# EUROPEAN SYMPOSIUM ON FOOD SAFETY

IAFP'S EUROPEAN SYMPOSIUM ON FOOD SAFETY
29-31 MARCH 2017
BRUSSELS, BELGIUM

## 29-31 MARCH 2017

BRUSSELS, BELGIUM

# PROGRAMME

HELD AT THE SQUARE – BRUSSELS MEETING CENTRE

ORGANIZED BY:

International Association for Food Protection®

www.foodprotection.org

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# IAFP EUROPEAN SYMPOSIUM ON FOOD SAFETY PROGRAMME-AT-A-GLANCE

| Room | Silver Hall | The Arc | Studios 311 - 312 | Studios 314 - 316 | Exhibit Hall |
|---|---|---|---|---|---|
| **Wednesday, 29 March 2017** | | | | | |
| Wednesday 8.30 - 10.30 | Opening Session *Silver Hall* | | | | |
| Wednesday 10.30 - 11.00 | Coffee/Networking Break *Exhibit Hall* | | | | Poster Session 1 – Antimicrobials; Beverages and Acid/Acidified Foods; Communication Outreach and Education; Dairy; Epidemiology; Food Chemical Hazards and Food Allergens; Food Defense; Food Law and Regulation; Food Processing Technologies; Food Safety Systems; Food Toxicology |
| Wednesday 11.00 - 12.30 | S1 - Employing Whole Genome Sequencing for Successful Traceback Investigation | S2 - Don't Dismiss Clostridia in Food (they are still there!): From Disease Burden to Prevention and Health Promotion | S3 - Foodborne Microbial Toxins, Virulence, and Host-pathogen Interactions | T1 - Intervention Strategies | |
| Wednesday 12.30 - 14.00 | Lunch *Exhibit Hall* | | | | |
| Wednesday 14.00 - 15.30 | RT1 - Putting the Limelight on Non-pathogenic Microbes | S4 - Source Attribution of Campylobacteriosis | S5 - Use of Molecular Techniques to Understand Mechanisms of Persistence and Removal of *E. coli* from the Food Supply Chain | T2 - Contamination, Source Tracking, Epidemiology and Regulation | |
| Wednesday 15.30 - 16.00 | Coffee/Networking Break *Exhibit Hall* | | | | |
| Wednesday 16.00 - 17.30 | S6 - How to Exploit Omics Data on Pathogen Behavior in Microbiological Risk Assessment: An Update on the Current Research | S7 - Dietary Exposure to Food Chemicals: Data Needs, Methods, and Case Studies | S8 - Prevalence, Properties and Control of *Listeria monocytogenes* in the Food Supply Chain | T3 - Laboratory and Detection Methods | |
| Wednesday 17.30 - 18.30 | Exhibit Hall Reception | | | | |
| **Thursday, 30 March 2017** | | | | | |
| Thursday 8.30 - 10.00 | S9 - Predictive Mycology Applied to Spoilage: From Data Collection to User-friendly Tools | S10 - The Race to Zero – Everybody Loses | | T4 - Molecular Characterisation of Microorganisms | |
| Thursday 10.00 - 10.30 | Coffee/Networking Break *Exhibit Hall* | | | | Poster Session 2 – General Microbiology; Laboratory and Detection Methods; Low-water Activity Foods; Meat, Poultry and Eggs; Microbial Food Spoilage; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Packaging; Preharvest Food Safety; Produce; Retail and Food Service Safety; Sanitation and Hygiene; Viruses and Parasites |
| Thursday 10.30 - 12.00 | S11 - Progress in Food Safety Education and Training – Learnings from Tailored Small Group Offerings to Running Massive Open On-line Courses | S12 - Novel Insight in Microbial Ecology within Food Processing with Next Generation Sequencing Methods | S13 - Cleaning and Disinfection Methods for Low-water Activity Foods | T5 - Food Safety and Microbiology | |
| Thursday 12.00 - 13.30 | Lunch *Exhibit Hall* | | | | |
| Thursday 13.30 - 15.00 | S14 - Foodborne Viruses: Detection, Risk Assessment, and Control Options in Food Processing | S15 - Identification of Emerging Risks in Food – Different Approaches to Achieve a Common Goal | S16 - Ensuring Food Safety of Meat Products by Use of High Pressure Processing (HPP): From Recent Research Initiatives to Commercial Developments | T6 - Modeling and Risk Assessment 1 | |
| Thursday 15.00 - 15.30 | Coffee/Networking Break *Exhibit Hall* | | | | |
| Thursday 15.30 - 17.00 | S17 - Predictive Microbiology for Process Validation, Biological Variability and FSMA Compliance | S18 - Innovative Non-thermal Technologies for Microbial Biofilm Decontamination on Biotic and Abiotic Surfaces | S19 - How to Manage Microorganism with a Complex Cycle of Life in Food Industry | T7 - Food Processing Technologies | |
| Thursday 19.00 - 22.30 | Thursday Evening Social *(additional registration and fee required)* | | | | |
| **Friday, 31 March 2017** | | | | | |
| Friday 8.30 - 10.00 | RT2 - Globalisation Challenges in Food Safety Management – Emerging Issues in Culture, Systems and Practice | S20 - Application of Bacteriophages as an Antimicrobial Intervention and Detection Strategy in the Food Industry | S21 - Differentiate the Real Culprits from the Presumed Ones: How Emerging Technologies Improve a Typical Day's Work in Routine Testing Labs | T8 - Modeling and Risk Assessment 2 | |
| Friday 10.00 - 10.30 | Coffee/Networking Break *Silver Hall Foyer* | | | | |
| Friday 10.30 - 12.45 | Closing Session *Silver Hall* | | | | |
| Friday 12.45 - 14.00 | Farewell Refreshments *Silver Hall Foyer* | | | | |


