for their diagnosis and therapy published by the *Eidgenössische Kommission für Sexuelle Gesundheit* (1) which include that antibiotic susceptibility testing should be performed whenever possible.

According to these recommendations all samples that tested positive for *Neisseria gonorrhoeae* by molecular assays in our laboratory were cultivated even if no culture had been requested. This allowed us to compare two groups of specimens: 1. Specimens cultured upon arrival in the laboratory (N=24), and 2. Specimens cultured only after they turned out to be positive by PCR (N=27), i.e. usually after storage for approx. 24 hours at 4°C. In group 1 16/24 (66.6%) and in group 2 16/27 (59.2%) were culture-positive. Further, 3/11 culture-negative specimens in group 2 had been stored for 48 to 72 hours, and one was from a patient previously treated with antibiotics. We conclude that PCR-positive specimens should always be cultured for gonococci even after storage of specimens under suboptimal conditions because recovery rates are not significantly lower than for those specimens immediately cultured upon arrival in the laboratory. With changing the storage temperature from 4°C to room temperature an even better outcome is expected.

(1) L. T. Trellu et al., Gonorrhoe: neue Empfehlungen zu Diagnostik und Behandlung, Schweiz Med Forum 2014, 14 (20)

Evaluation of the Seegene MTB assay for direct detection of Mycobacterium tuberculosis complex

C. Ritter 1, GV. Bloemberg 1

1 Institut für Medizinische Mikrobiologie, Universität Zürich, 8006 Zürich, Switzerland

In 2012 the Seegene Anyplex MTB/NTM assay for direct detection of Mycobacterium tuberculosis complex based on Real-Time PCR became available for the clinical laboratory. DNA extraction from respiratory samples was performed using the MICROLAB Nimbus IVD (Nimbus IVD). This is an easy-to-use automatic pipetting workstation with automatic nucleic acid extraction & PCR setup.

Using a selected set of 115 respiratory clinical samples, which were tested in parallel with the COBAS Taqman MTB assay (Roche) (1) and contained 33 MTB culture positive specimens. An overall concordance of 95.7 % was observed between the two assays. In addition, the Seegene Anyplex II MTB/MDR/XDRtest which became available recently was evaluated with a set of 33 MTB positive clinical specimens including 8 MDR/XDR specimens.

On basis of the results obtained we conclude that the Seegene Anyplex MTB/NTM assay has a high sensitivity and specificity for direct detection of MTB complex in clinical samples. Furthermore, we conclude that the Anyplex II MTB/MDR/XDR needs further investigation and possibly improvement of the assay.

1. Bloemberg GV, Voit A, Ritter C, Deggim V, Böttger EC. (2013) Evaluation of Cobas TaqMan MTB for direct detection of the Mycobacterium tuberculosis complex in comparison with Cobas Amplicor MTB. J Clin Microbiol. 51:2112-7

EXAMINATION OF 0104:H4 OUTBREAK AND NON OUTBREAK E.COLI STRAINS BY MASS SPECTROMETRY

D. Dressler 1, M. Andrian 2, O. Petrini 3

- 1 BioTeSvs GmbH. Esslingen, Germany
- 2 Ars Nova Software GmbH. Esslingen, Germany
- 3 POLE Pharma Consulting, Breganzona, Switzerland

Samples belonging to a large 2011 outbreak of O104:H4 Shiga-toxigenic *E. coli* have been analyzed by MALDI-TOF mass spectrometry after formic acid extraction (fae) and direct deposition (dsd) of samples. Toxigenic strains have been separated from non-toxigenic strains using the recently developed ABOS software and sensitivity, specificity and efficiency of the ABOS identification have been compared with those of a previous analysis.

ABOS analyses the overall peak pattern of every sample. Depending on the degree of similarity with reference samples unknown samples are classified as 0104:H4 or not. Based on one set of reference data of only 5 0104:H4 E.coli and 5 non outbreak *E.coli* the quality of ABOS analysis obtained with fae or dsd data as well as the impact of different threshold levels have been examined.

ABOS differentiation between O104:H4 and non outbreak strains showed specificity of 92.95%, sensitivity of 86.32% and efficiency of 88.66% at a threshold level of 8, with no differences in power of discrimination between fae and dsd sample data. Similar results have been generated also using a cut off level of 4, with fae and dsd samples having been correctly classified with an even higher specificity (100%) but lower sensitivity (74.21%) and slightly lower efficiency (83.33%). In contrast, the use of a threshold of 2 has shown much lower discriminatory quality.

On the basis of only 5 reference strains of each group ABOS was able to classify reliably all strains. Additionally, in concordance with earlier results, a number of discriminatory peaks have been identified that could be applied for strain classification.

Based on these results we believe that ABOS will be a helpful tool to quickly detect O104:H4 *E.coli* during outbreaks. Even unprocessed (dsd) samples are useful for the identification of these strains if a cut off level of at least 4 is used.

AMPLIFICATION EFFICIENCY MAY BE INFLUENCED BY THE SYSTEM USED FOR NUCLEIC ACID EXTRACTION

B. Nadesalingam 1, M. Altwegg 1

1 Bioanalytica, Lucerne, Switzerland

CE-IVD as well as lab-developed tests are often validated in combination with one particular extraction system but later used with a different one without revalidating the entire process. We have observed that this may cause serious problems for diagnostic applications when we compared different CE-marked Norovirus rtPCR reagents for their compatibility with the fully automated BD MAXTM (Becton Dickinson) extraction/amplification platform.

With easyMAGTM (bioMérieux)-extracted nucleic acids, amplification reagents from three different suppliers (Diagenode, rBiopharm, Cepheid) were adapted to the BD MAXTM using its *PCR only*-mode. For genogroup II all three achieved similar sensitivities while Cepheid reagents proved to be slightly less sensitive than the other two for genogroup I. Validation of the full BD MAXTM extraction/amplification process using the BD MAXTM Exk RNA-3 extraction reagents (Becton Dickinson) was therefore limited to Diagenode and rBiopharm reagents. Serial dilution of a strongly positive stool specimen resulted in almost identical sensitivities. However, when undiluted clinical specimens were analyzed in parallel we observed a different behavior of the two systems. In 3/6 specimens no inhibition was observed in both systems and the Ct values for the target were similar. One specimen was not inhibited but false negative with the Diagenode and fully inhibited with the rBiopharm reagents, whereas two specimens were partially inhibited with the Diagenode but fully inhibited with rBiopharm reagents. We assume that these results are most likely due to inhibitory components in the stool specimens that are not completely eliminated during the extraction process and which have non-identical inhibitory effects on the different enzymes used for amplification.

We conclude that for diagnostic applications the various steps should not only be validated individually but also as a whole process.

IN VITRO ACTIVITY OF INNOVATIVE METAL COMBINATION COATED CATHETERS AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

M.K.S. Ballo 1, S. Rtimi 2, J. Kiwi 2, C. Pulgarin 2, J.M. Entenza 3, A. Bizzini 3

- 1 Department of Fundamental Microbiology, UNIL, and EPFL, Lausanne, Switzerland
- 2 Institut des Sciences et Ingénierie Chimiques, EPFL, Lausanne Switzerland
- 3 Department of Fundamental Microbiology, UNIL, Lausanne, Switzerland

Infections arising from bacterial adhesion and colonization of intravenous catheters (IVCs) are a significant healthcare problem. Bacteria colonizing the skin at the catheter insertion site are the source of most IVCs infection. Measures for preventing IVCs infections include cutaneous antisepsis and rarely the use of antiseptic- or antibiotic-coated catheters. The efficacy of such preventive measures, however, is limited.

Hereby we report the development of a new metal nanoparticle-based catheter coating, and its antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The metal nanoparticles layer was applied to polyurethane catheters intended for intravascular human use. Since catheters introduced into the vascular system become rapidly covered by host plasma proteins, coated and uncoated catheters (1 cm in size) were pre-incubated in rat plasma at 4°C for 24h before being exposed to bacteria. Catheters were then immersed in 108 CFU/ml of MRSA ATCC 43300 and incubated at 37°C for 90 min. Catheters were then incubated in trypsin (0.05%, 60 min), vortexed (30 sec) and sonicated (100W, 5 min) and the resulting suspension diluted and plated for CFU counts.

The number of CFU bound to uncoated- and metal nanoparticle coated catheters was $5.0 \pm 0.3 \log_{10}$ CFU/cm² and < 1 log₁₀ CFU/cm², respectively (P= 0.0002). When they were pre-incubated in rat plasma, the CFU number bound to uncoated- and metal nanoparticle coated catheters were $6.4\pm0.3\log_{10}$ CFU/cm² and $2.9\pm0.56\log_{10}$ CFU/cm², respectively (P= 0.006).

Thus, new metal nanoparticle-impregnated catheters present a drastic reduction of MRSA ATCC 43300 and their activity seems not be affected by plasma coating. This finding suggests that those coated catheters might be of use to prevent IVCs infection.

DEVELOPMENT OF A NON-CULTURE BASED METHOD FOR THE FUNCTIONAL DETECTION OF ANTIBIOTIC RESISTANCE GENES; RESULTS FOR TETRACYCLINE

A. Allemann 1, M. Küffer 1, M. Hilty 1

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

Exchange of antibiotic resistant genetic information may easily occur in a niche with a complex microbiota like the nasopharynx. For instance, *tetM*, a common gene conferring resistance to tetracycline has recently been identified in a commensal bacterium (*Haemophilus parainfluenzae*)but is very frequently present in potential pathogenic *Streptococcus pneumoniae*. This gene is contained within conjugative transposons which may facilitate its spread among members of the microbiota. Therefore the investigation of antibiotic resistances within complex microbial communities may be more comprehensive than exclusive testing of distinct, potential pathogens.

We have developed a non-culture based method for the functional detection of antibiotic resistance genes directly from nasopharyngeal swabs. A genomic library has been prepared with extracted DNA from nasopharyngeal swabs from children with otitis media of which the pneumococcal antibiogram was known. Whole genomic DNA was amplified, sheared and repaired fragments were inserted into a vector before transformation into *E. coli.* Phenotypically resistant clones were recovered by functional screening and the gene conferring resistance was identified by sequencing.

Our method was optimized to clone DNA fragments of an average size of 2-9kb. For two out of two samples colonized with tetracycline resistant *S. pneumoniae*, we were able to recover the responsible gene (teth/l) by sequencing. For further validation of our non-culture based method, a collection of 10 nasopharyngeal swabs are currently investigated. All of them contain pneumococcal isolates with a known susceptibility to tetracycline. The results obtained with the standard culture based method and our method are currently compared and preliminary results look promising. In conclusion using this metagenomic approach, we were able to phenotypically reveal tetracycline resistance and recover the relevant gene by sequencing (i.e. teth/l). In the future, we could easily extend the functional screening to other antibiotics in order to investigate the resistome of the nasopharyngeal microbiota in more detail.

BETA-LACTAM TREATMENT OF mecC-POSITIVE STAPHYLOCOCCUS AUREUS EXPERIMENTAL ENDOCARDITIS

- S. Mancini 1, F. Laurent 2, R. Veloso 1, F. Vandenesch 2, P. Moreillon 1, J. Entenza 1
- 1 Department of Fundamental Microbiology, University of Lausanne, Switzerland
- 2 International Center of Infectious Research, University of Lyon, France

Methicillin-resistant $Staphylococcus\ aureus\ (MRSA)$, is a strain that has developed resistance to β -lactam antibiotics (e.g., methicillin, oxacillin, flucloxacillin, cefotoxin) through the acquisition of mecA, a gene encoding the penicillin binding protein 2a (PBP2a). For this reason, MRSA infections are generally treated with vancomycin, a member of the glycopeptide antibiotic class. Recently, mecC, a novel mecA homologue, was identified in bovine and human MRSA isolates (mecC-MRSA). However, the presence of mecC does not always correlate with resistance to β -lactams, which appears to be temperature dependent. Indeed, at $37^{\circ}C$ mecC-MRSA exhibit MICs of oxacillin close to those of methicillin-susceptible S. aureus (MSSA). Consistently, the activity of the mecC-product PBP2c appears to be thermosensitive, with a decline at $37^{\circ}C$. This raises the question as to whether, in regard of treatment, S. aureus strains harboring mecC should be considered just like mecA-MRSA, ordinarily treated with vancomycin, or like MSSA, generally treated successfully with β -lactams, such as flucloxacillin.

To address this issue, we investigated the sensitivities of mecC-MRSA towards β -lactams in vitro and in vivo using a rat model of endocarditis. To this end, five mecC-MRSA (two of animal origin and three human isolates) were used.

Consistent with previous reports, all the tested mecC-MRSA appeared resistant to oxacillin (MICs 4 μ g/ml) at 30°C while susceptible (MICs < 2 μ /ml) at 37°C. Moreover, flucloxacillin (human-kinetics of 2 g intravenously every 6h) successfully cured 80-100% of aortic valve vegetations induced by all the five different mecC-MRSA isolates in rats, a result which can be attributed to the body temperature of 37°C. These results suggest mecC-MRSA infections may successfully respond to treatment with β -lactams.

Confirmation of MRSA on culture isolates with the new Xpert® MRSA Gen 3 Assay

V. Hinic ¹, R. Frei ¹

1 Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland

Objectives: The Xpert® MRSA Gen 3 Assay performed on the GeneXpert® instrument (Cepheid, USA) is an automated PCR test for rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal swabs. Besides detection of SCC*mec* cassette types I-XI, this new and optimized test version detects also *mecA*-gene *a*nd the novel *mecA*-homologue named *mecC*. The test performance was evaluated on a challenging set of MRSA and non-MRSA strains.

Materials and Methods: The manufacturer's protocol for nasal swabs was adapted for testing bacterial isolates. 22 Staphylococcus spp. strains with phenotypically and/or genotypically well-characterized methicillin resistance/susceptibility profiles were included in the study. 4 mecA-positive MRSA (including one livestock-associated MRSA and one Zurich drug clone strain), 3 mecC-positive MRSA, 2 borderline oxacillin-resistant S. aureus (BORSA), 3 methicillin-sensitive S. aureus (MSSA), 1 methicillin-sensitive S. pseudintermedius, 3 methicillin-sensitive coagulase-negative staphylococci (CoNS) 2 methicillin-resistant CoNS and two 1:1 mixtures of an MSSA strain with a methicillin-resistant CoNS.

Results: Xpert® MRSA Gen 3 detected all *mecA*- and *mecC*-positive MRSA. All other strains were correctly categorized as negative. Even when MSSA and methicillin-resistant CoNS were mixed (simulation of the bacterial constellation possibly present in nasal swabs), the mixture was reported as true MRSA-negative.

Conclusions: Xpert® MRSA Gen 3 Assay successfully detected all *mecA* or *mecC* harbouring MRSA. In our small but challenging set of different staphylococcal strains, it also showed excellent specificity. Since this improved test version uses two independent targets for methicillin resistance detection, *S. aureus* strains with "empty" *SCCmec* cassette should not be reported as MRSA. This verification study suggests that the assay could be used for MRSA confirmation or exclusion in *S. aureus* culture isolates showing confusing phenotypic results. Since the assay has been primarily developed for MRSA detection from nasal swabs, further studies are needed to determine sensitivity and specificity of the test for MRSA detection directly from nasal swabs and other clinical samples.

DETECTION OF METHICILLIN-RESISTANCE IN COAGULASE-NEGATIVE STAPHYLOCCI: PERFORMANCE OF CEFOXITIN AND MOXALACTAM AND OPTIMIZATION OF THE DIAGNOSTIC WORKFLOW

G. Jost 1, G. V. Bloemberg 2, M. Hombach 2

- 1 Dianalabs, Geneva
- 2 Institut für Medizinische Mikrobiologie. Universität Zürich

Detection of methicillin resistance in coagulase-negative staphylococci (CoNS) is demanding, as false-negative and false-positive results are frequently encountered. The aim of this study was to analyze the performance of critical zone diameters of cefoxitin and moxalactam for the detection of methicillin-resistant CoNS and to derive an optimized diagnostic algorithm. Additionally, PBP2a agglutination and oxacillin MICs were analyzed, whereas mecA PCR was used as the gold standard. Analyzing 103 non-duplicate clinical CoNS isolates, sensitivity using the cefoxitin 2012 clinical breakpoints (CBPs) of the CA-SFM was higher (85%) than applying EUCAST/CLSI 2014 cefoxitin breakpoints (74%). CA-SFM 2012 moxalactam CBPs displayed highest sensitivity (93%) for mecA detection in CoNS. Species-specific cefoxitin breakpoints may be useful for Staphylococcus pettenkoferi as two cefoxitin-resistant, but mecA-negative isolates were found with MICs close to the CBP. A practical algorithm integrating both disk diffusion AND PBP2a agglutination OR molecular methods was developed, displaying sensitivity and specificity of 94-96% and 95% respectively, in the study population.

Lysosomal alkalinization sensitizes intracellular Staphylococcus aureus persister cells for antimicrobial therapy

N. Leimer ¹, C. Rachmühl ¹, M. Palheiros Marques ¹, F. Eichenseher ², K. Seidl ¹, MJ. Loessner ², RA. Schuepbach

- 1, AS. Zinkernagel 1
- 1 University Hospital Zurich, University of Zurich, Switzerland
- ² ETH Zurich, Zurich, Switzerland

Despite the availability of *in vitro* effective antibiotic therapy, *Staphylococcus aureus* infections often relapse in patients because they hide in privileged locations such as abscesses or in intracellular environments. Both sources feature compartments characterized by a low pH, and *S. aureus* small colony variants (SCVs) and/or persisters can frequently be recovered. We therefore assessed the effect of low pH on *S. aureus* colony phenotype and bacterial growth, using both *in vitro* assays and long-term *in vivo* infection models. We show that low pH can induce SCVs and non-replicating persisters, which are both capable of regrowth under physiological conditions. Within host cells, *S. aureus* was found to reside in lysosomes. Therapeutic neutralization of the low lysosomal pH employing either ammonium chloride, bafilomycin A1, or the anti-malaria-drug chloroquine significantly reduced SCVs in infected host cells. In a mouse systemic infection model, treatment with chloroquine also reduced the frequency of SCVs. Our findings demonstrate not only that acidic environments favor SCV formation, but also provide evidence that cell penetrating alkalinizing agents together with antibiotics may provide a useful therapeutic strategy for eradicating intracellular persisting staphylococcal reservoirs. This approach may also be extended to other intracellular hacteria

Label free SRM-based relative quantification of antibiotic resistance mechanisms in Pseudomonas aeruginosa isolates

Y. Charretier ¹, T. Cecchini ², C. Llanes ³, P. Bogaerts ⁴, S. Chatellier ⁵, J-P. Charrier ², T. Köhler ⁶, J. Schrenzel ¹

- 1 Geneva University Hospital, Geneva, Switzerland
- 2 bioMérieux SA, Marcy l'Etoile, France
- 3 Université de Franche Comté, Besançon, France
- 4 Université Catholique de Louvain, CHU de Mont-Godinne, Yvoir, Belgique
- 5 bioMérieux SA, La Balme les Grottes, France
- 6 University of Geneva, Geneva University Hospital, Geneva, Switzerland

Both acquired and intrinsic mechanisms play a crucial role in *Pseudomonas aeruginosa* antibiotic resistance. Many clinically relevant resistance mechanisms result from changes in gene expression namely multidrug efflux pumps overproduction, AmpC b-lactamase induction or derepression and/or carbapenem-specific porin OprD inactivation or repression. Changes in gene expression are usually assessed using RT-qPCR assays. Here, we evaluated label-free Selected Reaction Monitoring (SRM)-based mass spectrometry-based approach to directly quantify proteins implied in antibiotic resistance.

Reproducible assays were developed using a conventional bore chromatography coupled with an electrospray mass spectrometer operating in SRM mode. The SRM method allowed, within a single run, the multiplexed detection of 25 proteins, including *P. aeruginosa*-specific peptides, MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY(OprM) efflux systems, AmpC and OprD. Cutoffs for protein overproduction were established using thirty laboratory-derived mutants from the literature and verified using fifteen clinical strains. Concurrently, the same bacterial suspensions were analyzed by RT-qPCR.