# China International Food Safety & Quality Conference

November 1 – 2, 2017 • Beijing, P. R. China

**Come join 900+ food safety leaders to learn, discuss and discover the most recent developments in….**Surveillance and Epidemiology Information Risk Management
Food Traceability
Food Fraud: Vulnerability Assessment, Prevention and Analytical Detection
Creating and Implementing a Culture of Food Safety
Next Generation Methodologies in Chemical Risk Assessments for Food
Predictive Microbiology and Quantitative Microbial Risk Assessment
Sustainability and Food Safety Challenges
Managing Hygiene in Food Operations and Role of Non-Pathogen Limits in Standards
New Methods and Solutions in Rapid Testing and Detection
Real Time Testing Methods for the Food Industry
Modern Analytical Techniques and Testing of Contaminants in Food and Environmental Samples
Chemical and Microbiological Risk Assessment and Risk Management of the Food Supply Chain
Whole Genome Sequencing in Disease and Outbreak Investigation
Impact and Control Strategies for Antimicrobial Resistance
China's 13th Five Year Plan for Food Safety
Horizon 2020 EU - China Food Safety Progress Report
Food Allergens: Detection, Management & Prevention

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29-31 MARCH 2017
BRUSSELS, BELGIUM

# PROGRAMME

29–31 March 2017 – Brussels, Belgium

# Day 1 – Wednesday, 29 March

## 7.30 – 17.00 Registration Open

### 7.30 – 8.30 – Morning Coffee

### 10.00 – 18.30 – Exhibit Hours

**10.30 – 16.00 – Poster Session 1 – Antimicrobials; Beverages and Acid/Acidified Foods; Communication Outreach and Education; Dairy; Epidemiology; Food Chemical Hazards and Food Allergens; Food Defense; Food Law and Regulation; Food Processing Technologies; Food Safety Systems; Food Toxicology**

*Authors present during scheduled break times.*

## OPENING SESSION

*Silver Hall*
**Chair: Lilou van Lieshout**

**8.30** **Introduction to IAFP**
DAVID THARP, Executive Director, International Association for Food Protection, Des Moines, IA, USA

**8.45** **Introduction to IAFP's European Symposium**
LINDA J. HARRIS, University of California, Davis, CA, USA

**8.55** **Biocontrol of Foodborne Pathogens: The Pros and Cons**
JACQUES MAHILLON, University Catholic Louvain, Brussels, Belgium

**9.25** **Global Trends in Food Safety**
LINDA J. HARRIS, University of California, Davis, CA, USA

**9.55** **The Future of the One Health Approach: From Tracing Foodborne Pathogens and Spoilers to Mobile Genetic Elements and from Farm to Fork via the Environment**
MARC HEYNDRICKX, ILVO - Flanders Research Institute for Agriculture, Fisheries and Food, Melle, Belgium

**10.25** **Practical Announcements**
LILOU VAN LIESHOUT, ILSI Europe, Brussels, Belgium

### 10.30 Networking Coffee Break in the Exhibit Area

### S1 Employing Whole Genome Sequencing for Successful Traceback Investigation

*Silver Hall*
**Organizers and Convenors: Maria Hoffmann, Jesse Miller, Eric Stevens**
*Sponsored by IAFP Foundation*

**11.00** **Integration of Genomics Technologies in the Management of Food Safety and Outbreaks in Europe**
JAMINA-MIKA SUZUKI, European Commission, Brussels, Belgium

**11.30** **Identifying the Source of Foodborne Outbreaks: WGS, the New Sleuth on the Block**
KATHIE GRANT, Public Health England, Glasgow, United Kingdom

**12.00** **Implementing WGS-based Strain Characterization into Pathogen Surveillance: Introducing the Strategy of the Robert Koch Institute**
GUIDO WERNER, Robert Koch Institute, Wernigerode, Germany

### 12.30 Lunch Available in the Exhibit Area

### S2 Don't Dismiss Clostridia in Food (They are still there!): From Disease Burden to Prevention and Health Promotion

*The Arc*
**Organizers and Convenors: Georges Daube and Cristina Rodriguez**

**11.00** **Charting the Current Situation of Clostridia in Foods and the Environment: Prevalence, Pathogenicity, and Spoilage**
MIIA LINDSTRÖM, University of Helsinki, Helsingin Yliopisto, Finland

**11.30** **Clostridia Spread in Livestock Animals: Situation and Initiatives**
ALEXANDRA TABARAN, University of Agricultural Sciences and Veterinary Medicine, Cluj - Napoca, Romania

**12.00** **Clostridia in the Gut Microbiota and Their Implication in Food Allergies and Foodborne Diseases**
BERNARD TAMINIAU, University of Liege, Leige, Belgium

### 12.30 Lunch Available in the Exhibit Area

### S3 Foodborne Microbial Toxins, Virulence, and Host-pathogen Interactions

*311-312*
**Organizers and Convenors: Andreja Rajkovic, Luca Cocolin**

**11.00** **Mitochondrial Toxicity of *Bacillus cereus* Emetic Toxin with Intestinal and Liver Toxicological Endpoints**
ANDREJA RAJKOVIC, Ghent University, Ghent, Belgium

**11.30** **Impact of Abiotic and Biotic Parameters of the Human Gut on Enterohemorrhagic *Escherichia coli* Survival and Virulence**
STÉPHANIE BLANQUET-DIOT, Université Clermont Auvergne, Clermont-Ferrand, France

**12.00** **Microbe-microbe Interaction between *Staphylococcus aureus* and Lactic Acid Bacteria Resulting in a Reshuffle of the Microbial Metabolisms and Prevention of Staphylococcal Enterotoxin Production**
LUCA COCOLIN, University of Torino-DISAFA, Grugliasco, Italy

### 12.30 Lunch Available in the Exhibit Area

**T1** **Technical Session 1 – Intervention Strategies**
*Studio 314–316*
**Convenor: Florence Postollec**

**T1-01 11.00** **Cold Plasma Treatment for the Inactivation of *Salmonella* Enteritidis PT 30 on the Surface of Unpeeled Almonds**
CHRISTIAN HERTWIG, Kai Reineke, Nicolas Meneses, Oliver Schlüter, Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany

**T1-02 11.15** **The Impact of Drying on Foodborne Pathogens *Salmonella enterica* and *Cronobacter sakazakii***
EMILIE LANG, Stéphane Guyot, Pablo Alvarez-Martin, Jean-Marie Perrier-Cornet, Patrick Gervais, Unité Mixte de Recherche - Procédés Alimentaires et Microbiologiques (UMR PAM), France, Dijon, France

**T1-03 11.30** **Evaluation of the Hygienic Design of an Industrial Device for Drying Food Using Supercritical Fluids**
ILIJA DJEKIC, Simeon Bourdoux, Cynthia Akkermans, Gerard Hofland, Frank Devlieghere, Nikola Tomic, Andreja Rajkovic, University of Belgrade, Belgrade, Serbia

**T1-04 11.45** **Reduced Contamination of Pork Carcasses with Hygiene Indicator Bacteria, ESBL/AmpC-producing *Escherichia coli, Salmonella* spp. and *Yersinia enterocolitica* by Alternative Removal of the Pluck Set during Slaughter**
WAUTER BIASINO, Lieven De Zutter, Tanuja K.G.M. Gowda, Inge Van Damme, Ghent University, Merelbeke, Belgium

**T1-05 12.00** **The Glutamate Decarboxylase System in Bacterial Food Pathogens and Its Inhibition by Dicarboxylic Acids**
RUTH BARNES, Kimon Andreas Karatzas, University of Reading, Reading, United Kingdom

**T1-06 12.15** **Reverting Multidrug-Resistant Phenotypes of *Escherichia coli* Isolated from Cattle Using 1-(1-Naphthylmethyl)-Piperazine**
JOÃO ANES, Séamus Fanning, Daniel Hurley, Shabarinath Srikumar, University College Dublin, Dublin, Ireland

**12.30** **Lunch Available in the Exhibit Area**

**RT1** **Putting the Limelight on Non-pathogenic Microbes**
*Silver Hall*
**Organizers and Convenors: Christophe Dufour and Leon Gorris**