Both SRM and RT-qPCR were able to identify resistance related to efflux pumps at high expression levels, AmpC derepression or decreased OprD expression. Referring to efflux systems, we found a more robust relative quantification of antibiotic resistance mechanisms by SRM than RT-qPCR. Label-free SRM-based AmpC detection predictions showed a better consistency with the antibiogram data than RT-qPCR The label-free SRM approach showed agreement with disk diffusion assays of 88, 95, and 90% for piperacillin/tazobactam, ceftazidime and cefepime, respectively. Label-free SRM-based OprD detection was the best predictor for carbapenem resistance.

The SRM method shows results that are equivalent or even better than those provided by RT-qPCR. The mass spectrometry-based approach can be adapted to analyze complex resistance mechanisms by taking advantage of each contributory effect. As multidrug resistance of *P. aeruginosa* involves several resistance mechanisms, this multiplexed method should allow a more educated choice of antimicrobial treatment.

Phenotypic methods for detection of carbapenemases in Gram-negative bacteria

L. Azimi ¹, A. Rastegar Lari ¹, M. Talebi ¹, P. Owlia ², MR. Pourshafie ³, F. Fallah ⁴

- 1 Department of Microbiology, Iran University of Medical Sciences, Tehran, Iran
- 2 Molecular Microbiology Research Center, Shahed University, Tehran, Iran
- 3 Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran
- 4 Shahid Beheshti University of medical Sciences, Tehran, Iran

Carbapenemases production can cause multidrug resistance in Gram-negative bacteria. A simple rapid and accurate phenotypic test for detection of Gram-negative, carbapenemase-producing bacteria can be useful for diagnostic purposes. The aim of this study was to detect phenotypically and genotypically carbapenemases producers. In this study, 150 imipenem resistant Gram-negative bacteria were surveyed. Modified Hodge test, boronic acid, ethylenediamine tetraacetic acid and dipicolinic acid were used for detection of carbapenemases, KPC and Metallo-Beta-Lactamases. PCR was performed for detection of carbapenemases genes. Our results indicated 52.7%, 31.6% and 69.5% Modified Hodge Test, boronic acid and dipicolinic acid positive tests respectively. Non synergism effects were observed between imipenem and ethylenediamine tetraacetic acid. Sixty-nine strains were confirmed as carbapenemases-producers according to molecular tests. Comparing results of phenotypic and genotypic methods, we suggest that phenotypic methods can be used for primary screening of carbapenemases and PCR based methods remain as the gold standard for detection.

Sensitivity and specificity of MHT for detection of carbapenemases

NPV	PPV	Specificity	Sensitivity	
51%	69%	60%	61% carb	MHT for

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FIRST EVIDENCE FOR THE DISSEMINATION OF BLAOXA-48 IN THE COMMUNITY IN SWITZERLAND

K. Zurfluh * 1, M. Nüesch-Inderbinen 1, L. Poirel 2, P. Nordmann 2, H. Hächler 1, R. Stephan 1

- 1 Institute for Food Safety and Hygiene, University of Zurich, Switzerland
- 2 Medical and Molecular Microbiology, University of Fribourg, Switzerland

The emergence and worldwide spread of carbapenemase-producing *Enterobacteriaceae* is of great concern to public health services. The aim of this study was to investigate the occurrence of carbapenemase-producing *Enterobacteriaceae* in the community in Switzerland.

One thousand and eighty-six stool samples of healthy humans (staff members of a food-processing company which were screened for the occurrence of Salmonella) were collected in September 2014. After an initial enrichment-step, carbapenemase-producing *Entereobacteriaceae* were selected by a carbapenem-containing selective medium. Grown colonies from 11 samples were screened by PCR for the presence of *blavim*, *blakPc*, *bla* NDM and *bla*OXA-48. One OXA-48-producing *Escherichia coli* was detected. Phylogenetic grouping and multi-locus sequence typing (MLST) revealed that this strain belongs to D:ST38, a strain type which has been previously reported in UK, France, Lebanon and Egypt.

The results of this study show that OXA-48-producing *Enterobacteriaceae* have started to spread into the community in Switzerland and a continuous monitoring is necessary to better understand the spread in the human population as well as in animals and the environment.

EXTENDED-SPECTRUM & LACTAMASE-PRODUCING ENTEROBACTERIACEAE IN VEGETABLES IMPORTED FROM THE DOMINICAN REPUBLIC, INDIA, THAILAND AND VIETNAM

K. Zurfluh 1, M. Nüesch-Inderbinen 1, M. Morach 1, A. Zihler Berner 2, H. Hächler 1, R. Stephan 1

- 1 Institute for Food Safety and Hygiene, University of Zurich, Switzerland
- ² Cantonal Office of Consumer Protection Aargau, Aarau, Switzerland

To examine to what extent fresh vegetables imported to Switzerland represent carriers of ESBL-producing Enterobacteriaceae, 169 samples of different types of fresh vegetables imported to Switzerland from the Dominican Republic, India, Thailand and Vietnam where analyzed. Overall, 25.4% of the vegetable samples yielded one or more ESBL-producing Enterobacteriaceae, of which 78.3% were multidrug resistant. Sixty isolates were obtained: 26 Escherichia coli, 26 Klebsiella pneumoniae, 6 Enterobacter cloacae, 1 Enterobacter aerogenes and 1 Cronobacter sakazakii.

Twenty-nine CTX-M-15, 8 CTX-M-14, 7 CTX-M-55, 3 CTX-M-65, one each of CTX-M-1, CTX-M-3 and CTX-M-63, 5 SHV-2. 3 SHV-12 and one SHV-2a were found.

Four of the *E. coli* isolates belonged to epidemiologically important clones: CTX-M-15-producing B2:ST131 (1 isolate), D:ST405 (1 isolate), and D:ST38 (2 isolates). One of the D:ST38 isolates belonged to the extraintestinal enteroaggregative *E. coli* (EAEC) D:ST38 lineage. Two of the *K. pneumoniae* isolates belonged to the epidemic clones ST15 and ST147.

Occurrence of antibiotic-resistant pathogenic and commensal *Enterobacteriaceae* in imported agricultural foodstuffs constitutes a source of ESBL genes and a concern for food safety.

ESBL detection directly from urine samples with the novel isothermal amplification-based eazyplex® SuperBug CRE assay

V. Hinic 1, J. Ziegler 1, R. Frei 1

1 Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland

Objectives: The prevalence of multiresistant extended-spectrum beta-lactamase (ESBL)- producing Enterobacteriaceae has dramatically increased over the past years, particularly in urine samples. Direct and rapid detection of ESBL in urine of patients with suspected urosepsis would be of great clinical benefit. The eazyplex® SuperBug CRE assay (Amplex Biosystems GmbH, Germany) developed for use on Genie® II (Optigene, UK) instrument is based on isothermal amplification technique. Besides the most common carbapenemases KPC, NDM, VIM, OXA-48 and OXA-181, this assay detects ESBL types CTX-M-1 and CTX-M-9 group comprising >90% of all ESBL strains in our hospital. Although primarily developed for use with rectal swabs and bacterial cultures, we evaluated this assay directly on urine samples.

Materials and Methods: 20 consecutive Enterobacteriaceae strains with suspected ESBL production isolated from urine samples were tested with eazyplex® according to the manufacturer's instructions. Simultaneously, the corresponding urine samples were tested following our newly developed eazyplex® protocol. ESBL production was confirmed by use of conventional phenotypic methods.

Results: Out of 20 urine samples tested, 17 grew ESBL-producing *Enterobacteriaceae* (16 *E. coli*, 1 *K. pneumoniae*). eazyplex® correctly detected ESBL-encoding genes in all 17 ESBL-positive isolates and in the corresponding urine samples (12 CTX-M-1 and 5 CTX-M-9 group). The remaining 3 culture isolates and corresponding urine samples tested ESBL-negative in eazyplex® and phenotypic tests confirmed AmpC production (2 *E. coli*, 1 *E. cloacae*). Hands-on time for urine samples and culture isolates was 3 min. The turnaround time was 7-16 minutes

Conclusions: Our pilot study demonstrated that the eazyplex® isothermal amplification assay is able to detect the most common CTX-M ESBL types directly from urine samples within 16 min. These rapid results might be critical for the initiation of an adequate therapy in patients with urosepsis. Additionally, this test can be used for detection of the most common ESBL and carbapenemase genes in positive blood cultures, bacterial isolates and directly from rectal swabs.

RESISTANT POPULATION CUTOFFS (RCOFFS) AND EPIDEMIOLOGICAL CUTOFFS (ECOFFS): A NEW STATISTICAL APPROACH FOR CUT-OFF DETERMINATION TO CHARACTERIZE WILD-TYPE AND NON-WILD-TYPE POPULATIONS IN ANTIMICROBIAL SUSCEPTIBILITY TESTING

G. Valsesia ¹, M. Roos ², F. P. Maurer ¹, P. Courvalin ³, E. C. Böttger ¹, M. Hombach ¹

- 1 Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland
- ² Epidemiol., Biostatistics and Prevention Inst., University of Zurich, Zurich, Switzerland
- 3 Unité des Agents Antibactériens, Institut Pasteur, Paris, France

The epidemiological cut-off (ECOFF) has been defined as the largest inhibition zone diameter of a wild-type population. ECOFFs are proposed to discriminate between wild-type and non-wild-type isolates by EUCAST and CLSI and are used to define clinical breakpoints (CBPs). However, ECOFFs do not allow a reliable separation of wild-type and non-wild-type if the wild-type and the non-wild-type populations are overlapping. Analysis of disk diameter data of E. coli, K. pneumoniae, and E. cloacae populations with seven beta-lactam antibiotics indicated that the majority of wild-type and non-wild type populations overlap. Consequently, ECOFF-based classification of non-wild-type and wild-type isolates can lead to misclassification. The new concept of the "resistant population cut-off" (RCOFF), i.e. the largest inhibition zone of a non-wild-type population, is proposed as an additional cut-off to improve characterization of antimicrobial susceptibility patterns. RCOFFs allow identification of zone diameter ranges in which isolates should be subjected to additional testing, and may be used to define an intermediate zone in the process of CBP setting. A statistic approach based on Receiver Operator Characteristic (ROC) curves was applied for determination of ECOFFs and RCOFFs for wild-type E. coli, K. pneumoniae, and E. cloacae populations and various non-wild-type populations, including extended-spectrum beta-lactamase-positive (ESBL) E. coli, K. pneumoniae and E. cloacae populations, and AmpC-cephalosporinase-positive (AmpC) E. coli and K. pneumoniae populations. A broad set of ECOFF and RCOFF data were generated with this method, even for species-drug combinations for which EUCAST ECOFFs are currently not available. Moreover, area under the curve (AUC) values for each species-drug combination were generated by ROC curve analysis, which quantitatively reflect in vitro antimicrobial activity levels and which appear to correlate with clinical outcome. To conclude, systematic calculation of ECOFFs. RCOFFs, and AUCs has the potential to support clinical breakpoint setting and, thus, the quality of antibiograms reported to clinicians.

VERTICAL TRANSMISSION OF HIGHLY SIMILAR BLACTX-M-1-HARBOURING INCI1 PLASMIDS IN ESCHERICHIA COLI WITH DIFFERENT MLST TYPES IN THE POULTRY PRODUCTION PYRAMID

K. Zurfluh* 1, J. Wang 2, J. Klumpp 3, M. Nüesch-Inderbinen 1, S. Fanning 2, R. Stephan 1

- 1 Institute for Food Safety and Hygiene, University of Zurich, Switzerland
- 2 UCD Centre for Food Safety, University College Dublin, Ireland
- 3 Institute of Food, Nutrition and Health, ETH Zürich, Switzerland

Five sets of ESBL-producing *E. coli* collected longitudinally from five different flocks of broiler breeders, meconium of one-day-old broilers from these breeder flocks, as well as from these broiler flocks before slaughter were characterized by multi-locus sequence typing (MLST), phylogenetic grouping, PCR-based replicon typing (PBRT) and resistance profiling. The *blac*TX-M-1-harbouring plasmids of one set (pHV295.1, pHV114.1 and pHV292.1) were fully sequenced and subjected to comparative analysis.

Eleven different MLST sequence types (ST) were identified with ST1056 the predominant one, isolated in all five sets either on the broiler breeder or meconium level. Plasmid sequencing revealed that *bla*_{CTX-M-1} was carried by highly similar Incl1/ST3 plasmids that were 105 076 bp, 110 997 bp and 117 269 bp in size, respectively.

The fact that genetically similar Incl1/ST3 plasmids were found in ESBL-producing *E. coli* of different MLST types isolated at the different levels in the broiler production pyramid provides strong evidence for a vertical transmission of these plasmids from a common source (nucleus poultry flocks).

RENEWAL OF INTEREST IN THE UTILIZATION OF BACTERIOPHAGES

A. Demarta ¹, JC, Piffaretti ², R, Peduzzi ³

- 1 Laboratory of applied microbiology, SUPSI, DACD, Bellinzona, Switzerland
- 2 Interlifescience, Massagno, Switzerland
- 3 Fondazione Centro Biologia Alpina Piora, Bellinzona, Switzerland

Because of their ability to specifically kill bacterial cells, at the beginning of the previous century bacteriophages were frequently used to treat infections. However, the advent of antibiotics and a number of disadvantages related to the nature of phages, lead to forget their potential utility. Nowadays, the interest in bacteriophages is increasing again, mainly because of their potential use as therapeutic alternatives to treat infections caused by multi-resistant bacteria. In 1983, thanks to a suspension of phages isolated from wastewater, we experienced the healing of an ear infection caused by a resistant *Pseudomonas aeruginosa* strain that was not responding to any antibiotic available at that time.

Besides their therapeutic use, bacteriophages can be useful in several other situations. We report here a number of applications we have successfully experienced.

- We observed that in specific situations, the viral load of surface water could be better correlated with the
 presence of bacteriophages rather than with the content of classic bacterial indicators such as faecal coliforms
 or streptococci.
- We successfully used phages as biological markers in hydrogeological studies aimed to track waters through
 a karstic zone up to the reservoirs of drinking water, thus avoiding the use of dye-based tracers.
- We have been able to determine the source of contamination of a catchment basin.
- We have successfully used specific bacteriophages to lower the amount of *Aeromonas spp* in the water of fish breeding tanks.

These experiences confirm the therapeutic and biotechnological potential of bacteriophages.

Multicenter validation of standardized MALDI-TOF based typing of ESBL-producing *Escherichia coli* outbreak isolates

M. Oberle¹, R. Frei², F. Maurer³, G. Jost⁴, A Widmer⁵, B. Sakem^{1,6}, N. Wohlwend⁶, D. Jonas⁷, C. Ottiger¹, H. Fankhauser¹, A. Eqli²

- 1 Institut für Labormedizin, Kantonsspital Aarau
- 2 University Hospital Basel
- 3 University Hospital Zurich
- 4 Dianalabs Geneva
- 5 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel
- 6 labormedizinisches zentrum Dr Risch, Bern-Liebefeld
- 7 Universitätsklinikum Freiburg, Institut für Umweltmedizin und Krankenhaushygiene, Hygiene- & Molekularlabor

Introduction: Rapid determination of strain relatedness is crucial for outbreak control. Matrix-assisted laser desorption/ionization-time of flight mass-spectrometry (MALDI-TOF) has the potential to become a high-resolution and fast typing method. However, the reproducibility and performance of MALDI-TOF based typing has not been thoroughly assessed.

Methods: We have established a standard operating procedure (SOP) to conduct MALDI-TOF based typing. To test for inter-laboratory reproducibility, we distributed 12 extended spectrum beta-lactamase (ESBL)-producing *E. coli* strains from two independent outbreaks and two non-related strains to seven laboratories. All laboratories were blinded regarding the strain relatedness and followed the SOP for work-up and analysis. Every center used a Microflex MALDI-TOF system (Bruker) and the analysis was done in quadruplicates in flexAnalysis software (Bruker). In the overlay-view masses (m/z) that were specifically present or absent for individual isolates were included in a peak list. Confirmed peak lists from all centers were evaluated for the reproducibility of MALDI-TOF based typing results. Pulsed-field gel electrophoresis was used as gold standard.

Results: The participating centers could clearly identify specific peaks for the two outbreaks strains. All participants identified between 9 and 17 peaks for each strain, two of them were predominantly found. The first outbreak strain had a specific peak at 6537 m/z (median, IQR: 6536-6539) and the second at 9714 m/z (median, IQR: 9710-9714). The variability of these peaks determined by the various centers was below 4 Daltons. The reproducibility between centers for identification of the specific peaks was 100%.

Conclusion: Using a standardized procedure for MALDI-TOF based typing allows the identification of highly reproducible outbreak strain-specific peaks within ESBL-producing *E. coli* strains. The peak list analysis enabled the correct allocation of outbreak-related and non-related strains. Due to the high reproducibility, the specific peaks can be exchanged between centers.

DRUG RESISTANCE RATES OF INTESTINAL BACTERIAL PATHOGENS

N. Wohlwend ¹, P. Kuhnert ², L. Risch ¹, M. Risch ³, T. Bodmer ¹

- 1 labormedizinisches zentrum Dr Risch, Bern-Liebefeld, Switzerland
- 2 Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland
- 3 labormedizinisches zentrum Dr Risch, Schaan, Liechtenstein

Objectives: Bacterial infectious gastroenteritis is a widespread health concern. *Campylobacter* sp. and *Salmonella* sp. are important zoonotic agents in Western countries. In contrast, the primary reservoir of *Shigella* sp. is human. Usually, bacterial gastroenteritis is self-limiting, and current guidelines preserve specific antibiotic treatment to invasive infections or debilitated patients. We investigated the drug susceptibility patterns of *Campylobacter* sp., *Salmonella* sp. and *Shigella* sp. fecal patient isolates in order to assess the up-to-date drug resistance rates among the patient collective that is served by us.

Methods: From March 1, 2014 to February 28, 2015 the drug susceptilities of 263 human fecal patient isolates including *Campylobacter* sp. (n=225), *Salmonella* sp. (n=29) and *Shigella* sp. (n=9) were tested by Vitek-2 (bioMérieux (Suisse) SA) or disk diffusion according to EUCAST guidelines.

Results: A total of 83/225 (36.9%) *Campylobacter* sp. isolates were fully susceptible to all three antibiotic agents tested, i. e. ciprofloxacin (CIP), tetracycline (TET), and erythromycin (E). The respective resistance rates were 57.3% (CIP), 33.3% (TET), and 1.8% (E). A total of 15/29 (51.7%) *Salmonella* sp. isolates were fully susceptible to all four antibiotic agents tested, i.e. ampicillin (AM), cetfriaxone (CTR), ciprofloxacin (CIP) and trimethoprim-sulfamethoxazole (SXT). The respective resistance rates were 37.9% (AM), 24.1% (CIP), 6.0% (SXT) and 0.0% (CTR). None of the nine *Shigella* sp. isolates was fully susceptible: the corresponding resistance rates were 33.3%(AM), 44.4% (CIP), 88.9% (SXT) and 11.1% (CTR), respectively.

Conclusions: We found high resistance rates in fecal patient isolates of bacterial intestinal pathogens. In *Campylobacter* sp. the rates exceeded those previously reported in epidemiological studies of human clinical isolates and veterinary screenings in Switzerland.

Our data indicate the importance of systematic drug susceptibility testing of human bacterial intestinal pathogens for epidemiological and therapeutic reasons, particularly in patients presenting with invasive enteritis and in travellers.

Evaluation of the AID Carbapenemase line probe assay for rapid detection of carbapenem resistance genes in Enterobacteriaceae.

A. Braun-Kiewnick ¹, C. Meijerink ¹, C. Ritter ¹, E. C. Böttger ¹, M. Hombach ¹, G. V. Bloemberg ¹

1 Institut für Medizinische Mikrobiologie, Universität Zürich, 8006 Zürich, Switzerland

In recent years, the emergence and spread of diverse carbapenemases in *Enterobacteriaceae* has become a major challenge for health care systems. Carbapenemase-producing bacterial isolates pose a severe clinical problem, as non-susceptibility to beta-lactams is frequently accompanied by co-resistance to additional drug classes, e.g., aminoglycosides or quinolones. As a consequence, treatment options for carbapenemase producers are limited. Reliable identification of carbapenemase-producing *Enterobacteriaceae* is necessary for proper treatment and to limit their spread. The AID carbapenemase line probe assay is based on a multiplex PCR followed by reverse hybridization using sequence-specific oligonucleotide probes. The line probe assay was verified for accuracy of its probes using both PCR products from clinical *Enterobacteriaceae* isolates harbouring KPC, VIM, NDM, GIM, AIM, SPM, IMP, and OXA-48 genes or synthetic produced genes i.e. BIC, SIM, DIM, IMI-3, IMI-1, and NMC-4 cloned into pUC57-kan and transformed into *E. coli* DH5a. Sensitivity and specificity of the Carbapenemase line probe assay was determined testing a set of 151 clinical Enterobacteriaceae strains previously characterized phenotypically (including 113 strains negative for carbapenemase genes to evaluate the possibility of false positive signals). The results show that the Carbapenemase line probe assay is an accurate and easy to use test for the detection and differentiation of carbapenemase genes, which can readily be implemented in any diagnostic laboratory.