14.00 PANELISTS:

ROY BETTS, Campden BRI, Gloucestershire, United Kingdom

JAN DIJKSTERHUIS, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

CHRISTOPHE DUFOUR, Merieux NutriSciences, Cergy Pontoise Cedex, France

PETER MCCLURE, Mondelez International, Birmingham, United Kingdom

ELENI PANTIORA, UN World Food Programme, Addis Ababa, Ethiopia

**15.30** **Networking Coffee Break in the Exhibit Area**

**S4** **Source Attribution of Campylobacteriosis**
**Organizers and Convenors: Marianne Chemaly and Katell Rivoal**

**14.00** **Genomic Signatures of *Campylobacter* Adaptation and Source Attribution Studies**
SAMUEL SHEPPARD, The Milner Center for Evolution, University of Bath, Bath, United Kingdom

**14.30** **Gene-by-Gene Comparison of *Campylobacter jejuni* Genomes to Identify Host Segregating Epidemiological Markers for Source Attribution**
AMANDINE THEPAULT, ANSES, Ploufragan, France

**15.00** **Combining Case-control and Source-attribution Data: A Way to Reconstruct Campy's Journey along the Transmission Chain**
LAPO MUGHINI GRAS, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

**15.30** **Networking Coffee Break in the Exhibit Area**

**S5** **Use of Molecular Techniques to Understand Mechanisms of Persistence and Removal of Foodborne Pathogens from the Food Supply Chain**
*Studio 311–312*
**Organizer and Convenor: Kimon Andreas Karatzas**
*Sponsored by IAFP Foundation*

**14.00** **Understanding the Molecular Targets of Broccoli Extract on *Escherichia coli* through the Use of an Extensive Mutant Library**
KIMON ANDREAS KARATZAS, University of Reading, Reading, United Kingdom

**14.30** **Assessing the Antimicrobial Mode of Action of Novel Non-thermal Processes through the Use of *Escherichia coli* Mutants**
VASILEIOS VALDRAMIDIS, University of Malta, Msida, Malta

**15.00** **Understanding Stress Adaptation during the Transition of *Listeria monocytogenes* from Environment to Food to Host**
CORMAC GAHAN, University College Cork, Cork, Ireland

**15.30** **Networking Coffee Break in the Exhibit Area**

# Day 1 – Wednesday, 29 March

**T2** **Technical Session 2 – Contamination Source Tracking, Epidemiology and Regulation**
*314–316*
**Convenor: Sarah Cahill**

**T2-01 14.00** **Whole Genome Comparisons of *Listeria monocytogenes* Isolates: A Two-step Analysis Combining Whole Genome Mist and Whole Genome SNP**
Katleen Vranckx, KATRIEN DE BRUYNE, Hannes Pouseele, Applied Maths NV, Sint-Martens-Latem, Belgium

**T2-02 4.15** **Practical Application of Whole Genome Sequencing for *Listeria monocytogenes* Source Tracking in the Food Industry**
KATIA ROUZEAU-SZYNALSKI, Caroline Barretto, Catherine Ngom-Bru, Coralie Fournier, Johan Gimonet, Leen Baert, Nestec Ltd. Nestle Research Center, Lausanne, Switzerland

**T2-03 14.30** **The Global Importance of a Publically Available, Genomic Database for Environmental and Food Isolates**
ERIC STEVENS, FDA/CFSAN/ORS/DM, College Park, MD, USA

**T2-04 14.45** **FSMA (USA) Versus Hygiene Package (EU): Differences and Opportunities**
CLAUDIO GALLOTTINI, Franco Rapetti, Sara Trombetti, ITA Corporation, Miami, FL, USA

**T2-05 15.00** **Third-party Auditors in the Food Protection System: Comparing the Role of Third-party Auditors to Government Regulatory Agents**
ELIZABETH DRISCOLL, Ryerson University, Toronto, ON, Canada

**15.30** **Networking Coffee Break in the Exhibit Area**

**S6** **How to Exploit Omics Data on Pathogen Behavior in Microbiological Risk Assessment: An Update on the Current Research**
*Silver Hall*
**Organizers and Convenors: Luca Cocolin and Marcel Zwietering**

**16.00** **The Use of Metagenomics in Quantitative Microbiological Risk Assessment**
LUCA COCOLIN, University of Turin-DISAFA, Turin, Italy

**16.30** **The Use of Omics in Exposure Assessment**
HEIDY DEN BESTEN, Wageningen University, Wageningen, Netherlands

**17.00** **The Use of Omics in Hazard Characterisation**
TREVOR PHISTER, PepsiCo, Leicester, United Kingdom

**17.30 – 18.30** **Reception in the Exhibit Hall**

**S7** **Dietary Exposure to Food Chemicals: Data Needs, Methods, and Case Studies**
*The Arc*
**Organizer and Convenor: Cian O'Mahony**