Comparative genomic analysis of highly versus low virulent Streptococcus tigurinus strains

G. Resch ¹, A. Zbinden ², J. Entenza ¹, P. François ³

- 1 Department of Fundamental Microbiology, University of Lausanne, Switzerland
- 2 Institute of Medical Virology, University of Zurich, Switzerland
- 3 Genomic Research Laboratory, University of Geneva Hospitals, Switzerland

Streptococcus *tigurinus* is a causative agent of invasive infections such as infective endocarditis and was demonstrated to be highly virulent in a rat model of experimental endocarditis. However, differences in virulence in this model were observed with both *S. tigurinus* strains AZ_3a and AZ_14 that were highly virulent compared to the less virulent strain AZ_8. In this new study, comparative whole-genome sequencing approach was used to investigate genetic determinants that could explain these different *in vivo* phenotypes.

Phylogeny based on the alignment of 1840 core genes revealed that AZ_3a and AZ_14 strains were closely related. They diverged from a common ancestor with AZ_8 by acquisition of 214 genes amongst which numerous genes encoding for host-tissue destroying enzymes and carbohydrate metabolism were identified. As previously described in *Streptococcus agalactiae*, a possible concomitant role of these virulence and metabolic genes could explain in part the highly invasive phenotype. Another important discovery was the presence of several new LPXTG proteins that might well be important for bacterial adhesion to host tissues, i.e., the necessary first step to subsequent invasion. A new LPXTG protein located on a potential Integrative and Conjugative Element (ICE) inserted in the genome of AZ_14 and a potential large adhesin found on the genome of AZ_3a are currently under detailed investigation. Additional candidate genes for virulence and resistance were identified and are discussed herein. Finally, the presence of a genomic island harbouring several genes encoding for abortive phage infection proteins may explain why AZ_3a and AZ_14 carry less prophages than AZ_8 (2 and 0 versus 4, respectively). Accordingly, a total of 23 genes, mostly phage related, were acquired by the common ancestor to give rise to AZ_8. Whether a correlation exists between abundance of prophages and level of virulence remains to be elucidated.

BOVINE DORSAL ROOT GANGLIA NEURONS AS IN VITRO MODEL FOR LISTERIA MONOCYTOGENES BRAIN INVASION

A. Fadda 1, M. Bärtschi 2, H.R. Widmer 3, A. Oevermann 2

- 1 Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Switzerland
- 2 Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Switzerland
- 3 Department of Neurosurgery, Research Labolnselspital, Bern University Hospital, Switerland

The bacterium *Listeria monocytogenes* (LM) causes encephalitis targeting the brainstem (rhombencephalitis) in humans and ruminants, which is associated with high mortality rates. Previous studies indicated that LM invades the brain by migrating intra-axonally in cranial nerves and certain strains may have increased neurotropism.

To further test this hypothesis and to investigate molecular mechanisms of intra-axonal brain invasion, we are establishing a host specific *in vitro* model based on the culture of bovine dorsal root ganglia (DRG) neurons. DRG are sampled from slaughtered calves and various dissociation and culture protocols are assessed. The influence of the growth factors NGF, GDNF and IGF on neurite outgrowth and arborization is investigated. Cell cultures are infected with a LM strain isolated from a case of bovine rhombencephalitis in a gentamicin exclusion assay and colony forming units (CFU) are determined. Additionally, infected neuronal cultures are examined by immunofluorescence (IF).

Bovine DRG neurons cultured with this method are able to survive for more than 4 weeks. Cell cultures contain also contaminating cells including Satellite cells, Schwann cells and fibroblasts. DRG cultures are susceptible to LM infection. Bacteria mainly infect Schwann cells and Satellite cells, but are also found within neurons and their axons. Our results indicate that this model is promising for the investigation of neurotropism in LM strains and molecular mechanisms of brain invasion

Non specific transfer factors as immunotherapy in experimental Pseudomonas aeruginosa infection in rats

M. Al-Graibawi 1, A. Yousif 2, M. Abd Al-Rubaee 3, J. Darkhan 4

- 1 Zoonosis Unit, College of Vet. Medicine, Baghdad University, Baghdad, Iraq
- 2 Department of Internal & Preventive Vet. Medicine, Baghdad University, Baghdad, Iraq
- 3 Zoonosis Unit, College of Vet. Medicine, Baghdad University, Baghdad, iraq
- 4 Swiss Tropical and Public Health Institute, Basle, Switzerland

Pseudomonas aeruginosa is one of the most important opportunistic bacterial pathogens in humans and animals and a major cause of morbidity and mortality in compromised patients, with a high incidence of drug resistance. This has led researchers to investigate immunotherapeutic approaches based on either passive or active immunization to prevent and treat P. aeruginosa infections. Transfer factors (TF) are low molecular weight dialysable products from immune cells, which transmit the cell mediated immunity from sensitized donors to nonimmune recipients. The current experiment was carried out to investigate the efficiency of non specific TF extracted from colostrum of cows as immunotherapy to treat rats experimentally infected with P. aeruginosa. Transfer factor has been prepared from cow colostrums. Twenty rats P. aeruginosa free and not exposed previously to this organism were divided equally in two recipient groups. The first recipient group was inoculated intraperitionally (i.p.) with 2 ml (equivalent to 5 x108 cell/ml) of non specific transfer factorr and the second group with phosphate buffer saline pH 7.2 seven days post TF administration; all the recipients were challenged with one ml containing 2x107 cfu of virulent P.aeruginosa i.p. Rats inoculated with the non specific transfer factor showed a survival rate of 60%, while in the control group all rats died from bacteremia and septicemia within 3-5 days post infection, and P. aeruginosa was isolated from spleen and liver from dead rats. The results reported in this study demonstrated the ability of non specific transfer factor extracted from cow colostrums to reduce the mortality of rats against P. aeruginosa infection. Reviewing the available recent literatures, no report could be found concerning specific or nonspecific transfer factor as immunotherapy in *P. aeruginosa* infections.

Ethics: This study was approved by the Ethical and Research Committee of the College of Veterinary Medicine – University of Baghdad.

S. aureus biofilms: regulators of eDNA production

A. Fischer 1, P. François 1, M. Girard 1, M. Chanson 2, J. Schrenzel 1

- 1 Genomic Research Laboratory, Geneva University Hospital, Geneva, Switzerland
- 2 Laboratory of Clinical Investigation III, University of Geneva, Geneva, Switzerland

Staphylococcus aureus is a major human pathogen. Despite high incidence and morbidity, molecular mechanisms occurring during infection remain largely unknown. Under defined conditions, biofilm formation contributes to the severity of S. aureus-related infections. The presence of mecA in MRSA strains inhibits ica operon expression and favours proteinaceous biofilm formation. The structure and stability of biofilms is dependent on the release or the extracellular accumulation of sugar moieties (PIA regulated by the ica operon), but also on the presence of extracellular DNA (eDNA). eDNA provides structure and stability in mature biofilms and is degraded by DNase. eDNA facilitates cell attachment to surfaces and cell-to-cell interactions during early biofilm formation. In many bacteria, eDNA in the biofilm matrix originates from cell lysis (regulated by autolysins). But eDNA can also be actively secreted or exported by membrane vesicles.

Our aim is to understand eDNA regulation and roles during *S. aureus* biofilm formation. We focused on 27 clinical strains (26 MRSA and 1 MSSA) to study their biofilm phenotype and susceptibility to DNasel treatment. To investigate eDNA dependent matrix we deleted *gdpS*, a new actor in eDNA regulation recently identified by our group. We found that although expression of *ica* operon should be inhibited in MRSA, it might be involved in eDNA production. Using the laboratory strain SA113 and the clinical MSSA isolate UAMS-1, we confirmed *ica* involvement in eDNA production mediated by GdpS. However, its role seems to be strain dependent and needs to be clarified. To this end we plan to use the Nebraska transposon mutant library to identify genes involved in the production of eDNA and related to either *gdpS* and/or *ica* eDNA regulation. One of these genes could be RpiRc, a transcriptional regulator from the pentose phosphate pathway, linked to the TCA cycle itself involved in *ica* dependent biofilm formation.

Mutation by the Tn7-UAU cassette causes unexpected transcripts production in C. albicans responsible for reduced host fungal burden

A. Pierrehumbert 1, F. Ischer 1, D. Sanglard 1, AT. Coste 1

1 Institut de Microbiologie de l'université de Lausanne - CHUV

We initiated in our laboratory an *in vivo* screening of a *C. albicans* transcription factor (TF) mutant collection in a disseminated mouse model of infection. The screening of the Zn2Cys6 TF mutants led to the selection of a strain (BCY152) mutated for orf19.2646 by the insertion at both alleles of a Tn7-UAU cassette (1). This strain displayed a significant lower kidney fungal burden in mice as well as in *Galleria mellonella* larvae as compared to a wild-type parent. This phenotype was reversible when a wild type allele was re-introduced in the mutant, thus suggesting that the low fungal burden phenotype observed was due to the mutation in orf19.2646. However, an independent full homozygous deletion mutant of the same gene failed to recapitulate the low fungal burden phenotype in both hosts, thus indicating that orf19.2646 *per se* did not mediate tissue colonization in mouse or *G. mellonella* but rather that the Tn7 gene inactivation in BCY152 was responsible for the phenotype. Using a RACE analysis, we demonstrate here that full mRNA transcripts upstream and downstream of the Tn7-UAU cassette are unexpectedly produced in BCY152. Reintroduction in the full deletion mutant of sequences encoding both mRNA transcripts recapitulated a low fungal burden phenotype in *G. mellonella*. This study therefore demonstrates for the first time that generation of *C. albicans* mutants using a Tn7 transposons strategy might lead to erroneous phenotypes due to the occurrence of unexpected transcripts.

(1)Vandeputte P., et al. (2011), PlosOne, 6(10):e26962.

Adaptation of a Gaussia princeps Luciferase reporter system in Candida albicans for in vivo detection in the Galleria mellonella infection model

E. Delarze 1, F. Ischer 1, D. Sanglard 1, AT. Coste 1

1 Institut de Microbiologie de l'Université de Lausanne - CHUV

Mini-host models, and in particular the great wax moth *Galleria mellonella*, tend to replace murine models of fungal infection due to cost- but essentially ethical constraints. Thus, methods to study the infectious process in such a host have to be developed. In this study, we implemented the detection of *Candida albicans* cells expressing the *Gaussia princeps* luciferase in its cell wall (1) in infected larvae of *G. mellonella*. We first demonstrated that the detection of luminescence in the pulp of infected larvae was a highly reliable method for addressing drug efficacy and fungal burden assays. Moreover, since this method is faster than classical fungal burden assays, it could be applied to large scale studies. Then, using a simple CCD camera and a new soluble and non-toxic form of coelenterazine, we demonstrate that this system allows visualizing and following infection in living animals with a limit of detection of about 10³ cells per larvae. In addition, fluconazole treatment experiments over 72h in *G. mellonella* with the luciferase reporter strain were also performed. Thus, the luciferase system coupled with the use of a soluble coelenterazine is a simple method to follow *C. albicans* infection and to perform drug efficacy and *C. albicans* virulence assays in *G. mellonella*.

(1) Enjalbert B. et al., (2009), Infect Immun.,77(11):4847-58.

INDOOR MOLDS: IMPLEMENTATION OF MALDI-TOF MASS SPECTROMETRY DATABASE FOR POPULATION ANALYSIS IN DIFFERENT BUILDING ENVIRONMENTS

S. De Respinis 1, A.P. Caminada 1, A. Crenna 1, V. Guidi 1, M. Tonolla 1

1 Laboratory of applied microbiology, SUPSI, Bellinzona, Switzerland

Indoor environments are very rich in molds, their communities being strongly related to the air dampness. Identification of indoor molds is generally performed by classical microscopy, requiring the expertise of trained mycologists, or molecular techniques that are time consuming, laborious and expensive. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) can be a rapid and inexpensive system. to identify indoor molds. The MALDI-TOF MS databases are in general very performant for clinical bacteria, but very limited for the identification of environmental bacteria as well as clinical and environmental fungi. The primary objective of this project was to implement a MALDI-TOF MS database to obtain a robust and useful system for the analysis of indoor molds. Air sampling was performed in three different buildings in canton Ticino. After culturing on DG18 agar plates, the growing fungi were counted and varied between 57 and 331 CFU/m³, whereas on Rose Bengal culture medium their number was comprised between 40 and 245 CFU/m³. Most of the grown molds were sub-cultured, submitted to MALDI-TOF MS and the results subsequently analyzed by clustering. Some representatives of each cluster were analyzed by beta-tubulin partial gene sequencing and identified at the species level. Most of the strains belonged to the genera Aspergillus (Section Versicolores), Penicillium and Cladosporium. The species affiliations and quantifications depended on the building investigated, demonstrating the high variability of environmental molds. Type strains related or distant to the isolated and identified (beta-tubulin) indoor molds were cultured and analyzed by MALDI-TOF MS to create specific mass spectra and enable an identification at the species (when possible), or upper level (Section). The implemented MALDI-TOF MS database for indoor molds will further be tested on other indoor environments, to check its robustness as well as the community and population variability of molds in different buildings.

FUNGAL DETERMINANTS GOVERN THE HOST RESPONSE TO C. ALBICANS IN THE ORAL MUCOSA

F. Schönherr ¹, K. Trautwein-Weidner ¹, F. Kirchner ¹, S. Altmeier ¹, A. Gladiator ¹, C. Fragoso-Corti ², O. Petrini ³, S. LeibundGut-Landmann ¹

- 1 Institute of Microbiology at ETH Zürich and Institute of Virology at University of Zürich
- 2 Laboratorio di microbiologia applicata, SUPSI, Bellinzona, Switzerland
- 3 POLE Pharma consulting, Breganzona, Switzerland

The opportunistic fungal pathogen *Candida albicans* is a member of the normal human microbiota, but it can cause severe infections in immunocompromised individuals. It is generally believed that the host immune status determines the outcome of the interaction between the fungus and the host, resulting in either health or disease. 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.

In a mouse model of oropharyngeal candidiasis the IL-17 and neutrophil responses are strongly activated during infection with *C. albicans* strains SC5314 and both mechanisms account for the rapid control of the fungus. We explored how the capacity of the fungus to induce these characteristic responses can affect the infection dynamics. For this, we compared the host response to different clinical isolates of *C. albicans* and found that weak IL-17 and neutrophil responses resulted in persistent colonization of the oral mucosa by *C. albicans* and in delayed fungal clearance. However, IL-17 and neutrophils remained essential for preventing fungal outgrowth. These findings thus emphasize how important fungal determinants are to define the host-pathogen interaction *in vivo*, which regulates host colonization versus infection

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

A. Lohberger 1, T. Junier 2, Y. Ventura 3, N. Jeanneret 3, K. Jeanneret 3, R. Bshary 3, E. Verrecchia 4, P. Junier 3

- 1 University of Neuchâtel and University of Lausanne, Switzerland
- 2 University of Neuchâtel and Swiss Institute of Bioinformatics, Lausanne, Switzerland
- 3 University of Neuchâtel, Switzerland
- 4 University of Lausanne, 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 interaction with fungi by using the so-called "Fungal Highways" to disperse in porous unsaturated soil-like media. Dispersal allows the bacteria to reach nutrient sources, as their mobility is limited in these conditions. However, the direct fitness benefit for the transporting fungi remains unknown. Until now, a long-term benefit was shown for the ectomycorrhizal and saprophytic fungus *Morchella crassipes*, which acted as a farmer of the dispersing bacteria and storing bacterial carbon into resting structures.

We focus on the investigation of the benefit obtained by fungi during dispersal of bacteria. Experiments were all undertaken on skimmed-milk agar medium (SMA; protein as N source) to see how extracellular proteolytic activity is used to access organic nitrogen. In addition, the fungal enzymes responsible were characterized according to their activity.

We have determined that fungal proteolysis can be enhanced by the presence of *P. putida* even when fungal cells are removed. The enhanced activity requires living bacterial cells, which is also observed for other bacteria dispersing in the fungal mycelia. In conclusion, enhancement of proteolytic activity seems to be a direct fitness benefit for the interaction of *M. crassipes* with soil bacteria.

MALDI-TOF MASS SPECTROMETRY CAN DISCRIMINATE AMONG CLOSELY RELATED SPECIES OF FILAMENTOUS FUNGI

S. De Respinis 1, O. Petrini 2, M. Tonolla 1

- 1 Laboratory of applied microbiology, SUPSI, DACD, Bellinzona, Switzerland
- 2 POLE Pharma Consulting, Breganzona, Switzerland

The identification of moulds still relies heavily on morphological features, although sequencing has now been introduced in most laboratories for their reliable and comparatively quick identification. An accurate and correct fungal identification by morphology, however, is challenging for the non-mycologist who has no access to molecular techniques. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is now widely employed for the identification of clinically relevant bacteria, yeasts and increasingly also of filamentous fungi. MALDI-TOF MS has routinely been used in our laboratory to identify moulds in the genera Aspergillus, Cladosporium, Penicillium and Trichoderma, as well as the dermatophytes Epidermophyton, Microsporum, and Trichophyton. During database construction, MALDI-TOF MS results have routinely been compared to classical and molecular biology identification techniques. Validation studies have been carried out in particular on closely related or cryptic species to assess the performance of MALDI-TOF MS as compared to molecular methods. Here we present and discuss the outcome of studies carried out on: i) Aspergillus species of Sections Flavi, Fumigati and Versicolores, in particular the very closely related species Aspergillus parasiticus and A. sojae as well as A. flavus and A. oryzae; ii) the cryptic species Trichoderma asperellum and T. asperelloides; iii) sub-populations of the Trichophyton anamorph of Arthroderma benhamiae, iv) the anthropophilic and zoophilic populations of Trichophyton interdigitale; v) the very closely related species T. rubrum, "African" T. rubrum and T. violaceum. In general, MALDI-TOF MS results were comparable with those obtained by ITS sequencing; in some cases, however, MALDI-TOF MS is more performing and allows a quick and reliable identification of closely related and cryptic fungal species, otherwise not easily achieved by molecular methods.

FUNCTIONAL CHARACTERIZATION OF THE PNEUMOCYSTIS JIROVECII POTENTIAL DRUG TARGETS DHFS AND ABZ2 INVOLVED IN FOLATE BIOSYNTHESIS

A. Luraschi 1, OH. Cissé 1, M. Monod 2, M. Pagni 3, PM. Hauser 1

- 1 Institute of Microbiology, Lausanne University Hospital, Switzerland
- 2 Service of Dermatology, Lausanne University Hospital, Switzerland
- 3 Vital-IT group, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland

Pneumocystis species are fungal parasites colonizing mammal lungs with strict host specificity. Pneumocystis jirovecii is the human specific species and can turn into an opportunistic pathogen causing severe pneumonia in immuno-compromised individuals. This disease is nowadays the second most frequent life-threatening invasive fungal infection worldwide. The most efficient drug co-trimoxazole presents important side effects, and resistance towards this drug is emerging. The search of new targets for the development of new drugs is thus of utmost importance. The recent release of the P. jirovecii genome sequence opens a new era for this task. It can now be carried out on the actual targets, and no more on those of the relatively distant model Pneumocystis carinii, the species infecting rats. We focused on the folic acid biosynthesis pathway because (i) it is widely used for efficient therapeutic intervention, and (ii) it involves several enzymes that are essential for the pathogen and have no human counterparts. In this study, we report the identification of two such potential targets within the genome of P. jirovecii, the dihydrofolate synthase (dhfs)and the aminodeoxychorismate lyase (abz2). The function of these enzymes was demonstrated by the rescue of the null allele of the orthologous gene of Saccharomyces cerevisiae.

THE ABILITY OF CANDIDA GLABRATA TO CROSS EPITHELIAL CELL BARRIERS: INFLUENCE OF HYPERACTIVITY OF THE TRANSCRIPTION FACTOR PDR1

- L. Bottinelli 1, L.A. Vale-Silva 1, D. Sanglard 1
- 1 Institute of Microbiology, University Hospital Center and University of Lausanne

The incidence of life-threatening candidiasis has increased over the last decades. Available treatment options are far from ideal and candidiasis is treated with azole antifungals, among a few other drugs. Candida glabrata has been on the rise and it is the second most common Candida species causing disseminated infection, after C. albicans. C. glabrata is intrinsically less susceptible to azole drugs and quickly develops secondary resistance, typically relying on drug efflux by ABC transporters regulated by the transcription factor CqPdr1. Gain-of-function (GOF) mutations in CqPDR1 lead to a hyperactive state and thus ABC transporters upregulation. Our lab has characterized a collection of C. glabrata clinical isolates in which azole resistance correlates with increased virulence in vivo (1). Contributing phenotypes were the evasion from adhesion and phagocytosis by macrophages and increased adhesion to epithelial cells (2). An interesting follow-up question is whether CgPDR1 may also influence the ability of C. glabrata to cross epithelial cell barriers. To address that problem, we set up a yeast cell transmigration assay across epithelial cell barriers in vitro, using either human colorectal adenocarcinoma (Caco-2) or human epidermoid carcinoma (A-431) cells. The system was validated using C. albicans, which crossed the epithelial barriers in high numbers, and the non-pathogenic Saccharomyces cerevisiae, which was not able to cross. We found that C. glabrata also crosses the epithelial cell layer, although in lower numbers than C. albicans. The study is ongoing, with preliminary data suggesting that CqPDR1 hyperactivity does not influence transmigration. We are also addressing the co-infection with C. glabrata and C. albicans, mimicking what is often found in the clinical setting, as well as the influence of azole drugs. With this study we expect to elucidate important questions related to the pathogenicity of C. glabrata.

- 1. S. Ferrari et al. 2009, PLoS Pathog 5:e1000268.
- 2. L. Vale-Silva et al. 2013, Infect Immun 81, 1709-1720.

Differential secreted protease genes expression in pathogenic dermatophytes in vitro versus infection

N. De Coi 1, D. Thuong Van 2, M. Pagni 2, M. Feuermann 3, M. Monod 1

- 1 Department of Dermatology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
- 2 Vital-IT University of Lausanne, Center of Integrative Genomics, Lausanne, Switzerland
- 3 Swiss Prot, University Medical Center, rue Michel Servet 1, 1211 Geneva, Switzerland

Dermatophytes are highly specialized filamentous fungi causing the majority of superficial mycoses in humans and animals. These pathogenic fungi exclusively invade and multiply within the epidermal stratum corneum, nails or nair. During *in vitro* growth on keratin, dermatophytes secrete keratolytic activity mediated by various endo-and exoproteases, many of which have been isolated and characterized in the last decade. To investigate the individual role of these secreted proteases in dermatophytosis a guinea pig infection model was established for the zoophilic dermatophyte *Arthroderma benhamiae*, which causes highly inflammatory cutaneous infections in humans and rodents. Total RNA was extracted from infected guinea pig skin, as well as RNA extracted from *in vitro* cultures as control. High throughput sequencing revealed a distinct *in vivo* protease gene expression profile in the fungal cells, which is surprisingly different from the pattern elicited during *in vitro* growth on keratin. Instead of the major in vitro expressed proteases others were activated specifically during infection. These enzymes are therefore suggested to fulfil important functions that are not exclusively associated with the degradation of keratin. Most notably, the gene encoding the serine protease subtilisin 6, which is a known major allergen in the related dermatophyte *Trichophyton rubrum* and putatively linked to host inflammation, was found as the most strongly upregulated gene during infection. In addition, our approach identified other candidate pathogenicity related factors in *A. benhamiae*, such as genes encoding key enzymes of the glyoxylate cycle and an opsin-related protein.

Host genetic polymorphisms and Invasive mold infections in the Swiss Transplant Cohort Study

A. Wójtowicz ¹, T. Doco Lecompte ², M. Gresnigt ³, S. Bibert ¹, F. van de Veerdonk ³, C. van Delden ², P-Y. Bochud ¹, STCS. members ⁴

- 1 University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland
- 2 University Hospitals of Geneva, Geneva, Switzerland
- 3 Radboud University Medical Center, Nijmegen, The Netherlands
- 4 Due to space limitation all the remaining authors are listed below*

O Manuel¹, L Joosten⁷, S Rüeger¹, C Berger², K Boggian³, A Cusini⁴, C Garzoni^{4,6}, H Hirsch⁵, N Khanna⁵, M Weisser⁵, NJ Mueller², M Pascual¹, PR Meylan¹, J Steiger⁵, Z Kutalik¹

¹Lausanne, ²Zürich, ³St. Gallen, ⁴Bern, ⁵Basel, ⁶Lugano, Switzerland; ⁷Nijmegen, The Netherlands

Polymorphisms in immune genes have been associated with risk of invasive aspergillosis among onco-hematological patients. We explored the role of those polymorphisms in susceptibility to invasive mold infections in 1101 solid organ transplant (SOT) recipients from the Swiss Transplant Cohort Study (STCS). DNAs were isolated from patient's blood and polymorphisms were genotyped by using a custom-made Illumina® Golden Gate assay or Competitive Allele-Specific PCR (KASP™) system. Association between genotypes and the endpoint were assessed by the log-rank test and by Cox regression models after adjustment for all relevant covariates. The risk of invasive mold was independently associated with homozygocity for polymorphisms in *IL1B* (*rs16944*, HR=4.75, 95%CI 2.01-11.2, P=0.0004), *DEFB1* (*rs1800972* HR=4.18, 95%CI 1.28-13.6, P=0.02) and *PTX3* (*rs3816527* HR=2.12, 95%CI 1.14-3.95, P=0.02), respectively, together with recipient older age, CMV replication/disease, acute cellular rejection, and use of mycophenolate mofetil regiment. Presence of two copies of rare allele of *rs16944* was associated with reduced *Aspergillus*-induced IL-1β and TNF-α secretion by PBMCs. In conclusion, *PTX3*, *IL1B* and *DEFB1* polymorphisms are independent risk factors for mold infections after solid organ transplantation.

DISCRIMINATION OF CLOSELY RELATED POPULATIONS BY MALDI-TOF MASS SPECTROMETRY USING THE ABOS SOFTWARE: TRICHOPHYTON RUBRUM SENSU LATO AS A MODEL

- S. De Respinis 1, D. Dressler 2, M. Tonolla 1, O. Petrini 3
- 1 Laboratory of applied microbiology, SUPSI, DACD, Bellinzona, Switzerland
- 2 BioTeSvs GmbH, Esslingen, Germany
- 3 POLE Pharma Consulting, Breganzona, Switzerland

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is increasingly employed for the identification of filamentous fungi but the available software does not always allow discriminating closely related or cryptic species. The dermatophytes Trichophyton rubrum sensu stricto, T. soudanense (also called the "African" T. rubrum) and T. violaceum have been shown to be very closely related at the molecular level. The MALDI-TOF MS software was also not able to discriminate these species. We therefore used the ABOS software to try to separate them. Using a combination of statistical and geometric methods ABOS compares all parameters of all sample data and calculates differences to given references. Samples that show similarities within certain thresholds are grouped with the respective reference. All masses are included in the analysis, their relative relevance being mostly unclear or unknown. ABOS lists as possible markers for strains those masses that are most significant to detect differences or similarities. We tested two types of culture media, i.e. Sabouraud Dextrose Agar with (SDA) or without (SGC2) gentamicin/chloramphenicol. 174 (100 of T. rubrum sensu stricto, 64 of African T. rubrum and 10 of T. violaceum) spectra from strains grown on SDA and 136 (94 of T. rubrum sensu stricto, 33 of African T. rubrum and 9 of T. violaceum) grown on SGC2 were used. ABOS separated very well T. soudanense from the complex T. rubrum sensu lato/T. violaceum but did not distinguish satisfactorily T. violaceum from T. rubrum. Work is presently ongoing to test whether other strategies, including cultures on other media, may allow a satisfactory separation of the three groups with this software.

IMPACT OF FUNGAL MYCELIA ON THE DISPERSAL AND ACTIVITY OF **OXALOTROPHIC BACTERIA IN SOILS**

S. Bindschedler 1, A. Simon 1, EP. Verrecchia 2, P. Junier 1, LY. Wick 3

- 1 Laboratory of Microbiology, University of Neuchâtel, Switzerland
- 2 Biogeosciences laboratory, University of Lausanne, Switzerland 3 Bioavailability group, UFZ-Leipzig, Germany

The oxalate-carbonate pathway (OCP) is a process occurring in terrestrial environments that has major biogeochemical implications. In soils, oxalate produced either by plants or fungi turns into the poorly soluble calcium oxalate (Ca-ox; K_{sp} 10^{-8.5}). However Ca-ox does not accumulate. It is oxidised by oxalotrophic bacteria, leading to a local pH increase. Although CaOx is biochemically transformed by bacteria, it has been shown that for effective CaOx turnover and subsequent pH increase in soils, the presence of fungi is required. We hypothesized that in unsaturated environments fungi may promote CaOx bioaccessibility and biotransformation by allowing oxalotrophic bacteria to disperse on their mycelia ("fungal highway"-hypothesis).

To challenge this hypothesis we assessed the pH change, CaOx turnover and microbial abundance in two-compartmented soil microcosms. Compartment 1 contained a donor soil consisting of an OCP-active microbial community. Four treatments were applied to either exclude fungi, bacteria, both or none. Compartment 2 was separated from compartment 1 with an air gap and contained a target sterile acidic soil that was amended or not with CaOx. The highest pH increase and CaOx turnover in compartment 2 were observed when fungi, bacteria and CaOx were simultaneously present. This indicates that mycelial networks increased CaOx bioaccessibility to bacteria and thus promoted their oxalotrophic activity. In conclusion, our results demonstrate that fungal highways must be considered as relevant dispersal routes for bacteria, leading to the stimulation of environmental functions. Importantly, this highlights the relevance of the often underestimated role of fungi-bacteria interactions in biogeochemical processes.

TAKING THE "FUNGAL HIGHWAY": METABOLISMS AND GENES INVOLVED IN FUNGAL-DRIVEN BACTERIAL DISPERSAL IN NATURAL ECOSYSTEMS

A. Simon 1, A. Al-Dourobi 1, S. Bindschedler 1, LY. Wick 2, J. Zopfi 3, D. Job 1, EP. Verrecchia 4, P. Junier 1

- 1 University of Neuchâtel, Switzerland
- 2 Helmholtz Centre for Environmental Research UFZ, Leipzig, Germany
- 3 University of Basel, Switzerland
- 4 University of Lausanne, Switzerland

Bacterial motility in water-unsaturated soil is restricted. Previous experiments *in vitro* however have demonstrated that bacteria may take the 'fungal highway', i.e. are able to actively move along fungal hyphae. In order to get insights into such fungal-bacterial interactions *in situ*, we developed a collumn system designed to collect and cultivate fungi and associated migrator bacteria at varying growth conditions. After validation in the laboratory, our column system was placed into a soil known to exhibit metabolic fungal-bacterial interactions within the oxalate-carbonate pathway. After 4 to 8 days, the columns were removed, bacteria and fungi that grew in the columns identified, and the bacteria analyzed for their ability to use different carbon sources, fix dinitrogen and encode the type-III secretion system (as possible indicator for migrating activity along hyphae). We identified *Fusarium* sp. and *Chaetomium* sp. as fungi, and *Achromobacter* sp., *Acinetobacter calcoaceticus*, *Ochrobactrum* sp., *Olivibacter* sp., *Pseudomonas* sp., *Stenotrophomonas* sp. and *Variovorax* sp. as associated migrator bacteria. We found that the ability of bacteria to move along their fungal hosts was medium-dependent. Most of the bacteria were able to fix dinitrogen. This points to a possible trophic exchange between bacteria and fungi. Except for one bacterial strain, genes encoding type-III secretion systems were not found in the isolated bacteria.

Our findings propose that fungal hyphae are not an inert path taken by motile bacteria. 'Fungal highways' interactions seem to be mediated by trophic exchanges between fungi and bacteria, and this selection of migrator bacteria might be based on their metabolic capabilities.

The next step of this study, using a molecular biology approach, will help us to assess the diversity of microorganisms involved in 'fungal highway' interactions. Columns have been placed in various Swiss soils, and pyrosequencing has been performed on the soil, attracting and target media of the columns.

DESULFITOBACTERIUM HAFNIENSE TCE1 MEMBRANE PROTEOMICS

G. Buttet 1, R. Hamelin 2, F. Armand 2, C. Holliger 1, J. Maillard 1

- 1 Laboratory for Environmental Biotechnology, ENAC-IIE-LBE, EPFL, Lausanne, Switzerland
- 2 Proteomic Core Facility, SV PTECH PTP, EPFL, Lausanne, Switzerland

Chlorinated hydrocarbon pollution threatens nature and human health due to its toxic and carcinogenic potential. The underlying biodegradation process, organohalide respiration (OHR), is a bacterial anaerobic respiration in which the chlorinated compounds, such as chloroethenes, are used as terminal electron acceptors. Respiratory metabolism such as OHR requires the presence of an electron transport chain located in the cytoplasmic membrane which allows proton translocation and establishes a proton motive force across the membrane. Redox proteins and other non-protein electron shuttles are usually combined in the membrane to accomplish that task. Menaquinones have been shown to be involved in the electron transfer between the hydrogenase and the reductive dehalogenase but no complete electron transport chain has yet been elucidated in OHR bacteria.

A preliminary analysis allowed obtaining soluble and membrane-associated proteins from cells of $Desulfitobacterium\ hafniense\ TCE1$ that were cultivated on H_2 as electron donor and tetrachloroethene as electron acceptor. So far, we identified in total 1500 different proteins out of a theoretical maximum of 5452 proteins. For many predicted enzyme complexes, we mainly detected the non-membrane components. As extracting and detecting membrane proteins is still a challenging task, we are currently establishing a new strategy to better access the membrane proteome by mass spectrometry-based proteomics, which involves protein fractionation, membrane protein enrichment, and combination of protein extraction and digestion methods. The results of current work will be presented.

RECOMBINANT CHEMORECEPTORS IN E. COLI AND DESIGN OF CHIMERIC METHYLACCEPTING CHEMOTAXIS PROTEINS (MCPS)

V. Maffenbeier 1. JR. van der Meer 1

1 Université de Lausanne, Lausanne, Switzerland

Chemotaxis describes the ability of living cells to move towards or away from specific compounds. It involves a sophisticated signal transduction pathway which starts with binding of a chemical ligand to a membrane bound methylaccepting chemotaxis protein (MCP), which is then translated into a directional change of flagellar rotation. The system is quite well understood from Escherichia coli. MCPs are common and well conserved within Bacteria and Archaea and cluster according to their length, but very little information is available on the ligand-binding specificities of most MCPs. A few studies have shown that foreign MCPs expressed in *E. coli* can be incorporated into the signaling pathway and establish a chemotactic response. The modular architecture of MCPs has further allowed the creation of chimeric MCPs in which the ligand binding and response domains from different MCPs are fused to alter their response. This interesting technique can potentially be used to design bacterial bioreporters with new target specificities.

ANALYSIS OF SAMPLES FROM BOVINE ABORTION BY 16S RIBOSOMAL DNA AMPLICON SEQUENCING

S. Vidal 1, V. Perreten 1, S. Rodriguez-Campos 1

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

Abortion in ruminants is of worldwide concern due to the zoonotic potential of the pathogens involved and the economic impact on agriculture. The etiology of abortion can be very complex and the majority of abortions are not epizootic but sporadic. Few infectious agents are examined in routine abortion diagnosis and especially opportunistic pathogens are not included in routine analysis. Next generation sequencing (NGS) technology has become a valuable tool to study the diversity of bacterial communities based on 16S rDNA amplicon sequencing. To improve our knowledge of the bacterial community involved in bovine abortion, we studied 33 placentae and 48 fetal abomasal contents by Illumina MiSeq 16S rDNA sequencing technology (Microsynth, Switzerland).

DNA extraction was carried out using the QIAamp Mini Kit (Qiagen GmbH). For amplicon sequencing primers targeting variable regions V3-V5 of the 16S rDNA were used.

NGS data analysis identified an average of 30 representative operational taxonomic units (OTUs) for placenta samples (0.3% *Actinomycetes*, 5.6% *Bacteroidetes*, 16.3% *Firmicutes*, 2.8% *Fusobacteria* and 74.6% *Proteobacteria*) and 26 representative OTUs for abomasum samples (0.0009% *Actinomycetes*, 2.1% *Bacteroidetes*, 12.3% *Firmicutes*, 0.006% *Fusobacteria* and 85.6% *Proteobacteria*). The most abundant family found in both organs was the *Pseudomonaceae*. The results revealed opportunistic pathogens of interest such as *Campylobacter fetus* subsp. *fetus*, *Trueperella pyogenes* and *Streptococcus pluranimalium*.

Microbiome studies offer new perspectives in veterinary research which have the potential to reveal the implication of unsuspected opportunistic infections, e.g. by *Pseudomonaceae*. Increased knowledge of the microbiome may improve broadness of abortion diagnostics. Furthermore, it may shed light on the relation between the placental and fetal microbiome

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.C.J. Turlings ⁴, R. Campos Herrera ⁴, M. Maurhofer ², C. Keel ¹

- 1 Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland
- 2 Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland
- 3 Institute for Plant Production Sciences, Agroscope Changins, Changins, Switzerland
- 4 University of Neuchâtel, Neuchâtel, Switzerland

In Switzerland there is a lack of knowledge about the activity of bacteria expressing plant-beneficial traits in agricultural soils. In the context of the recent National Research Programme 68 "Sustainable use of soil as a resource" (www.nfp68.ch), we evaluate how key plant-beneficial bacteria can serve as bio-indicators of soil health. Of particular interest are root-colonizing Pseudomonas protegens and Pseudomonas chlororaphis that display both fungicidal and insecticidal activities and can be used to control fungal pathogens and pest insects. We constructed GFP-tagged reporter strains of these biocontrol agents, 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 optimized a soil extraction protocol and a flow cytometry approach. Our first data reveal pronounced differences in antifungal gene expression in the different Swiss soils. Another important aspect of our NRP 68 contribution is to develop novel environment-friendly methods to control crop diseases and pests. To this end, we test combined applications of pseudomonads with other beneficials. In a first field experiment, 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 fitness of wheat plants assessed. Ironically, the experimental field was invaded by Oscinella frit, an insect notorious for causing damage to cereal crops. Nevertheless, we observed that plants treated with the two bacterial species were less damaged. Insights into how the phytobeneficial activity of these beneficial organisms can be rendered more efficient could help Swiss farmers in the search for new agricultural strategies.

DART: DISTRIBUTION OF ANTIBIOTIC RESISTANT MICROORGANISMS IN MUNICIPAL WASTEWATER TREATMENT PLANTS EQUIPPED WITH A TERTIARY STEP TO REMOVE MICROPOLLUTANTS

- D. Ravasi 1, R. König 2, P. Principi 2, C. Corti-Fragoso 1, A. Demarta 1
- 1 Laboratory of Applied Microbiology, SUPSI, Switzerland
- 2 Bio Environmental Technologies, ICIMSI, SUPSI, Switzerland

The emergence of antibiotic and antimycotic resistant species of bacteria and yeasts represents a threat for human health and environmental welfare. Wastewater treatment plants (WWTPs) are major conveyors of micropollutants. Here, under the pressure of sub-inhibitory concentrations of micropollutants, microorganisms can mix, mutate, and exchange resistance mechanisms. Efforts are increasing to develop effective measures to prevent the release of micropollutants and resistant microorganisms into the environment. In Switzerland, WWTPs will be asked progressively up to 2025 to implement tertiary polishing steps to remove micropollutants. Different processes and technologies have been evaluated as potential options to eliminate micropollutants. However, at present, the effects of such processes on the distribution of antibiotic-resistant microorganisms in municipal WWTP outputs are not yet completely understood.