**16.00** **Approaches to Dietary Exposure for Chemicals in Food: Data Needs and Modelling Techniques**
CIAN O'MAHONY, Creme Global, Grand Canal Quay, Ireland

**16.30** **Aggregate Exposure to Vitamin A from the Diet, Personal Care Products and Cosmetics**
SARAH TOZER, Procter and Gamble, Egham, United Kingdom

**17.00** **Contaminants in Tea: Exposure and Risk Assessment Approaches to Ensure Consumer Safety**
THERESA NEELY, Unilever, London, United Kingdom

**17.30 – 18.30** **Reception in the Exhibit Hall**

**S8** **Prevalence, Properties, and Control of *Listeria monocytogenes* in the Food Supply Chain**
*Studio 311–312*
**Organizers and Convenors: Kimon Andreas Karatzas, Marjon Wells-Bennik**
*Sponsored by IAFP Foundation*

**16.00** **A Three-year National Survey of *Listeria monocytogenes* Prevalence in the Irish Food Chain: Implications for Food Safety**
CONOR O'BYRNE, National University of Ireland, Galway, Galway, Ireland

**16.30** **A Novel Targeted Approach in Disinfection and Decontamination through Inhibition of Specific Stress Resistance Mechanisms in *Listeria monocytogenes***
KIMON ANDREAS KARATZAS, University of Reading, Reading, United Kingdom

**17.00** **Hurdles to Prevent Outgrowth of *Listeria monocytogenes*: Evaluation of Factors in Gouda Cheese**
MARJON WELLS-BENNIK, NIZO Food Research, Ede, Netherlands

**17.30 – 18.30** **Reception in the Exhibit Hall**

**T3** **Technical Session 3 – Laboratory and Detection Methods**
*314–316*
**Convenor: Patrice Arbault**

**T3-01 16.00** **Enabling Accurate Measurements of Staphylococcal Enterotoxin A (SEA) in Food by Use of a Comprehensively Characterised Calibrant Solution**
REINHARD ZELENY, Sébastien Boulo, Amalia Muñoz, Heinz Schimmel, Dominique Baiwir, Gabriel Mazzucchelli, Isabelle Mutel, Yacine Nia, European Commission, Geel, Belgium

**T3-02 16.15** **Development and Application of Peptide Nucleic Acid Fluorescence *In Situ* Hybridization for the Specific Detection of *Listeria monocytogenes***
RUI ROCHA, José Mário Sousa, Laura Cerqueira, Maria João Vieira, Carina Almeida, Nuno Filipe Azevedo, LEPABE, University of Porto, Porto, Portugal

**T3-03 16.30** ***Campylobacter* Strain Choice and Food Matrix Strongly Affect $LOD_{50}$ Results**
WILMA HAZELEGER, Ida Jongenburger, Wilma Jacobs-Reitsma, Heidy den Besten, Wageningen University and Research, Wageningen, Netherlands

**T3-04 16.45** **Monitoring the Quality of the Foods with Real-time Sensors for the Detection of Pathogenic Bacteria**
Grazia Lupoli, Danila Mosconi, Stanislao Maria Di Amato, Giancarlo Barraco, CLAUDIO GALLOTTINI, ITA Corporation, Miami, FL, USA

**T3-05 17.00** **Impact of Chronic Exposure of Low Concentrations of Microbial Depsipeptide Cereulide on Mitochondrial Disruptions in Caco-2 Cells**
MARLIES DECLEER, Sarah De Saeger, Andreja Rajkovic, Ghent University, Ghent, Belgium

**T3-06 17.15** **Quantitative Detection of Deoxynivalenol in Multiple Grain Commodities Using Envirologix DON-Flex, a Rapid Lateral Flow Assay**
ANNA RICE, Serguisz Polakowski, Cheryl Bailey, Keith Tanguay, Jamie Welch, Brendan Gow, Terry Goddard, EnviroLogix, Inc., Portland, ME, USA

**17.30 – 18.30 Reception in the Exhibit Hall**

**7.30 – 16.00 Registration Open**

# Call for Submissions for IAFP 2018 and IAFP's European Symposium on Food Safety

## Submission Deadline:

### 16 January 2018 – Technical and Poster Abstract Submissions

### 3 October 2017 – Symposia and Roundtable Proposals

Questions regarding submissions can be directed to Tamara Ford
Phone: +1 515.276.3344 or +1 800.369.6337
E-mail: tford@foodprotection.org