The DART project evaluates the fate of antibiotic/ antimycotic-resistant microorganisms in a WWTP equipped with a tertiary step for micropollutant removal, i.e., adsorption on powdered activated carbon (PAC), combined with a solid/ water separation through dissolved air flotation (DAF). The aims are to understand if the tertiary treatment affects the distribution of resistant bacteria and yeasts and if it can decrease the risk of the release of these microorganisms into surface waters. A laboratory scale plant with a Sequencing Batch Reactor (SBR) configuration was set up, with operational parameters following a real scale plant in Bioggio (Switzerland), which also supplied the sludge. Escherichia coli and Candida albicans were isolated from the Bioggio WWTP and their resistance to different antibiotics/ antimycotics determined. Antibiotic/ antimycotic-resistant isolates were fluorescence-tagged and inoculated into the model. The presence of labeled cells collected at different phases in the SBR treatment was quantitatively evaluated by epifluorescence microscopy and flow cytometry. The microbial community present in the WWTP was compared to the microbial community present in the SBR by denaturing gradient gel electrophoresis (DGGE) and pyrosequencing.

BIOPATINA TREATMENT FOR THE STABILIZATION OF CONTEMPORARY BRONZE ARTWORKS

M. Albini 1, P. Letardi 2, L. Mathys 1, P. Junier 1, E. Joseph 1

- 1 Laboratory of Microbiology, Institute of Biology, University of Neuchâtel, Switzerland
- 2 CNR, Institute of Marine Sciences, Genoa, Italy

To create chromatic effects on their surface, contemporary bronze artworks are often artificially patinated. To this purpose, foundries usually propose a large color palette applying chemicals directly on bare metals. Those layers result however to be incoherent with the underneath metal surface, powdery and unstable. Without further protection, that causes a color loss due to their washout. In addition, the heavy metals and toxic chemicals composing these layers are leached out in the soils and environment. Therefore it is important to stabilize them, increasing their cohesion within the underneath metal. The BIOPATINA project exploits unique properties of a specific fungal strain in order to convert existing corrosion patinas into more stable copper oxalates. In fact, thanks to their insolubility and stability even under acidic conditions, copper oxalates provide long-term stabilization to the treated objects and low aesthetical alteration. In particular, this strain can convert almost 100% of copper hydroxysulfates and hydroxychlorides into copper oxalates. In the current study bronze coupons of three different alloys and with 5 different artificial patinas were used. Results after the biopatina treatment showed a low color variation and a stabilization of the corrosion process compared to surfaces left untreated or covered with a standard protective wax. Ageing procedures are also ongoing in order to evaluate the long-term behavior of the biopatina treatment. In parallel, biopatina treatment efficiency was validated on real cases. Preliminary results on the aging monitoring and examples from the Sculpture Park "Legende d'Automne" (Lausanne) will be showed here.

BIOTECHNOLOGICAL APPLICATIONS TO WOOD CULTURAL HERITAGE

- C. Jacquet 1, M. Rebord 1, F. Danza 2, Y. Braendle 1, M. Tonolla 2, P. Junier 1, E. Joseph 1
- 1 Laboratory of Microbiology, University of Neuchâtel, Neuchâtel, Switzerland
- 2 Laboratory of applied microbiology, SUPSI, Bellinzona, Switzerland

Archaeological wood objects compose a significant part of our cultural and historical heritage. However wood, as organic material, may suffer from biological, physical or chemical damages. Depending on the burial environment conditions, two main degradation phenomena are observed once objects are exposed to oxygen after excavation. For example, fungal attack can be attested when wood must be kept wet for dendrochronology studies. Another important issue is severe wood acidification when wood originally buried in anoxic environment is contaminated with iron and sulfur compounds. The aim of this study is to exploit bacterial metabolic activities to prevent wood decay in these two cases and to propose innocuous and ecofriendly alternative methods for the conservation of waterlogged wood.

In order to prevent fungal growth on wet wood, biocontrol based on bacterial metabolites are studied. In particular, Pseudomonas fluorescens and Bacillus subtilis, which present antifungal capabilities, have been selected as model organisms. Confrontation tests with ten fungal strains will be now conducted in order to obtain extracts that can be characterized further in order to identify bioactive compounds.

In addition, to ensure the long-term protection of the waterlogged wood, bioextraction of iron and sulfur compounds will be developed. To this purpose, wood artificially degraded (by impregnation in iron- and sulfur-containing solutions) is incubated in anaerobic conditions with sulfur-oxidizing bacteria, such as phototrophic purple sulfur bacteria (*Thiodictyon syntrophicum*, *Chromatium okenii*), *Thiobacillus denitrificans* and *Acidovorax facilis*. In addition, extraction tests will be conducted with bacterial siderophores in order to chelate iron(III). SEM-EDX, FTIR, XRD and Raman analyses will allow to confirm the efficiency of the developed methods. The preliminary results will be presented within this contribution.

CAN BACTERIA AND FUNGI BE USED TO PRESERVE ARCHAEOLOGICAL IRON OBJECTS?

- L. Comensoli 1, J. Maillard 2, P. Junier 1, E. Joseph 1
- 1 University of Neuchâtel, Neuchâtel, Switzerland
- 2 Swiss Federal Institute of Technology, Lausanne, Switzerland

Without any conservation-restauration intervention, archaeological iron objects are affected by corrosion that leads to an irreversible deterioration. The main issue with this metallic substrate is the chlorine content within the corrosion layers that, reacting with H_2O and O_2 , causes flacks, cracks and loss of shape of the objects after excavation. Conservation-restauration methods available are expensive, time consuming and often employ toxic substances. Therefore, the aim of this study is to develop alternative conservation-restauration methods for iron artefacts using selected microbes.

For this purpose, anaerobic iron reduction and the formation of stable iron minerals in the presence of chlorine was investigated in two strains (LBE and TCE1) of *Desulfitobacterium hafniensis*. Fungal capacity of reducing iron and translocating and/or accumulating chlorine was also studied.

Reduction of different iron sources was tested for soluble (iron citrate) or solid iron phases (powdered iron compounds from real archaeological objects) using lactate as electron donor. Spectrophotometric analyses were conducted to ascertain iron reduction and bacterial growth was followed by quantitative PCR. Analyses by Scanning Electronic Microscopy allowed studying the iron and chlorine absorption by fungi, and finally, Fourier Transform Infrared (FTIR) and Raman Spectroscopies were used to identify the bio-minerals produced.

The results show that microbes have the potential to stabilize iron objects by both reduction into more stable minerals and by removing chloride from the object.

EVALUATION OF BIOMINERALIZATION PROPERTIES OF BACTERIA FOR THE REMOVAL OF CHLORIDE SPECIES AND STABILIZATION OF IRON ARTEFACTS

WM. Kooli 1, P. Junier 1, E. Joseph 1

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

In the field of 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 different salts containing chloride ions contaminate the corrosion crust surrounding the objects. As a result a loose of shape can be observed on the objects.

Since decades, research on iron conservation has focused on interventions aiming to control these alterations. However, conventional methods of iron conservation present some disadvantages (i.e. toxicity, cost, durability). Therefore, exploiting microbial metabolisms that are environmentally friendly processes could be an alternative for the stabilization of iron artefacts. To this purpose, this study focuses on the capacities of the halophilic *Shewanella loihica*, to stabilize iron corrosion, while preventing further damage on the artefacts. In particular, this can be achieved by forming stable iron compounds with a low molar volume. The porosity is hence increased, allowing the release of chloride ions that can be then extracted from the object, for example by osmotic diffusion in an immersion bath.

The first experiments performed aimed at evaluating the iron reduction abilities of *S. loihica* and investigating iron minerals that can be produced from soluble and amorphous iron(III) phases. SEM-EDX analyses demonstrated that different compounds precipitated depending on the iron(III) phase and culture conditions used. In particular with iron(III) citrate, an amorphous compound containing sulfur and iron that could be pyrite and the presence of a crystal phase, probably vivianite, were identified. On the contrary with iron(III) chloride, black precipitates were observed and could be due to the formation of amorphous siderite or magnetite. Further tests are now planned in order to identify the minerals formed through molecular spectroscopic techniques or X-ray diffraction.

ASSESSING THE SOIL MICROBIAL INTERACTOME

M. Dubey 1, J. R. van der Meer 1

1 University of Lausanne, 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 may to a large extent depend on the types of interactions that the microorganism is developing with other existing microbes, such as neutralism, commensalism, syntrophism or competition. Deciphering the rules governing such organised establishments is a strenuous task.

Our project is inclined towards underlining the principles of success of establishing pure cultures in complex microbial ecosystems such as contained within soil. Here we focus on the development of a high-throughput co-cultivation approach that might enable us to study the species "interactome", the identification of species combinations that decide favourable/non-favourable community fitness. The on-going study involves the use of agarose micro-beads as growth chambers. The co-cultivation technique immobilise cells allowing species-species interaction study by microscopy and flow cytometry. Such "interactome" help detecting possible species pairs, negative or positive, that can be studied in more detail separately. The resulting knowledge not only provides ample data in designing functional synthetic communities but also construct new avenues for "synthetic ecology".

THE BIODETERIOGENIC BACTERIA OF THE SAINT ROCCO ORATORY (PONTE CAPRIASCA, TICINO)

L. Mazza 1, B. Boffini 2, N. Minoretti 3, A. Demarta 4

- 1 Scuola Superiore MedicoTecnica, Locarno, Switzerland
- 2 Liceo Cantonale di Bellinzona, Bellinzona, Switzerland
- 3 Conservazione-restauro, SUPSI, DACD, Lugano, Switzerland
- 4 Laboratory of applied microbiology, SUPSI, DACD, Bellinzona, Switzerland

Artworks and historical monuments of cultural interest are exposed to environmental pollutants, climatic factors and physical and chemical treatments that may facilitate microbial growth, which may cause chemical, physical and aesthetical damages. Actions designed to limit undesirable microbial growths are possible after knowledge of the colonizing bacterial populations. For this purpose, the identification of the potential biodeteriogenic bacteria is necessary.

Ten different samples were taken from the Oratory of Saint Rocco (Ponte Capriasca, Ticino). Each sample was representative of a type of macroscopic alteration that was supposed to be of biological origin. The identification was made using basic microbiology techniques such as macroscopic examination of the microbial growths on specific media and Gram staining, as well as molecular techniques, specifically PCR and sequence analysis.

The study emphasized the complexity of the system under investigation. Cyanobacteria were not seen in any samples but it was possible to identify a very varied heterotrophic flora that included bacteria such as *Methylobacterium*, *Bradyrhizobium* and *Sphingomonas spp.* already described as biodeteriogens.

Multiple semi-continuous chemical detection by bacterial bioreporters in a microfluidics chemostat

- S. Beggah 1, N. Buffi 2, P. Renaud 2, JR. van der Meer 1
- $_{\rm 1}$ Department of Fundamental Microbiology, University of Lausanne, Switzerland
- 2 Laboratory of Microsystems, EPFL, Switzerland

Whole-cell bioreporters have been successfully applied for the detection of different target chemicals, but the development of robust and reliable bioreporter assays remains challenging. In particular, storage of bioreporter cells in such a way that they maintain and display immediate activation potential is a longstanding issue.

Here we propose a strategy that consists in culturing reporter cells in a PDMS-based microfluidic chemostat. Cells are continuously grown in a nL-reactor with integrated valves operated by pressurized channels in a second PDMS layer. The overflow of cells leads to a continuous supply of actively growing cells, which can be exploited for bioreporter assays.

To demonstrate the feasibility of a chemostat-driven biosensor-unit, we used an *Escherichia coli* EGFP-reporter strain for the detection of arsenic, a harmful chemical contaminating water supplies across the world. We show how reporter cells continuously grown in the miniaturized reactor remain in physiologically active state and how part of them can be released from the reactor and transferred to a measurement cage where they are trapped and exposed to an arsenic aqueous sample. Cells stored on chip for one week remain clearly inducible and, as the trapping is reversible, multiple measurements can be performed on the same device.

A case-study of valorization of whey from cheese producers in the Alps

N. Storelli 1, P. Principi 2, R. König 2, M. Mattogno 2

- 1 Lab. of Applied Microbiology, Uni. of applied sciences of southern Switzerland, Bellinzona
- 2 SUPSI, Bio-Environmental Technology (BET) lab

Several small and mid-sized dairy and cheese manufacturers are located, especially in Ticino, in remote alpine area. The localization in pristine areas allows for high quality dairy production locally and internationally appreciated and demand for civil responsibility in terms of waste management. In fact, even if the production activities are limited to three/four months per year and the annexed wastes are quantitatively scarce, the dairy wastes have high organic contents. Project Piora (2013) has been a case study for waste treatment feasibility in alpine isolated small facilities provided by BET group.

Within the Piora project, the high energetic potential of the cheese whey (CW) was analyzed more in detail in laboratory. The good availability, the chemical characteristics in terms of high energy content, the need for treatment before environmental discharge, and -up to now- the under-exploitation as a resource, make CW one of the best feedstock for energy recovery. This energy is commonly recycled by AD, a microbiological process performed in the absence of oxygen to stabilize organic matter while producing biogas. Bio-methane potential (BMP) tests comparing CW with others commonly used organic waste confirmed the CW high potentiality in biogas production. In order to increase the BMP, the CW was pre-treated using Chitosan (fat coagulate) and the most of the fat was removed by DAF. The pre-treated CW showed an higher and faster biogas production compared to the common CW. Moreover, we decided to focalize our attention on the microbiology of the process to further improve the biogas yield from the CW. We divide the first part of the AD from the last methanogensis step. The metabolic characteristics of both group of microorganisms are first analyzed in laboratory and then applied to the realization of 2 independent bioreactors. The resulting two-stages bioreactor would develop the best growing conditions for each metabolic pathways.

Ribosomal protein biomarkers provide root nodule bacterial identification by MALDI-TOF MS

D. Ziegler ¹, J. Pothier ², J. Ardley ³, V. Pflüger ⁴, M. Tonolla ⁵, G. Vogel ⁴, J. Howieson ³, X. Perret ¹

- 1 University of Geneva, Sciences III, Microbiology Unit, CH-1211 Geneva, Switzerland
- 2 Zurich University of Applied Sciences (ZHAW), CH-8820 Wädenswil, Switzerland
- 3 Centre for Rhizobium Studies, Murdoch University, Murdoch, Australia
- 4 Mabritec AG, Lörracherstrasse 50, CH-4125 Riehen, Switzerland
- 5 University of Applied Sciences of Southern Switzerland, CH-6500 Bellinzona

The accurate identification of soil bacteria that form nitrogen-fixing associations with legume crops is challenging given the phylogenetic diversity of root nodule bacteria (RNB), also called rhizobia. The labour-intensive and time-consuming 16S rRNA sequencing and/or multilocus sequence analysis (MLSA) of conserved genes so far remained the favoured molecular tools for characterization of RNB. With the development of mass spectrometry (MS) as an alternative method to rapidly identify bacterial isolates, we initially showed that Matrix-Assisted Laser Desorption Ionisation (MALDI) Time-Of-Flight (TOF) could accurately characterise symbiotic bacteria found inside plant nodules or grown in/on laboratory cultures (Ziegler et al. 2012). As correct identification of bacterial isolates via MALDI-TOF MS requires comprehensive spectral databases, we begun to collect reference spectra for rhizobial strains that were selected to represent the major genera of RNB. Here we report on the creation and testing of one RNB-specific database module consisting in the reference spectra of 116 symbiotic and tumorigenic strains (Ziegler et al. 2015). As the genome of each of these selected rhizobial strains was sequenced, we also showed that for each of the strains a subset of 13 ribosomal protein masses derived from genome data was sufficient for obtaining a reliable identification of nodule isolates. These results confirmed that data gathered from genome sequences can be used to expand empirical spectral libraries to aid the accurate identification of bacterial species by MALDI-TOF MS.

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THE INTERPLAY BETWEEN THE AMOEBA ACANTHAMOEBA CASTELLANII AND THE HUMAN PATHOGEN VIBRIO CHOLERAE

C. Van der Henst ¹, T. Scrignari ¹, C. Maclachlan ¹, M. Blokesch ¹

1 Ecole Polytechnique Fédérale de Lausanne (EPFL), Station 19, 1015 Lausanne, Switzerland

Vibrio cholerae is a Gram-negative bacterial pathogen, which is responsible for the severe diarrheal disease cholera. The occurrence of the bacterium in the aquatic environment represents a key epidemiological aspect of the disease as it increases the risks of cholera outbreaks. The current view about facultative bacterial pathogens suggests that virulence determinants evolved in the natural environment where they provide a fitness advantage for the pathogen. To better understand and potentially even predict cholera outbreaks, it is of prime importance to decipher the environmental life style of V. cholerae.

Among eukaryotic predators, protists such as amoebae play major roles with respect to the regulation of bacterial populations. The amoeba *Acanthamoeba castellanii* represents an interesting model for the interplay with *V. cholerae* since both organisms are members of aquatic environments. *A. castellanii* shows a biphasic life cycle between a metabolically active/feeding form (trophozoite) and a stress-induced dormant/resistant form (cyst).

In this study, we tested the ability of *V. cholerae* to survive the predation exerted by *A. castellanii* and to use the amoeba as a host for intracellular proliferation. We monitored the *A. castellanii*-colonizing bacteria in real time using live-cell confocal microscopy. We observed that *V. cholerae* shows different survival strategies that are specific for either the trophozoite or the cyst stage. Based on our observations we proposed a model of the complex life cycle between *V. cholerae* and *A. castellanii*. Next, we tested diverse mutant strains in this host-pathogen interaction model and observed impairment at different steps of the *V. cholerae* life cycle.

The data provided in this study redefines V. cholerae as a facultative intracellular pathogen. Moreover, the ability of

V. cholerae to use a natural bacterial predator as a host might contribute to its environmental fitness and the maintenance of virulence determinants.

Human pathogens in the wild - how Vibrio cholerae interacts with the amoeba Acanthamoeba castellanii

C. Van der Henst 1. M. Blokesch 1

1 Ecole Polytechnique Fédérale de Lausanne (EPFL), Station 19, 1015 Lausanne, Switzerland

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Metabolic and physiologic investigations of phototrophic purple sulfur bacteria in vitro and in situ through flow cytometry

F. Danza 1, M. Tonolla 1

1 SUPSI, DACD, Laboratory of Applied Microbiology, Bellinzona, Switzerland

Phototrophic Purple Sulfur Bacteria (PSB) are described as anaerobic anoxygenic photo-organisms that utilize reduced sulfur compounds like sulfide as electron donor and light as energy source. In batch cultures the predominant photolithoautotrophic metabolism is stimulated through light irradiation and H₂S supply. However the PSB natural environment, like the chemocline of Lake Cadagno, is subjected to continuous fluctuations. Consequently PSB need alternative metabolic strategies. Therefore, inclusion bodies of elemental sulfur and reduced carbon polymers such as polyhydroxybutyrate (PHB) are essential. Aim of this study was to investigate the dynamic and ecological significance of inclusion bodies for PSB Thiodictyon syntrophicumCad16 and Chromatium okenii. The application of flow cytometry (FC) for the characterization of physiological behavior of natural pigmented cells is a powerful technique allowing a rapid evaluation of PSB cell activity and dynamic description of intracellular inclusion bodies formation and depletion. Sideward and forward light scatter (SSC, FSC) values are linked with sulfide-oxidation and PHB biosynthesis reactions. Intracellular sulfur globules S(0) during light- oxidation of H₂S increase the internal cell complexity with a consequent rise of the SSC value. Decrease of ŠSČ during dark period corresponds to a reduction of intracellular sulfur inclusions. A similar light/dark dependence is observed with FC for PHB. These observations suggest the relevance of inclusions for PSB metabolism during dark period. Moreover a rapid kinetic reactions evaluation is also possible. Under laboratory conditions C. okenii showed a faster H₂S oxidative activity compared to other tested PSB strains. Similarly C. okenii rapidly reacted to sulfide addition during in situ experiment. Through FC characterization of PSB culture we have a tool for rapid description of PSB population activity in situ. The idea is to describe in situ the fate of a single PSB population over long time, considering the influence of external biotic and abiotic factors.

CHARACTERIZATION OF THE MOLECULAR ACTION OF THE CHAPERONE PCET ON THE REDUCTIVE DEHALOGENASE PCEA

C. Grob 1, F. Palmieri 1, G. Buttet 1, J. Maillard 1

1 Laboratory for Environmental Biotechnology (LBE), ENAC, EPFL, Lausanne, Switzerland

Chlorinated compounds are widespread soil and groundwater pollutants. Only few bacteria have the ability to degrade these compounds via organohalide respiration (OHR) in which the chlorinated compounds such as tetrachloroethene are used as terminal electron acceptors. In this process, a key enzyme, the reductive dehalogenase PceA, has been identified and characterized. It is a complex redox enzyme that is transported across the cytoplasmic membrane by the Twin-arginine translocation (Tat) pathway. In members of the bacterial genera Desulfitobacterium and Dehalobacter, it has been found that the gene pceT, which is in the direct vicinity of pceA, encodes for a protein showing sequence similarity to the Trigger Factor, a general molecular chaperone.

The goal of this study is to characterize the PceT-PceA protein interaction in order to better understand the molecular action of PceT on the maturation of PceA.

Several biochemical approaches are used to achieve this goal. First, isothermal titration calorimetry (ITC) between PceT and the Tat signal peptide of PceA provided useful information about the stoichiometry and the binding affinity of this interaction. At the same time, heterologous expression of the signal peptide of PceA fused to the fluorescent protein mCherry was monitored by fluorimetry, fluorescence microscopy and Western blot to study the Tat-dependent translocation of the fusion protein to the periplasmic space of *E. coli.* The effect of PceT on the translocation of the fusion protein was also assessed.

Biogeography of the western Swiss Alps: teasing apart the effect of the environment on the soil bacterial communities

E. Yashiro ¹, E. Pinto ², A. Buri ², D. Ciccarese ¹, H. Niculita-HIrzel ³, A. Guisan ², JR, van der Meer ¹

- 1 Department of Fundamental Microbiology, University of Lausanne, Switzerland
- 2 Department of Ecology and Evolution, University of Lausanne, Switzerland
- 3 Institut universitaire romand de santé au travail, Switzerland

The possible effects of climate change on biodiversity is of major concern for scientists, policy-makers, and laypeople. The wide elevational gradients and topographical heterogeneity in the Alps present a unique opportunity to study the effects of climate and land-use changes on this biodiversity. Indeed, the mean annual temperatures in the Swiss Alps have increased by 0.57°C per decade, while the northern hemisphere has increased by 0.25°C per decade. Over the last decade, an ongoing project at the University of Lausanne has extensively investigated plant-plant and plant-insect interactions within a 700 km² area of the Western Swiss Alps, and used these data to model and predict niche-based migration patterns in a possible future with a changed climate. However, despite the exhaustive scientific resources available for macroorganisms and abiotic processes, there is currently no systematic data available on the microbial diversity associated with the study sites. In order to fill this knowledge gap and to allow us to study the alpine biodiversity from a more holistic perspective, we have begun to investigate the bacterial community diversity in the alpine top-soils across an elevational gradient of 500-3000 m at the same sites where plant and insect data have been previously collected. Here we will present first results of soil bacterial community analysis across more than 100 sites and the correlations of soil bacteria occurrence with environmental data and plant species distributions. Initial work on the biogeographic study of functional genes related to the alpine nitrogen cycle will also be presented and related to the findings from the bacterial 16S rRNA data.

BISTABLE INTEGRATIVE AND CONJUGATIVE ELEMENT TRANSFER IN PSEUDOMONAS IS CONTROLLED BY LOCAL NUTRIENT LEVELS

F. Delavat 1, S. Pelet 1, J.R. van der Meer 1

1 DMF, University of Lausanne, Switzerland

Integrative and Conjugative Elements (ICEs) are mobile DNA, which are normally integrated in the host genome, but seldomly excise, form a circular intermediate, and conjugate to recipient cells. Here, they re-integrate into the genome at one or more specific sites. In an ICE model called ICE*clc*, originally discovered in *Pseudomonas knackmussii* B13, a subpopulation of 3 to 5% of cells in stationary phase becomes "transfer competent" (tc) for conjugation of ICE*clc*. The fate of individual cells that activate the ICE*clc* to pathway is poory understood. Here we focus on the development of new tools which enable the study of ICE*clc* excision and transfer in real-time at the single cell level.

ICE*clc* variants were created that carry the *egfp* gene downstream of the *intB13* gene for integrase. Because ICE *clc* excision and recombination at the ends causes replacement of the promoter upstream of *intB13*, we expected that cells in which ICE*clc* is excised would be distinguishable by their EGFP expression. Specific recipients were constructed, which would "light up" when ICE*clc* integrates in their genome. Time-lapse microscopy of individual *Pseudomonas putida* cells carrying such ICE*clc* indicated three different EGFP expression levels. Further time-lapse microscopy experiments of *P. putida* donor and recipient cells indicated that the highest EGFP levels in donors was associated with cells actually transferring ICE*clc*. In contrast, the lowest EGFP expression occurred in cells in which ICE*clc* remains silent, and intermediate EGFP expression was found for cells which start the to pathway. Interestingly, therefore, donor cells which activate ICE*clc* in stationary phase do not immediately excise it. Probably, only to cells having access to sufficient nutrient are able to engage ICE*clc* excision, while most to cells depleted in nutrient are not. Our results thus illustrate the unique steps in ICE transfer dependent in a broader ecological context.

PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF LISTERIA MONOCYTOGENES STRAINS ISOLATED DURING 2011-2014 FROM DIFFERENT FOOD MATRICES IN SWITZERLAND

R. Ebner 1, R. Stephan 1, D. Althaus 1, S. Brisse 2, M. Maury 2, T. Tasara 1

- 1 Institute for Food Safety and Hygiene, University of Zurich, Zurich, Switzerland
- 2 Institut Pasteur, Microbial Evolutionary Genomics, Paris, France; CNRS, Paris, France

One hundred and forty two *L. monocytogenes* strains isolated from different food matrices in Switzerland between 2011 and 2014 were characterized with respect to their genotypic and phenotypic properties. Analyzed strains originated from various meat, milk, plant-associated food products and production environments as well as from other types of foods including fish, seafood, and ready to eat (RTE) products. The collection included serotype 1/2a (64%), 4b (15%), 1/2c (12%), 1/2b (7%) and 3c (3%). The strains were genetically diverse representing 61 MLST sequence types (ST) including 24 new STs. The most frequent clonal complexes (CC) were CC9 (15%) and CC121 (12%). PCR screening detected presence of the stress survival islet (SSI-1) in 50 % of the strains. Phenotypic resistance to benzalkonium chloride (BC) was detected in 18% of the strains. The BC resistance genetic determinants *qacH* and *bcrABC* were detected in 80% and 12% of the strains, respectively. Most (n=129) of the strains isolated from Swiss food matrices exhibited poor biofilm formation capacity and there were no correlations detected between strain serotypes, genotypes and biofilm production.

FUNCTIONAL CHARACTERIZATION OF THE WADDLIA TRANSCRIPTION FACTOR EUO

M. de Barsy ¹, A. Frandi ², G. Panis ², L. Theraulaz ², T. Pillonel ¹, P. Viollier ², G. Greub ¹

- ₁ Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne
- 2 Dept. Microbiology and Molecular Medicine, University of Geneva, Switzerland

Waddlia chondrophila belongs to the Chlamydiales order which includes the Chlamydiaceae family, whose members are important human and animal pathogens. Growing evidence suggests the role of W. chondrophila in human miscarriage and animal abortion. All Chlamydiales members share a similar developmental cycle divided in three stages. During the early stage, elementary bodies (EBs) enter host cells and convert into reticulate bodies (RBs), then RBs divide by binary fission (mid stage) and finally RBs convert back into EBs that are released after host cell lysis (late stage). Euo is a well characterized transcription factor (TF) in Chlamydia that was first identified as an early gene. Recent studies showed that Euo regulates σ_{66} - and σ_{28} - dependent late genes such as omcAB and omcAB and omcAB respectively.

In order to gain insight if the regulon and regulation of Euo is conserved between different members of the *Chlamydiales*, we explored the temporal expression of *Waddlia* Euo at the transcriptional level and protein level and its promoter specificity. We showed that *euo* is transcribed during the late phase of the *Waddlia* developmental cycle and that the Euo protein is present in the early phase. We showed that Euo binds 19 promoters and we dissected the binding site preference for three of these promoters. We identified a common Euo target box. Finally, we showed that *Waddlia* Euo target genes are mainly transcriptionally activated in mid-stage and we suggest that Euo represses their expression.

Our data suggest that, in contrast to *Chlamydia*, *Waddlia euo* is a late gene and that Euo protein is produced during the early stage of the developmental cycle in order to repress the expression of mid-genes.

INVESTIGATING THE ROLE OF PSEUDOMONAS PROTEGENS EXTRACELLULAR POLYSACCHARIDES IN HOST COLONIZATION AND PATHOGENICITY

- C. Terrettaz 1, P. Kupferschmied 1, M. Péchy-Tarr 1, T. Chai 1, M. Maurhofer 2, C. Keel 1
- 1 Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland
- ² Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland

Extracellular polysaccharides represent an important fraction of bacterial secreted molecules. They are essential constituents of the bacterial cell envelope and its associated matrix and are crucial for biofilm formation. Consequently they have various important functions in maintaining cellular integrity and protecting the cell against harsh environmental conditions, mechanistic shocks, and toxic compounds. Extracellular polysaccharides are also critical for the virulence of pathogenic bacteria as exemplified by the opportunistic Gram-negative pathogen Pseudomonas aeruginosa in which both membrane-anchored O-polysaccharides (outermost components of the lipopolysaccharide molecule) and biofilm polysaccharides are involved in chronic infections of patients. Major biofilm components in P. aeruginosa comprise the exopolysaccharides alginate, Psl and Pel, each of them exerting specific functions and contributing to the high complexity of the extracellular matrix. Remarkably, complete gene clusters encoding the biosynthesis of alginate, Psl and Pel are also present in the genome of Pseudomonas protegens. We also identified the gene clusters required for O-polysaccharide synthesis (see abstract Kupferschmied et al.) as well as two additional polysaccharide biosynthesis clusters in this species. P. protegens group bacteria are effective plant root colonizers with biocontrol activity against soil-borne fungal pathogens and in addition display also potent oral and systemic insecticidal activity against agricultural pest insects. We have begun to investigate the role of these polysaccharides in the insect pathogenicity of P. protegens and in the bacterium's capacity to competitively colonize insect and plant hosts. To this end we follow a targeted mutational analysis approach. We expect to obtain novel insights into the function of the extraordinary diversity of extracellular polysaccharides of *P. protegens* in host colonization and virulence. First findings will be presented.

COULD AEROMONAS BACTERIOPHAGES BE INVOLVED IN HORIZONTAL GENE TRANSFER?

M. Mauri 1, D. Demarta 1

1 Laboratory of applied microbiology, Bellinzona, Switzerland

Bacteria belonging to the genus Aeromonas are usually sensitive to most of the antibiotics used in clinical settings but in the last years there was an increase of antibiotic resistant isolates. Since the rise of antibiotic resistant pathogens is a worldwide problem, studies on how antibiotic resistant genes could spread among bacteria are mandatory. We chose Aeromonas as a model to investigate the dissemination of resistance genes in the aquatic environment. Besides conjugation and transformation, bacteriophages seem to play an important role in the horizontal gene transfer. The aims of this study are to investigate the presence of resistance genes in lytic and lysogenic Aeromonas bacteriophages and to verify whether these genes could be transduced to host bacteria. Aeromonas strains were isolated from different aquatic environment (hospital wastewater, wastewater treatment plant, river upstream and downstream the outlet of the WWTP and alpine lake) and their resistance profiles were determined by disc diffusion test. The presence of resistance genes and of transposon 21 (tn21) were tested by PCR. We were able to recover 17 lysogenic bacteriophages by inducing with mitomycin C 231 Aeromonas strains. Moreover, 21 lytic phages previously isolated from water, were tested for their capability to infect our strains. All the phages suspensions were purified and concentrated by PEG/NaCl: DNA was extracted from phages using a phenol-chloroform method prior to perform specific PCR amplifications of resistance genes. In two suspensions of lysogenic bacteriophages we were able of amplifying the resistance determinants present in the donor strain (i.e. tn21, dfr22 and sul1). Thus, lysogenic bacteriophages of Aeromonas can integrate resistance genes from their hosts and that they could therefore be involved in the horizontal gene transfer of resistant determinants. At present, we are using these lysogenic phages to perform transduction experiments on propagation hosts. The 21 suspensions of lytic phages did not carry genes for antibiotic resistance; nevertheless we are testing their ability to incorporate resistance genes from donors and to transfer them into recipient hosts.

Epigenetic control of a bacterial cell cycle

D. Gonzalez 1, J. Collier 1

1 University of Lausanne, Switzerland

DNA methylation is involved in a diversity of processes in bacteria, including maintenance of genome integrity and regulation of gene expression. Here, using *Caulobacter crescentus* as a model, we exploit genome-wide experimental methods to uncover the functions of CcrM, a DNA methyltransferase conserved in most *Alphaproteobacteria*. Using single molecule sequencing, we provide evidence that most CcrM target motifs (GANTC) switch from a fully methylated to a hemi-methylated state when they are replicated, and back to a fully methylated state at the onset of cell division. We show that DNA methylation by CcrM is not required for the control of the initiation of chromosome replication or for DNA mismatch repair. By contrast, our transcriptome analysis shows that >10% of the genes are misexpressed in cells lacking or constitutively over-expressing CcrM. Strikingly, GANTC methylation is needed for the efficient transcription of dozens of genes that are essential for cell cycle progression, in particular for DNA metabolism and cell division. Many of them are controlled by promoters methylated by CcrM and co-regulated by other global cell cycle regulators, demonstrating an extensive cross talk between DNA methylation and the complex regulatory network that controls the cell cycle of *C. crescentus* and, presumably, of many other *Alphaproteobacteria*.

The Candidatus Thiodictyon syntrophicum strain Cad16T genome revised

S. Lüdin ¹, F. Danza ¹, P. Schneeberger ², J. F. Pothier ³, M. Wittwer ⁴, M. Tonolla ¹

- 1 University of Geneva, Microbiology Unit, Geneva, Switzerland
- 2 Swiss TPH, Department of Epidemiology and Public Health, Basel, Switzerland 3 Zurich University of Applied Sciences ZHAW, Wädenswil, Switzerland
- 4 Spiez Laboratory, Bacteriology, Federal Office for Civil Protection, Spiez, Switzerland

Background: The meromictic aloine lake Cadagno (Switzerland) is a model ecosystem for the study of phototrophic sulphur bacteria (PSB) communities since it provides a stable vertical physiochemical gradient over time. High concentration of sulfate favors the development of microbial populations directly involved in the sulfur cycle. Members of the family Chromatiaceae such as Chromatium okenii. Lamprocystis purpurea and the genus Thyocystis and Thiodictyon have been extensively studied by 16S rRNA sequence analysis and have been isolated

The CO₂ fixation capacity of Candidatus "Thiodictyon syntrophicum" has been studied in vivo and in vitro. Despite "T. syntrophicum" only represents 2% of the total PSB community, it provides an estimate of 25% of the total primary production in the chemocline. Further studies on the "T. syntrophicum" sulphur metabolism have led to the discovery of novel carotene ketolases and the autotrophic dicarboxylate/4-hydroxybutyrate cycle, normally found in archaea.

Study Outline: As a basis for further proteogenomic studies of the sulphur metabolism, we sequenced the "T. syntrophicum" 7.3 Mb genome. Here we describe the features of "T, syntrophicum", in combination with the draft genome and a preliminary annotation. We thereby especially focused on the enzymatic pathways involved in sulphur metabolism and trafficking.

For the de novo assembly and finishing of the T. syntrophicum genome we used a hybrid approach combining shorter IonTorrent PGM and MiSeg reads with Single Molecule. Real-Time (SMRT) PacBio reads. To complete the genome assembly and scaffolding different hierarchical and hybrid assemblers were used (MIRA, SPADES, CL and HGAP).

New insights into the two regulatory proteins TfoX and TfoY of Vibrio cholerae

L.C. Metzger 1, S. Borgeaud 1, M. Blokesch 1

1 Global Health Institute, School of Life Sciences, École polytechnique fédérale de Lausanne

In the aquatic environment, the facultative human pathogen *Vibrio cholerae* lives closely associated with zooplankton and their molted exoskeletons. Such exoskeletons contain chitin, which induces natural competence for transformation. Natural competence is a mode of horizontal gene transfer and describes a developmental state in which bacteria are able to take up free DNA from the surrounding and to recombine this DNA into their own genome. In *V. cholerae* the initiation of this developmental program requires certain signaling molecules and the interconnection of multiple regulatory pathways. In particular the production of the main regulator TfoX is of prime importance. This regulator is required for the expression of the so so-called competence genes even though the protein lacks any prominent DNA-binding motif. Instead, TfoX and its homologs display a two-domain architecture and both domains are highly abundant in the prokaryotic world. Still, no biological function has been assigned to these domains. Interestingly, a second homolog of TfoX exists in all *Vibrio* species (TfoY) for which no phenotype has been determined yet. Here, we present new insights into these two proteins and show that TfoY is dispensable for natural transformation of *V. cholerae* but involved in the bacterium's motility control network. As a summary, we propose a model that shows a novel and unusual mode of action for TfoX and TfoY.

OmpA family proteins and Pmp-like autotransporter: new adhesins of Waddlia chondrophila

C. Kebbi Beghdadi ¹, A. Domröse ², E. Becker ², O. Cisse ¹, J. H. Hegemann ², G. Greub ¹

1 Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne 2 Inst für Funkt. Genomforschung der Mikroorganismen, H.-Heine Universität, Düsseldorf

Waddlia chondrophila is an obligate intracellular bacteria belonging to the Chlamydiales order, a clade that also includes the well-known classical Chlamydia responsible for a number of severe human and animal diseases. Waddlia is an emerging pathogen causing adverse pregnancy outcomes in humans and abortion in ruminants. Adhesion to the host cell is an essential prerequisite for survival of every strict intracellular bacteria and, in classical

Chlamydia, this step is partially mediated by polymorphic outer membrane proteins (Pmps), a family of highly diverse autotransporters that represent about 15% of the bacterial coding capacity. Waddlia chondrophila genome however only encodes one putative Pmp-like protein. Using a proteomic approach, we identified several bacterial proteins potentially implicated in the adhesion process and we characterized their expression during the replication cycle of the bacteria. In addition, we demonstrated that the Waddlia Pmp-like autotransporter as well as OmpA2 and OmpA3, two members of the extended Waddlia OmpA protein family, exhibit adhesive properties on epithelial cells. We hypothesize that the large diversity of the OmpA protein family is linked to the wide host range of these bacteria that are able to enter and multiply in various host cells ranging from protozoa to mamalian, fish and insect

Kinetics of Pseudomonas aeruginosa CzcRS two-component system induction

V. Ducret 1, F. Laroche 2, G. Vilmart 2, K. Perron 1

- 1 Microbiology Unit, Dept of Botany and Plant Biology University of Geneva, Switzerland
- 2 Section of Mathematics, University of Geneva, Switzerland

Two-component systems are well known mechanisms allowing bacteria to respond quickly and specifically to changing environmental conditions and stresses. These systems consist of a sensor protein, involved in the detection of a stimulus, that activates by phosphorylation a response regulator protein. This response regulator is often a transcriptional factor that modulates gene expression in order to respond to the external stimulus.

In the laboratory, we studied the CzcRS two-component system of *Pseudomonas aeruginosa* involved in the response to an excess of zinc or cadmium. Using a series of qRT-PCR experiments, Western blot analysis and GFP reporter assays, we investigated the timing of induction of CzcRS and its target gene upon different zinc treatments. We found a strong discrepancy between the CzcR response regulator mRNA levels and the CzcR protein amounts, suggesting that this two-component system alone may not explain the fast metal stress response. In addition, the analysis of several mutants affected in zinc responses indicated that other regulators might be involved in the initial step of CzcRS induction.

Altogether, our data permits to precisely determine the induction kinetics of this *Pseudomonas aeruginosa* two-component system, allowing the precise deciphering of this complex metal response regulatory circuit. These results also open the way for the mathematical modeling of such a two-component system induction.