International Association for Food Protection®

# Day 2 – Thursday, 30 March

**7.30 – 8.30 – Morning Coffee**

**10.00 – 16.00 – Exhibit Hours**

**10.30 – 16.00 – Poster Session 2 – General Microbiology; Laboratory and Detection Methods; Low-water Activity Foods; Meat, Poultry and Eggs; Microbial Food Spoilage; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Packaging; Preharvest Food Safety; Produce; Retail and Food Service Safety; Sanitation and Hygiene; Viruses and Parasites**

*Authors present during scheduled break times.*

**S9** **Predictive Mycology Applied to Spoilage: From Data Collection to User-friendly Tools**
*Silver Hall*
**Organizers and Convenors: Mariem Ellouze and Elissavet Gkogka**
*Sponsored by IAFP Foundation*

**8.30** **Predictive Mycology: History and Importance of Data Collection**
PHILIPPE DANTIGNY,UUniversity of Western Brittany, France

**9.00** **Mathematical Models and Probabilistic Approaches Quantifying the Influence of Formulation, Processing, and Environmental Factors on Mould Growth: Application in Shelf-life Assessment of a Food Commodity**
JEANNE-MARIE MEMBRÉ, UMR1014 SECALIM, INRA, Oniris, Nantes, France

**9.30** **Sweetshelf: A User-friendly Tool to Predict the Growth of Yeasts and Moulds in Intermediate Moisture Food**
AN VERMEULEN, Ghent University, Ghent, Belgium

**10.00** **Networking Coffee Break in the Exhibit Area**

**S10** **The Race to Zero – Everybody Loses**
*The Arc*
**Organizer and Convenor: Anthony Flood**
*Sponsored by IAFP Foundation*

**8.30** **Chasing Zero: Holy Grail, Marketing or Necessity**
BERT PÖPPING, FOCOS, Alzenau, Germany

**9.00** **Approaches for Prioritizing and Evaluating Trace Contaminants**
GABRIELE SCHOLZ, Nestle, Lausanne, Switzerland

**9.30** **Communicating with Consumers: How to Talk about Food Risk**
NINA MC GRATH, European Food Information Council (EUFIC), Brussels, Belgium

**10.00** **Networking Coffee Break in the Exhibit Area**

**T4** **Technical Session 4 – Molecular Characterisation of Microorganisms**
*Studio 314–316*
**Convenor: Luca Cocolin**

**T4-01 8.30** **Molecular Characterization of Shiga-toxin Producing *Escherichia coli* in Food Products Marketed in Romania**
SORIN DANIEL DAN, Alexandra Tabaran, Marian Mihaiu, Oana Lucia Reget Reget, Alina Dana Magdas, University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, Cluj Napoca, Romania

**T4-02 8.45** ***Listeria monocytogenes SigB*-induced Hypersensitivity against Oxidative Stress is Mediated by a Down Regulation of Catalase Expression**
MARCIA BOURA, Kimon Andreas Karatzas, University of Reading, Reading, United Kingdom

**T4-03 9.00** **Phenotypic and Pan-genomic Characterisation of *Salmonella enterica* Serovar Uganda, an Uncommon Foodborne Pathogen**
DANIEL HURLEY, Maria Hoffmann, Ellen Wall, Eric Brown, Marc Allard, Salim Mattar, Séamus Fanning, University College Dublin, Dublin, Ireland

**T4-04 9.15** **Estimated Infectivity of Human Norovirus in Environmental Water Samples by an *In Situ* Capture RT-qPCR Method**
PENG TIAN, David Yang, Qianqian Li, Dapeng Wang, ARS, USDA, Albany, CA, USA

**10.00** **Networking Coffee Break in the Exhibit Area**

**S11** **Progress in Food Safety Education and Training: Learnings from Tailored Small Group Offerings to Running Massive Open On-line Courses**
*Silver Hall*
**Organizers and Convenors: Leon Gorris and Marcel Zwietering**
*Sponsored by IAFP Foundation*

**10.30** **What Food Safety Knowledge and Skills Will Employers Expect When Recruiting Professionals to Work in the Food Industry?**
PIER SANDRO COCCONCELLI, Universita Cattolica del Sacro Cuore, Cremona, Italy

**11.00** **Examination Challenges for Teachers of Food Safety: Methods vs. Knowledge and Skills Gained**
STEPHEN FORSYTHE, Nottingham Trent University, Nottingham, United Kingdom

**11.30** **On-line Courses in Food Safety – SPOCs and MOOC**
MARCEL ZWIETERING, Wageningen University, Wageningen, Netherlands

**12.00** **Lunch Available in the Exhibit Area**
***Sponsored by Diamond V***

**S12** **Novel Insights into the Microbial Ecology of Food Processing Using Next Generation Sequencing Methods**
*The Arc*
**Organizer and Convenor: Trond Møretrø**
*Sponsored by IAFP Foundation*

**10.30** **Residential Bacteria in the Food Industry: Why? Who? So What?**
TROND MØRETRØ, Nofima, Norwegian Food Research Institute, Aas, Norway

**11.00** **High-resolution Exploration of Microbial Consortia in Food Processing Environments**
FRANCESCA DE FILIPPIS, University of Naples Federico II, Portici (NA), Italy

**11.30** **Elucidating Contamination Routes of Meat Spoilage Bacteria with Next Generation Sequencing Methods**
JOHANNA BJÖRKROTH, University of Helsinki, Helsinki, Finland

**12.00** **Lunch Available in the Exhibit Area**
***Sponsored by Diamond V***

**S13** **Cleaning and Disinfection Methods for Low-water Activity Foods**
*Studio 311–312*
**Organizer: Karin Blacow**
**Convenor: Roger Scheffler**

**10.30** **Technologies and Associated Challenges Related to Cleaning and Disinfection in Dry Environments**
KARIN BLACOW, Commercial Food Sanitation, Amsterdam, Netherlands

**11.00** **Cleaning Methods for Low-water Activity Food, the Successful and Less Successful**
COLLETTE GIRVIN, Kellogg Company, Dublin, Ireland

**11.30** **Verification and Validation of Sanitation Controls – What Should We Do?**
PAULINE TITCHENER, Neogen Europe, Auchincruive, United Kingdom

**12.00** **Lunch Available in the Exhibit Area**
***Sponsored by Diamond V***

**T5** **Technical Session 5 – Food Safety and Microbiology**
*314–316*
**Convenor: Anett Winkler**

**T5-01 10.30** **Insight into the Variables Affecting Bacterial Transference during the Washing Process of Fresh-cut Lettuce and Spinach in Simulated Reused Fresh-cut Produce Wash Water**
CRISTINA PABLOS, Aitor Romero, Javier Marugán, Rey Juan Carlos University, Mostoles, Spain

**T5-02 10.45** **The Lack of Tools to Track *Bacillus thuringiensis*-(Bt) based Insecticide Isolates from Farm to Fork**
Anne-Gabrielle Mathot, Emeline Cozien, Pierre Gehannin, Rodolphe Vidal, Nadine Henaff, FLORENCE POSTOLLEC, ADRIA Food Technology Institute - UMT14.01 SPORE RISK, Quimper, France

**T5-03 11.00** **Characterization of Microorganisms Isolated from Biofilms in Food Companies: Identification and Biofilm-forming Properties**
SHARON MAES, Son Nguyen Huu, Thijs Vackier, Marc Heyndrickx, Hans Steenackers, Alex Verplaetse, Katleen Raes, Koen De Reu, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium

**T5-04 11.15** **The Importance of Strain Selection to the Conduct of Challenge Testing and Assessment of Food Spoilage**
Francesca Valerio, Anne-Gabrielle Mathot, Marie-Laure Divanac'h, Emeline Cozien, Noémie Desriac, Nadine Henaff, Véronique Huchet, FLORENCE POSTOLLEC, ADRIA Food Technology Institute – UMT14.01 SPORE RISK, Quimper, France

**T5-05 11.30** **Early Detection of *Campylobacter* Using Air Sampling and VOC Analysers**
TIM GIBSON, Stan Curtis, Ben Curtis, Lynn McIntyre, Frank Vriesekoop, Sarah Hardy, Simon Lock-Pender, Andrew Stacey, RoboScientific Ltd, Leeds, United Kingdom

**T5-06 11.45** **Attribution of *Listeria monocytogenes* Human Cases to Food and Animal Sources in Northern Italy**
VIRGINIA FILIPELLO, Lapo Mughini-Gras, Silvia Gallina, Ettore Amato, Mirella Pontello, Lucia Decastelli, Marc Allard, Eric Brown, Sara Lomonaco, University of Turin, Grugliasco, Italy

**12.00** **Lunch Available in the Exhibit Area**
***Sponsored by Diamond V***

**S14** **Foodborne Viruses: Detection, Risk Assessment, and Control Options in Food Processing**
*Silver Hall*
**Organizer and Convenor: Lilou van Lieshout**
*Sponsored by ILSI Europe*

**13.30** **Pros and Cons of Methods of Detection for Viruses in Foods**
ALVIN LEE, Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, USA

**14.00** **Translating Risk Assessment of Viruses in Foods into Practice**
ELISSAVET GKOGKA, Arla Strategic Innovation Centre, Brabrand, Denmark

# Day 2 – Thursday, 30 March

**14.30** **Effect of Processing Technologies to Control Viruses in Foods**
SOPHIE ZUBER, Nestlé Research Center, Lausanne, Switzerland