SEQUENCING THE OBLIGATE INTRACELLULAR RHABDOCHLAMYDIA SP. WITHIN ITS TICK HOST IXODES RICINUS

T. Pillonel 1, C. Bertelli 1, L. Pilloux 1, G. Greub 1

1 Center for Research on Intracellular Bacteria, Institute of Microbiology, CHUV, Lausanne

Members of the phylum Chlamydiae were long restricted to a single genus of closely-related human and animal pathogens such as Chlamydia trachomatis. However, the chlamydial biodiversity is likely underestimated. Investigations on the prevalence of Chlamydiae in ticks originating from 172 sites throughout Switzerland revealed the presence of chlamydial 16S ribosomal RNA (16S rRNA) encoding gene in as much as 6.4 percent of the 8534 analyzed pools of ticks. One third of the sequences were phylogenetically related to the Parachlamydiaceae family, another third to the Rhabdochlamydiaceae family. The last third of the 16S rRNA sequences presented less than 90% identity with known chlamydial families. Thus, ticks may be a reservoir and vector of bacteria belonging to the order Chlamydiales. As Rhabdochlamydiaceae species are poorly studied and because no representative strain is available in culture, we sequenced the DNA directly from one pool of ticks presenting a high concentration of 16S rRNA highly similar to Rhabochlamydia crassificans.

The genome was sequenced using the MiSeq system with paired-end 150 bp reads. Over 24 million reads were obtained, containing approximately 40% of *Ixodes ricinus* sequences that were filtered out by mapping to the available draft genome of *Ixodes scapularis* and *Ixodes ricinus*. The genome assembly resulted in an estimated genome size of 1.8 Mbp with a GC content of 36%. Comparative analysis of ribosomal RNAs as well as 8 conserved genes confirmed its distant phylogenetical relationship with members of other chlamydial families (87.94 – 91.78% 16S rRNA identity) and its classification in a family called *Rhabdochlamydiaceae*.

Preliminary analysis allowed the identification of common factors involved in host-pathogens interaction such as the type III secretion system. Further investigations of this first genome within the family Rhabdochlamydiaceae will give us further insights into the biology of this bacterium with peculiar cell morphologies.

THE INTERPLAY BETWEEN THE DNA-UPTAKE MACHINERY AND THE TYPE VI SECRETION SYSTEM OF VIBRIO CHOLERAE

N. Matthey 1, M. Blokesch 1

1 Global Health Institute, EPFL, Switzerland

In evolutionary terms, horizontal gene transfer (HGT) constitutes an important driving force for the acquisition of new genes and functions allowing rapid bacterial adaptation to specific niches. Natural competence for transformation, which is a mode of HGT, implies the direct uptake and integration of exogenous DNA.

Vibrio cholerae is the causative agent of cholera. This pathogen is an aquatic bacterium that lives in the ocean as a free-living bacterium or associated with zooplankton. Natural competence for transformation of Vibrio cholerae is activated by the master regulator TfoXupon high cell density. This regulator is induced on chitinous surfaces, such as the exoskeletons of zooplankton. The DNA-uptake machinery, which is regulated by TfoX, is composed of a type 4 pilus (T4P) responsible of the incorporation of exogenous DNA into the periplasm, and other competence proteins that drive the inner membrane translocation. Interestingly, the type VI secretion system (T6SS) cluster is among the competence genes positively regulated by TfoX. The T6SS, often described as an inverted bacteriophage, is used to target and kill cells through the delivery of effectors proteins. The effector proteins encoded by the T6SS cluster are coupled with their corresponding immunity genes preventing self-destruction. This indicates that prey cells having the same set of effectors-immunity genes as the predator cells are protected against their attacks. Here, we illustrate that that that tho induction leads to the activation of the T6SS and killing of non-immune prey. Moreover, based on live-cell imaging we show that the successful attack provokes the release of genomic DNA from the prey, which is absorbed by the competent predator. Therefore the T6SS improves the acquisition of exogenous DNA in V. cholerae. We are currently assessing the co-regulation of the T6SS and the T4P, and we are testing whether the T4P or another pilus could facilitate the killing process.

Expression of 81655 operon and localization of orf81655 promoter region in Pseudomonas knackmussii B13 presumes its role in ICEclc transfer

S. Susler 1, F. Delavat 1, A. Vucicevic 1, V. Sentchilo 1, JR. van der Meer 1

1 University of Lausanne, Department of Fundamental Microbiology, 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, ICEc/lc 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 ICE*clc* in transfer remains obscure. One such example is comprised by a 14 kb cluster on ICE*clc* (provisorily named the *81655* operon), whose expression is among the highest detected so far for ICE*clc* genes. Here we investigate the role of the *81655* operon on ICE*clc* transfer and try to understand the nature of its extremely high expression.

By using gene replacement techniques, we find that several of the genes within the *81655* operon are directly involved in ICE*clc* 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 hybridisations 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 ICE*clc* showed expression only in cells containing ICE*clc* and only in a small fraction of cells. Expression coincided in cells which simultaneously expressed other ICE*clc* promoters, such as the *intB13* integrase promoter. This suggested that the *81655* is part of the same bistable regulon that typifies ICE*clc* transfer competence formation. RNAseq, fluorescent in situ hybridization and promoter deletion mapping confined the *81655* promoter to a specific region, which showed similar sequence motifs as other bistable ICE*clc* promoters. Mutation analysis of the 5' UTR region of the *81655* mRNA transcript is performed to understand why transcription from this operon is extremely high but translation is rather poor.

MICROBIAL FUEL CELL WITH ANODE STRUCTURE MIMICKING CICADA WING

M. Sugnaux 1, S. Wu 2, Q. Ren 3, J. Brugger 4, F. Fischer 1

- 1 University of Applied Sciences and Arts Western Switzerland, HES-SO, Sion, Switzerland
- 2 Beijing Jiaotong University, Beijing, P.R. China
- 3 Swiss Federal Laboratories for Materials Science and Technology, St. Gallen, Switzerland
- 4 Ecole Polytechnique Fédérale Lausanne, EPFL, Switzerland

Nano-structured surfaces provide higher surface area than the corresponding flat surfaces. To study the power-surface relationship within a microbial fuel cell, electrodes with Au nano-structures were used in comparison with flat Au surfaces in our study. Electrodes with Au nanopillar and nanoflower surface architectures were fabricated by templated electrodeposition technology. The nanopillars are ~ 50 nm in diameter, 50-300 nm in height with density of ~ 100 pillars/µm². The nanoflowers have a ring-like structure with diameter of 100 nm, height of 50 nm and density of 50 rings/µm². Escherichia coli K12 was used as a model microorganism. In a bi-cathodic microbial fuel cell biofilm formation was monitored and compared with a simultaneously inserted gold surface that was flat and without any nanostructure. The *E. coli* formed biofilm on the nano-structured electrode and the cells were shown to be viable by SYTO9/propidium iodide staining, whereas no biofilm was found on the unstructured flat surface. Power current plots from double chambered microbial fuel cell experiments showed that nano-structured surfaces resulted in high power maxima of 1986 mW/m², whereas the flat surface showed background power characteristics as a result from contact with planktonic cells and media. Beside the proposed nanopillar respiration mechanism also electro catalytic oxidation of secondary metabolites was a likely contributor to observed power levels. Our results demonstrate that nano-surfaces provide higher surface area for cells to adhere and also act as electron sink.

Comprehensive identification of regulatory network interactions in Chlamydiae

A. Frandi 1, M. De Barsy 2, L. Theraulaz 1, G. Greub 2, P. Viollier 1

- 1 University of Geneva, Geneva, Switzerland
- 2 University Hospital Center, CHUV, Lausanne, Switzerland

While only the 2% of the coding capacity of chlamydial genomes is devoted to transcriptional regulation, free-living bacteria usually encode tenfold more classical transcription factors (TFs). Because of this low TFs multiplicity, Chlamydiae present an ideal systme to explore a minimal regulatory genome. The 10 conserved TFs encoded in the chlamydial pan-genome likely not only govern cellular homeostasis but also the complex developmental programs that direct the interconversion of the chlamydial morphotypes, the elementary bodies (EBs) and reticuate bodies (RBs).

Our aim is to explore the spatio-temporal expression, the functions(s) and the regulatory network orchestrated by the 10TFs of the *chlamydial* pan-genome using *Waddlia chondrophila* as a model system.

We purified all 10 conserved TFs and raised antibodies against them. Using immunoblotting, we determined the steady-state levels of the TFs as function of the developmental cycle and correlated protein levels with transcript abundance as measured by qRT-PCR per chlamydial cell. We also determined the target motifs for each TF in order to compare the conserved set of genes harboring the target motif within the chlamydial pan-genome.

We defined three groups, based on the time at which TF transcript levels peak: late (48-72 hours post infection hpi, EBs), mid (16-24 hpi, late RBs), and early (3-8 hpi, early RBs). The three groups comprise respectively: 2 (late: Euo and AtoC), 7 (mid: ParB, YtgC, DnaA1, DnaA3, PhoB, HrcA, and Wcw_1223) and 1 (early: NrdR) TFs. The W. chondrophila Euo transcript peaks early in the developmental cycle, reaching a plateau which is maintained also in late developmental stages. The other TFs showed temporal control of their transcripts, as for instance Wcw_1223 and DnaA3 which peak at the end of the early phase and their transcript levels are maintained constant until disappearing in late phase. The transcript for HrcA exhibits a sharp peak at the end of the mid phase contrary to the HrcA protein whose levels remain constant even after the transcripts are no longer detectable, suggesting that HrcA is a very stable protein.

Collectively our experiments provide a first glimpse at the TFs that regulate the extraction of genetic information from the *Waddlia* genome and set the stage for decoding the underlying transcriptional circuitry of the *chlamydial* pan-genome.

Regulatory interplay between cell cycle control and phosphate starvation in Caulobacter crescentus

M. Delaby ¹, G. Panis ¹, C. Fumeaux ¹, P. Viollier ¹

1 Department of Microbiology & Molecular Medicine, Faculty of Medicine, University of Geneva

Caulobacter crescentus like many alpha-proteobacteria undergoes an asymmetric cell division, giving two progeny cells with distinct morphologies and fates: the sessile replicating stalked (St) cell and the adventurous quiescent swarmer (Sw) cell that resides in G1-like non-replicative state and must differentiate into the St cell before proceeding to division. In Caulobacter, cell cycle progression, differentiation and chromosomal replication are believed to be controlled by a cyclical complex genetic circuit comprising four essential and conserved transcriptional regulators that sequentially activate cell cycle-controlled promoters. Our work is focused on these regulators, the enigmatic GcrA protein, and we are now using genetic and genomic approaches to investigate these functional interactions further. Unexpectedly, we previously discovered that GcrA is in fact dispensable, finding that $\Delta gcrA$ cells are viable, but slow-growing, non-motile and elongated, with the latter mostly due to an insufficiency in key cell division proteins. By using suppressor genetics, we identified independent mutations in both phosphate (pho) uptake and phosphate signaling pathways as $\Delta gcrA$ suppressors, pointing to an unknown relationship of the phosphate starvation response and the GcrA regulon. While we found that GcrA-dependent promoters are indeed affected in ΔphoB cells, lacking the transcriptional regulator PhoB, ChIP-Seq failed to reveal binding of PhoB to these GcrA-dependent promoters, suggesting that the suppression is indirect and likely affects another pathway. We are now testing for such possible indirect effects using epistasis and ChIP-seg experiments to understand how pho signaling impinges on GcrA-dependent cell cycle control in Caulobacter.

TRACKING BIOTRANSFORMATION OF HEXACHLOROCYCLOHEXANES BY COMPOUND-SPECIFIC ISOTOPE ANALYSIS

I. E. Schilling 1, T. B. Hofstetter 1, H.-P. E. Kohler 1

₁ Eawag, Swiss Federal Institute of Aquatic Sience and Technology, Dübendorf, Switzerland

Hexachlorocyclohexanes (HCHs) were used frequently in agriculture and medicine due to their pesticidal activity. The persistence of HCH led to their frequent detection as diffuse pollution and point sources. In this work, we explore the use of compound-specific isotope analysis for assessing the extent and pathway of HCH biodegradation. To quantify the C- and Cl-isotope effects of biodegradation by substitution and elimination reactions, we use experimental systems with purified LinA enzymes. First experiments show that even at low turnover, C-isotope fractionation can be detected. We also observe a consistent C-isotope fractionation in the reaction product trichlorobenzene. Current work focuses on model systems, with which leaving group Cl-isotope effects can be quantified from the inorganic reaction product (chloride).

RESPONSE TO NITROGEN LIMITATION IN BURKHOLDERIA PHYMATUM AND COMPETITION BETWEEN BETA-RHIZOBIA FOR LEGUMES INFECTION

M. Lardi 1, G. Purtschert 1, M. Sanchez-Contreras 1, A. Pedrioli 1, Y. Liu 1, C. Ahrens 2, L. Eberl 1, G. Pessi 1

- 1 Institute of Plant Biology, Department of Microbiology, UZH Zurich, Switzerland
- 2 Institute for Plant Production Sciences, Agroscope, Wädenswil, Switzerland

The rhizobium-legume symbiosis is of major ecological and economic importance and accounts for two-thirds of the nitrogen fixed globally. Until recently, all known example of symbiotic relationship between legumes and prokaryotes were confined to the phylogenetically diverse α -rhizobia (α -proteobacteria). This changed with the discovery of certain β -proteobacteria of the genera Cupriavidus and Burkholderia (β -rhizobia), which are also capable of establishing nitrogen-fixing symbiosis with legumes. At present very little is known about the molecular determinants underlying the successful establishment of the symbiosis between legumes and β -rhizobia.

In the soil and rhizosphere, rhizobia are subject to changing environmental conditions including nutritional stresses. Under nitrogen limited conditions it has been shown that β-rhizobial strains such as *Burkholderia phymatum* STM815 are the most competitive symbionts.

In this study we use RNA-Sequencing to investigate the transcriptome of *B. phymatum* STM815 in response to nitrogen limitation. Among the genes significantly responding to nitrogen limitation, we found several genes for nitrogen assimilation but also genes coding for proteins involved in polyhydroxybutyrate (PHB) accumulation as well as exopolysaccharide (EPS) synthesis. Preliminary RNA-Seq experiments on bean root nodules suggest that genes involved in EPS synthesis are also upregulated in bacteroids.

We next investigated the capacity of seven β-rhizobial strains to compete for nodulation of different legumes and found *B. phymatum* to be the most competitive strain on *Vigna unguiculata* (cowpea) and *Burkholderia tuberum* was the most successful strain on *Macroptilium atropurpureum* (siratro). *Phaseolus vulgaris* (bean) was found to be preferentially nodulated by *B. tuberum* and *B. phymatum* while *Mimosa pudica* (mimosa) by *B. phymatum* and *Burkholderia mimosarum*. The identification of genes important for competiveness would allow us to better understand natural selection for high nitrogen fixation capacity.

DISTRIBUTION OF LISTERIA MONOCYTOGENES SEQUENCE TYPES IN CLINICALLY HEALTHY RUMINANTS AND THEIR ENVIRONMENT

M. Dreyer ¹, A. Thomann ², J. Frey ², A. Oevermann ¹

- 1 NeuroCenter, Division of Neurological Sciences, Vetsuisse Faculty, University of Bern
- 2 Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern

Listeria (L.) monocytogenes is an important ruminant pathogen causing rhombencephalitis, which is amongst the most important CNS diseases in these species and associated with high mortality rates. Clinico-epidemiological observations suggest that L. monocytogenes strains vary in virulence and organ tropism. To test this hypothesis we began a study to determine the distribution of genetic subtypes in healthy ruminants and their environment in order to compare them to clinical isolates of L. monocytogenes.

Environmental and fecal samples are collected in ruminant farms (with and without listeriosis outbreaks) throughout Switzerland. Questionnaires on farm management and feeding habits are handed out during each farm visit. A selective One-Broth Listeria enrichment followed by incubation on *Brilliance* Listeria agar plates is performed. Identification of *L. monocytogenes* is confirmed using the MALDI-TOF-MS and obtained isolates are subtyped in ongoing MLST analyses by determining the sequence variation in 7 housekeeping gene loci. Based on their allelic profile, a sequence type (ST) is assigned to each isolate.

L. monocytogenes were identified in 70% (16/23) of farms and in 24% (38/161) of samples. These were found in all analyzed sources, being most common in soil, floors and feed bunks, but rare in faeces and silage. Twenty-two isolates belong to lineage I, which contains most ruminant rhombencephalitis and severe human infection strains, and 23 isolates to lineage II. The currently typed isolates belong to STs 1, 4, 5, 6, 26 and 412, which except -ST5-have also been found in ruminant clinical infections.

Current results indicate that *L. monocytogenes* are not abundant in Swiss ruminant farms. In contrast to previous studies, silage contamination and fecal shedding are sparse. Sequence types of environmental and clinical isolates overlap, but the comparison of prevalence of STs between the ruminant environment and clinical infection requires the analysis of additional environmental isolates.

Circulating strains of Human Polyomaviruses WU from Patients with Respiratory Tract Infection in Kuwait

S. Essa 1

1 Kuwait University, Faculty of Medicine, Microbiology Department

Introduction: Human polyomavirus WU (WUV) is a newly discovered respiratory virus and limited data are available on the circulating strains. Here we investigated the circulating WUV strains among hospitalized patients with respiratory tract infections (RTI).

Methods: Samples were screened by nested PCR using two sets of primers. Positive samples were sequenced and subjected to phylogenetic analysis. Patients with RTI were screened during a four years period from April 2010 to March 2014.

Results: From four hundred fifty nine hospitalized children and adult patients with RTI, 17 patients (3.7%) were diagnosed with WUV infections. Genomic WUV DNA were sequenced and phylogenetic trees were constructed. The circulating strains belong to type Ia and the third one belongs to the type IIIb. This study enabled us to identify the circulating WUV genotypes among patients with viral RTI in Kuwait.

Conclusions: This finding allows us to develop an understanding of the circulating WUV genotypes among these patients. Precise classification and typing procedures will be important in the future for classifying WUV isolates and investigating what role these genetic groups play in human biology and disease.

Assessing the formation and role of coronavirus-induced replicative structures

P. Vkovski 1. V. Thiel 1

1 Federal Institute of Virology and Immunology, Mittelhäusern and Bern, Switzerland

Coronaviruses (CoV) are enveloped viruses possessing a large positive-sense single-stranded RNA genome. After entry and the release of viral RNA in the cytosol, the expression of 16 non-structural proteins forms the replication and transcription complex (RTC). Of particular interest, the membrane-spanning proteins nsp3, nsp4 and nsp6 divert membranes and anchor the RTC to ER-derived double-membrane vesicles (DMVs) and convoluted membranes that accumulate in the perinuclear region of the cell. Such membrane modifications and compartmentalization of the RTC is a hallmark of CoV replication.

It was shown that co-expression of SARS-CoV nsp3, nsp4 and nsp6 results in the formation of membrane structures similar to DMVs observed in SARS-CoV-infected cells. Additionally, a recent study described a novel potent CoV inhibitor (K22) specifically preventing the formation of DMV clusters and membrane-bound RNA synthesis. Remarkably, K22-resistant viruses containing substitutions in nsp6 are able to form DMVs, although the latter are reduced in number and structurally impaired.

We developed a similar co-expression system for hCoV-229E nsp3, nsp4 and nsp6 and are now assessing the effects of K22 and nsp6 mutants on membrane modifications and DMV formation in the absence of virus infection. Additionally, K22 will serve as a novel tool to study the role of DMVs in escaping the host innate immune response. Indeed, if RNA intermediates are not shielded from cytoplasmic pattern recognition receptors, resistance mutants could provide insights into the mechanisms of RNA sensing during infection by monitoring the recruitment of immune mediators to the sites of RNA replication.

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN SWISS CATS AND DOGS

JRK. Wipf 1, V. Perreten 1

1 Institute of Veterinary Bacteriology, University of Bern, Switzerland

Domestic animals such as cats and dogs may be colonized with methicillin-resistant *Staphylococcus aureus* (MRSA), which can cause infections after surgical interventions.

Twenty-three MRSA strains were isolated from domestic cats (n=9) and dogs (n=14) after ambulatory examination or hospitalization in five different veterinary clinics (VC 1, 2, 3, 4, and 5) in Switzerland between June 2008 and September 2014. The isolates were obtained from various infection sites (purulence, skin lesions [n=13]; surgical transplants, intra-operative swabs [n=8]; tracheobronchial exudate [n=1]; urine [n=1]). The isolates were tested for antibiotic resistance phenotypes and characterized using MLST, PFGE, *spa*, and SCC*mec* typing as well as using microarrays for virulence and antibiotic resistance genes.

Eighteen of the 23 strains belonged to the CC22-MRSA, a clonal group pandemic in humans. Of those, 17 belonged to ST22-t032-IV (VC 1, 2, 3, 4) and one to ST22-t01214-IV (VC 2). Three isolates belonged to ST398-t011-IV (VC 1, 4), a livestock-associated MRSA lineage, and two single isolates of ST5-t002-II (VC 3) and ST1-t001-IV (VC 5) were also detected. All strains were Panton-Valentine leucocidin (PVL) negative, while γ-hemolysin (hlgA, hlgB, hlgC) associated genes, enterotoxin genes (seg, sei, seln, seln, seln, seln, seln, and β-hemolysin converting phage genes (sak, chp, scn) were frequently detected. A predominant pattern of resistance to β-lactams (mecA, blaZ) and fluoroquinolones (amino acid substitutions in GyrA [S84L] and GrlA [S80F]) was found in all but one isolate.

The majority of the infections in dogs and cats were caused by a single MRSA clone (ST22-t032-IV) suggesting nosocomial spread of MRSA in Swiss veterinary settings. The isolates were mainly resistant against fluoroquinolones and β-lactams (including cephalosporins), classes of antibiotics widely used in companion animals. The presence of MRSA in pets emphasizes the need for stricter infection controls in veterinary medicine.

EPIDEMIOLOGICAL LINK BETWEEN FAECAL CARRIAGE OF SBSEC AND AFRICAN SPONTANEOUSLY FERMENTED DAIRY PRODUCTS AND COLORECTAL ADENOCARCINOMA IN KENYA

D. W. M. Kaindi1, W. Kogi-Makau1, J. Hattendorf2 G. N. Lule3, A.M. Mwangi1, B. Kreikemeyer4, P. Renault5, B. Bonfoh6, E. Schelling2, J. Zinsstag2, C. Lacroix7, L. Meile7, C. Jans7

- 1 Department of Food Science, Nutrition and Technology, University of Nairobi, Nairobi, Kenya
- 2 Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland
- 3 School of Medicine, University of Nairobi, Nairobi, Kenya
- 4 Institute of Medical Microbiology, Virology, and Hygiene, Rostock University Medical Centre Rostock, Germany.
- 5 Institut National de la Recherche Agronomique, UMR 1319 MICALIS, Jouy-en-Josas, France
- 6 Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Adiopodume, Côte d'Ivoire
- 7 Laboratory of Food Biotechnology, ETH Zurich, Zurich, Switzerland

Streptococcus infantarius subsp. infantarius (Sii) shows promising traits for application as indigenous African dairy starter culture but, due to its position within the opportunistic pathogenic Streptococcus bovis/Streptococcus equinus complex (SBSEC), its pathogenicity and epidemiology require elucidation. The objective of this study was to determine the prevalence of SBSEC in human volunteers, to elucidate the association between spontaneously fermented dairy products (FDP) and the presence of SBSEC/Sii in persons with or without colorectal adenocarcinoma (CRA).

A hospital-based study was conducted at Kenyatta National Hospital (Kenya) from June 2013 to March 2015, approved by ethics committees of Kenya and Switzerland. Adult participants were recruited among colonoscopy patients. Demographic and socio-cultural information, dietary habits, physical activity, proximity to livestock and morbidity events were captured. Blood, rectal swab/faeces and polyp tissue samples were collected. SBSEC members were isolated on Mitis-Salivarius agar medium and incubated at 37°C under aerobic conditions. Molecular identification was based on 16S rRNA and *groEL* sequencing and MLST.

A sample size of 271 was achieved yielding 1142 isolates. CRA was diagnosed in 51 patients (19%). Prevalence of SBSEC was 26.7 % and 16.2 % in persons with CRA and without CRA, respectively (**OR** 1.9; 95% CI 0.5-7.0), but this association was not statistically significant. All Blood cultures were negative for bacterial growth. Age above 50 years was a significant risk factor of CRA (**OR** 2.5; 95% CI 1.4 – 5.9). FDP consumption was 14.9% and 18.6% in persons with CRA and w/o CRA, respectively, (**OR** 0.8; 95% CI 0.3 to 1.8).

Conclusively, age and fecal carriage of SBSEC were potential risk factors for CRA. However, no indication suggested that FDP consumption is associated with a higher risk of CRA. Comparative analyses of strains across Africa using genomics and elucidation of pathogenicity mechanisms will be needed to further elucidate the epidemiology of *Sii*.

EMERGING VIRUSES: OPTIMIZATION OF METHODS FOR THE MONITORING OF MOSQUITO-BORNE VIRUSES IN SWITZERLAND

V. Guidi¹, D. Ravasi¹, AP. Caminada¹, J. Portmann², E. Flacio³, G. Licheri³, F. Pace³, L. Engeler³, M. Tonolla^{1,3}, O. Engler²

- 1 Laboratory of applied microbiology, SUPSI, DACD, Bellinzona, Switzerland
- 2 Spiez Laboratory, Federal Office for Civil Protection, Spiez, Switzerland
- 3 Mosquito Working Group, Canobbio, Switzerland

In the past two decades new exotic viruses have appeared or re-appeared in Europe possibly due to increasing global trade and travel, as well as environmental and climate changes. Many of these emerging or re-emerging viruses may represent a real threat to public health. Among them, viruses transmitted by mosquito vectors, such as West-Nile virus, Chikungunya virus, and Dengue virus can be found. An efficient surveillance of vectors and emerging viruses would allow preventing their spread across Switzerland. The project aims to optimize the methodologies for an efficient monitoring of new emerging and re-emerging viruses in Switzerland known to be pathogenic to humans and animals, and that are potentially transmitted by mosquitoes. The sampling of mosquitoes was carried out in Canton Ticino. Aedes albopictus mosquitoes were sampled using Biogents Sentinel™ (BG)-traps coupled with CO₂ and placed in inhabited areas. Blood fed Culex mosquitoes were collected using two different Gravid traps typologies (CDC Gravid Trap 1712 and Frommer Updraft Gravid Trap 1719), baited with either hay infusion or hav infusion supplemented with a commercial lure, and run over two consecutive nights. Collection of Culex mosquitoes was repeated in four different regions with a total of 131 traps used. The sampled mosquitoes were frozen-stored until laboratory analysis. The analytical methodology for an effective detection of pathogenic viruses in mosquitoes was optimized by testing different homogenization and RNA extraction protocols, and their applicability to various mosquito pool sizes. Mengovirus was added to mosquito pools and used as real-time PCR target for the optimization of the analytical protocol. The best cost-effective PCR protocol enabling the analysis of a higher number of mosquitoes is subsequently applied to test the collected mosquitoes for emerging viruses.

MALDI-TOF MS ANALYSIS OF EGGS IN THE SURVEILLANCE SYSTEM OF AEDES ALBOPICTUS AND MOSQUITO SPECIES NEW FOR SWITZERLAND

E. Flacio 1, L. Engeler 1, B. Feijoó Fariña 1, V. Pflüger 2, M. Tonolla 3

- 1 Gruppo cantonale di Lavoro Zanzare, Canobbio, Switzerland
- 2 Mabritec AG. Riehen, Switzerland
- 3 Laboratory of Applied Microbiology, University of applied sciences of southern Switzerlan

Eggs of Ae. albopictus and Ae. geniculatus were mainly found on the wooden paddles from oviposition traps used in the surveillance system of Aedes albopictus (Stegomyia albopicta). The differentiation of these species is generally based on egg morphology with a further confirmation by hatching, but in an extensive survey such as the one ongoing since 2000 in Ticino the hatching of eggs of all positive paddles of the oviposition traps is not feasible due to the high number of samples and the long time needed for the analyses. On the other hand, new invasive species are appearing in Europe (e.g. Ae. japonicus is spreading Northern of the Alps and Ae. koreicus in Italy and Belgium) and their eggs are morphologically indistinguishable from those of Ae. albopictus.

In the last 2 years the Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry (MALDI-TOF MS) technique was integrated in the surveillance system, complementary to the morphological egg examination. This allowed confirming the morphological differentiation of Ae. albopictus and Ae. geniculatus eggs. Thanks to this technique, two new species for Switzerland (Ae. cretinus and Ae. koreicus)were discovered and Ae. japonicus was found for the first time in Canton Ticino.

HIGH PREVALENCE AND DIVERSITY OF CHLAMYDIALES DNA WITHIN IXODES RICINUS TICKS SUGGEST A ROLE OF TICKS AS RESERVOIR AND VECTORS OF CHLAMYDIA-RELATED BACTERIA

L. Pilloux 1, S. Aeby 1, R. Gaümann 2, C. Burri 1, C. Beuret 2, G. Greub 1

- 1 Institute of Microbiology of the University Hospital Center and the University of Lausanne
- 2 Spiez Laboratory, Federal Office for Civil Protection, Spiez, Switzerland.

The Chlamydiales order is composed of nine families of strict intracellular bacteria able to infect and replicate within a wide range of eukaryotic cells. Some of them such as C. trachomatis, C. pneumoniae, and C. psittaci are established human pathogens, whereas others such as Waddlia chondrophila and Parachlamydia acanthamoebae emerge as new pathogens of both humans and animals. However, despite their medical importance, their biodiversity and ecology remain to be studied.

Since arthropods and particularly ticks are well known to be vectors of numerous infectious agents like viruses and bacteria, in our study we investigated the prevalence of *Chlamydiae* in ticks. Practically, 62889 *Ixodes ricinus* ticks, pooled in 8534 pools, were sampled at 172 collection sites throughout Switzerland. By using a pan-*Chlamydiales* qPCR, these samples were investigated for the presence of *Chlamydiales* DNA. Results show a pool prevalence of 6.4%, (543/8534) and an estimated prevalence in individual tick of 0.89%. Among these 543 positive pools, we obtained sequencing data for 359 pools, allowing classification of *Chlamydiales* DNA at the family-level lineage. We observed a high biodiversity since six of the nine families belonging to the *Chlamydiales* order have been detected. The commonest were the *Parachlamydiaceae* (33.1%), the *Rhabdochlamydiaceae* (29.2%), and so many "unclassified *Chlamydiales*" (31.8%). Interestingly, thanks to recent progress in sequencing technologies, the huge amount of *Chlamydiales* DNA recover from ticks offers us the unique opportunity to obtain genomic data about fastidious and/or still uncultured *Chlamydiales*. Thus taken together, these data suggest that ticks could represent a potential reservoir and vector for *Chlamydiales* bacteria, and might provide us the unique opportunity to increase our knowledge about *Chlamydiales* biodiversity.

Vector capacity traits of Aedes japonicus and Culex pipiens for West Nile virus

- S. Wagner ¹, A.C. Schönenberger ¹, V. Guidi ², H.C. Tuten ¹, S. Becker ³, E. Veronesi ¹, C. Silaghi ¹, A. Mathis ¹
- 1 Institute of Parasitology, University of Zürich, Switzerland
- 2 Laboratory of Applied Microbiology, University of Applied Sciences of Southern Switzerland
- 3 Bernhard-Nocht-Institute, Hamburg, Germany

West Nile virus (WNv) has spread in Southern and Eastern Europe over the last decade. Assessing the risk for WNv transmission by mosquitoes in Switzerland, this study was initiated to determine (1) population dynamics, (2) host preferences, and (3) vector competence of Swiss mosquitoes. Mosquitoes were collected from ovitraps, breeding sites and CDC traps (baited with CO₂ and iGU lure) at natural and suburban locations on both sides of the Alpine crest over two consecutive years. Host preferences were assessed with horse- and chicken-baited traps, and by blood meal analysis from blood-fed mosquitoes collected at the Zürich Zoo. Vector competence for WNv was assessed for Swiss mosquitoes under a fluctuating 'mid-summer' temperature regime (17-31 °C; typical heat period in Zürich in August). A total of 122'841mosquitoes were collected. The most abundant species at the location Zürich were Culex pipiens and Aedes japonicus, mainly trapped in midsummer. A total of 385 blood-fed mosquitoes were collected at the Zoo (90.6% by mechanical aspiration) and 1081 females through animal-bait experimentsunder field conditions. Culex pipiens collected with both horse- and chicken-baited traps, and Aedes japonicus trapped only with horse-baited traps, tested positive for mammalian or avian blood in the Zoo. Vector competence studies were done by feeding field-collected mosquitoes with WNv-spiked blood. Viral RNA of WNv was detected in pools (n=5) of mosquito head/thorax and saliva by RT qPCR. Aedes japonicus and Cx. pipiens were positive for the two WNv strains tested ("New York" and "Italy"). Viral RNA was detected among a significantly larger number of head/thorax pools infected with strain "New York" than strain "Italy". Overall, our studies provide a basis for assessing the risk of virus transmission under local conditions, suggesting Ae. japonicus and Cx. pipiens as potential candidates for WNv transmission in Switzerland.

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Llanes	C.	P024			Palheiros Marques	M.	P023		
Loessner	MJ.	P023			Palmieri	F.	P072		
Lohberger	Α.	P043			Panis	G.	O09	P076	P089
Looser	V.	OA 001			Parel	C.	P007		
Lüdin	S.	P080			Péchy-Tarr	М.	P077		
Lule	G.N.	P097			Pedrioli	Α.	P092		
Luraschi	Α.	P045			Peduzzi	R.	P031	OA 004	
Luraschi	M.	OA 006			Pelet	S.	P074	071 001	
					Pellegri	G.	OA 006		
M					Perret	X.	P068		
Maclachlan	C.	P069			Perreten	V.	P054	P096	
Maffenbeier	V.	P053			Perron	K.	P005	OA 005	OA 002
Maillard	J.	P052	P059	P072	Perron	K.	P083	OA 004	OA 002
Mancini	S.	P020			Pessi	G.	P092	OA 004	
Marklewitz	M.	M14			Petrini	0.	P016	P042	P044
Martins	D.	O09			Petrini	0.	P049	. 0-12	1 011
Mascher	F.	P055			Pflüger	V.	P068		
Mathis	A.	P101			Pflüger	V.	P099		
Mathys	L.	M18	P057		Pierrehumbert	Α.	P039		
Matthey	N.	P085			Piffaretti	JC.	P031		
Mattogno	M.	P065			Pillonel	T.	006	P076	P084
Maurer	F.P.	P029	P032		Pilloux	L.	P084	P100	1 004
Maurhofer	M.	O07	P055	P077	Pilo	P.	M13	1 100	
Mauri	M.	P078			Pincus	D.	012		
Maury	M.	P075			Pinto	E.	P073		
Mauvais	S.	P003			Pirozmand	L. A.	P001		
Mazza	L.	P062			Poirel	L.	P026		
McCallin	S.	O02			Portmann	J.	P098		
Meijerink	C.	P010	P034		Pothier	J.	P068	P080	
Meilie	L.	P097			Pourshafie	MR.	P025	1 000	
Menzi	C.	O02			Pradervand	S.	O19		
Metzger	L.C.	P081			Pranghofer	S.	P012	P014	
Metzner	K.J.	M07			Principi	P.	P056	P065	
Meuli	M.	014			Pulgarin	C.	P018	. 000	
Mika	M.	P004			Purtschert	G.	P092		
Minoretti	N.	P062			i di todicit	J.	. 002		
Mirzaee	M.	P001			0				
Monnin	V.	012			Q				
Monod	M.	P045	P047		Qi	W.	P004		
Morach	M.	P027			Que	YA.	M16	P005	OA 003
Moreillon	P.	O02	P020						
Morf	L.	P004			R				
Mueller	L.	O06			Raabe	M.	OA 001		
Mwangi	A.M.	P097			Rachmühl	C.	P023		
-					radimun	J.	1 020		

					_				
Raffoul	W.	P005			Thomas	V.	O06		
Rastegar Lari	Α.	P025			Thuong Van	D.	P047		
Ravasi	D.	P056	P098		Tonolla	М.	O16	P041	P044
Rebord	Μ.	M18	P058		Tonolla	М.	P049	P058	P068
Ren	Q.	P087			Tonolla	M.	P071	P080	P098
Renaud	P.	P063	P097		Tonolla	M.	P099		
Resch	G.	M16	O02	P035	Tonolla	M.	OA 004	OA 005	O22
Resch	G.	OA 003			Torres	C.	O01		
Rezzonico	R.	OA 006			Tran	TVD.	O19		
Risch	L.	P033			Trautwein-Weidner	K.	P042		
Risch	M.	P033			Tritten	M-L.	P003	P007	P008
Ritter	C.	P015	P034		Tschumi	A.	014		
Rodriguez	R.	O05			Turlings	T.C.J.	P055		
Rodriguez-Campos	S.	P054			Tuten	H.C.	P101		
Roggo	C.	O10							
Roos	M.	P029			U				
Rtimi	S.	P018			-	_			
					Ulli	C.	OA 001		
S					V				
Sakem	B.	P032			-		D0.40		
Sanchez-Contreras	М.	P092			Vale-Silva	L.A.	P046		
Sander	P.	014			Valipor	М.	P001		
Sanglard	D.	019	P039	P040	Valsesia	G.	P029		
•	D. D.		F039	F040	van Belkum	A.	012		
Sanglard		P046			van de Veerdonk	F.	P048		
Sarraj	Α.	P003			van Delden	C.	P048		
Sartori	D.	OA 005			Van der Henst	C.	P069	P070	
Schelling	E.	M13	P097		van der Meer	J.R.	M26	O10	P053
Schilling	I.E.	P090			van der Meer	J.R.	P061	P063	P073
Schmieder	S.	O21			van der Meer	J.R.	P074	P086	
Schneeberger	P.	P080			Vandenesch	F.	P020		
Schneider	L.	P012			Veloso	R.	P020		
Schoenherr	F.	P042			Venail	P.	M25		
Schönenberger	A.C.	P101			Ventura	Υ.	P043		
Schrenzel	J.	P024	P038		Veronesi	E.	P101		
Schuepbach	RA.	P023						D050	D054
Schulthess	B.	P010			Verrecchia	EP.	P043	P050	P051
Schwemmle	М.	M05			Verweij	P.E.	M09		
Scrignari	Т.	P069			Vidal	S.	P054		
Seidl	Γ. Κ.	P023			Vilmart	G.	P083		
	S.	P009			Viollier	P.	O09	P076	P088
Selvanayakam					Viollier	P.	P089		
Senn	L.	O04	Dooo		Vkovski	P.	P095		
Sentchilo	V.	M26	P086		Vogel	G.	P068		
Siegrist	H.H.	P003	P007	P008	Vögtlin	A.	M20		
Silaghi	C.	P101			von Garnier	C.	P004		
Simon	A.	P050	P051		Voordouw	MJ.	017		
Smits	T.H.M.	O07			Vucicevic	A.	M26	P086	
Stanley	C.E.	O21							
Stephan	R.	O15	P026	P027	W				
Stephan	R.	P030	P075		= =		D404		
Storelli	N.	P065			Wagner	S.	P101		
Straub	C.	O03	P009		Wang	J.	P030		
Sugnaux	M.	P087			Weber	A.	OA 002	OA 004	OA 005
Sulser	S.	M26	P086		Wick	LY.	P050	P051	
Culcul	0.	0	. 000		Widmer	H.R.	P036		
Т					Widmer	A.	P032		
=					Wipf	JRK.	P096		
Talebi	M.	P025			Wittwer	M.	P080		
Tamminen	M.	M23			Wohlwend	N.	P032	P033	
Tasara	T.	P075			Wójtowicz	A.	P048		
Tayyrov	A.	O21			Wu	S.	P087		
Terrettaz	C.	P077			Ÿ				
Teruzzi	T.	O16			='	_	D0=0		
Theraulaz	L.	P076	P088		Yashiro	E.	P073		
Thiel	V.	M06	O05	P095	Yerly	S.	M11		
Thomann	A.	P093			Yousif	A.	P037		
					Ythier	M.	OA 003		
				107					

Z									
Zarazaga	M.	O01			Zirkel	F.	M14		
Zbinden	Α.	P035			Zopfi	J.	P051		
Ziegler	D.	P068			Zuccaro	A.	M22		
Ziegler	J.	O03	P028		Zuchuat	S.	OA 002		
Zihler Berner	Α.	P027			Zurfluh	K.	O15	P026	P027
Zinkernagel	AS.	P023			Zurfluh	K.	P030		
Zinsstag	J.	M01	M13	P097					

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