**15.00** **Networking Coffee Break in the Exhibit Area**

**S15** **Identification of Emerging Risks in Food: Different Approaches to Achieve a Common Goal**
*The Arc*
**Organizer: Raquel Garcia Matas**
**Convenors: Sarah Cahill, Tobin Robinson, Carmen Savelli**

**13.30** **Application of Food Safety Early Warning Systems: Industry Perspective**
JOHN O'BRIEN, Nestlé Research, Lausanne, Switzerland

**13.50** **Networks of Knowledge – Sharing of Information and Expertise**
ANA AFONSO, European Food Safety Authority (EFSA), Parma, Italy

**14.10** **Testing New Methodologies for Identification of Emerging Chemical Risks in Food**
JAN OLTMANNS, Forschungs-und Beratungsinstitut Gefahrstoffe GmbH (FoBiG), Freiburg, Germany

**14.30** **Panel Discussion**

**15.00** **Networking Coffee Break in the Exhibit Area**

**S16** **Ensuring Food Safety of Meat Products by Use of High Pressure Processing (HPP): From Recent Research Initiatives to Commercial Developments**
*Studio 311–312*
**Organizer and Convenor: Sandrine Guillou**
*Sponsored by IAFP Foundation*

**13.30** **Understanding the Behavior of *Listeria monocytogenes* in High Pressure Processed Meat Products: Resources for Process Validation**
SARA BOVER-CID, IRTA, Monells, Spain

**14.00** **Combined Use of High Pressure Processing (HPP) and Biopreservation to Preserve Meat Products: Screening, HPP-inactivation and Regrowth of Three Lactic Acid Bacteria**
SANDRINE GUILLOU, Oniris, Nantes, France

**14.30** **High Pressure Processing Comercial Developments: Global Market, Equipment and Applications in the Meat Industry**
CAROLE TONELLO-SAMSON, Hiperbaric, Burgos, Spain

**15.00** **Networking Coffee Break in the Exhibit Area**

**T6** **Technical Session 6 – Modeling and Risk Assessment 1**
*Studio 314–316*
**Convenor: Annemarie Pielaat**

**T6-01 13.30** **Is It Safe to Use Tap Water to Prepare Infant Formula in France?**
GÉRALDINE BOUÉ, Luiza Wasiewska, Enda Cummins, Sandrine Guillou, Jean-Philippe Antignac, Jeanne-Marie Membré, UMR1014 SECALIM, INRA, Oniris, Nantes, France

**T6-02 13.45** **Risk Factors Selection, Criteria Assessment, and Final Weighting for the Canadian Food Inspection Agency's Establishment-based Risk Assessment Model**
Manon Racicot, Romina Zanabria, Mathieu Cormier, Julie Arsenault, Cecile Ferrouillet, Marie-Lou Gaucher, Ann Letellier, Anna Mackay, Ashwani Tiwari, Solomon Aklilu, Ryan Currie, Mansel Griffiths, Richard Holley, Tom Gill, Sylvain Charlebois, SYLVAIN QUESSY, University of Montreal, Saint-Hyacinthe, QC, Canada

**T6-03 14.00** **Insects Fed with Former Foodstuffs for Feed Production: What are the Risks to Public and Animal Health?**
LINDA KOX, Netherlands Food and Consumer Product Safety Authority, Office for Risk Assessment and Research Programming, Utrecht, Netherlands

**T6-04 14.15** **Evaluation of the Microbial Risk of Storage at Ambient Temperature of Artisanal Rice Pie**
ELS VAN COILLIE, Koen De Reu, Geert Van Royen, Claire Verraes, Marc Heyndrickx, Lieve Herman, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium

**T6-05 14.30** **Burden of Disease of Barbecued Meat: Who is at Risk?**
LEA SLETTING JAKOBSEN, Stylianos Georgiadis, Bo Friis Nielsen, Anders Stockmarr, Elena Boriani, Lene Duedahl-Olesen, Tine Hald, Sara Pires, National Food Institute, Technical University of Denmark, Lyngby, Denmark

**T6-06 14.45** **Conceptual Framework for a Cumulative Risk Assessment of Biogenic Amines in Foods**
D. Sylvain Dabadé, LIESBETH JACXSENS, Bruno De Meulenaer, Ghent University, Ghent, Belgium

**15.00** **Networking Coffee Break in the Exhibit Area**

**S17** **Use of Predictive Microbiology for Process Validation Encompassing Biological Variability**
*Silver Hall*
**Organizer and Convenor: Laure Pujol**

**15.30** **How to Validate in a Variable World: Use Data, Lots of Data, Both from Experiments and from Literature**
MARCEL ZWIETERING, Wageningen University, Wageningen, Netherlands

**16.00** **Comparison of Thermal Resistance of *Clostridium botulinum* and Its Surrogate Strain *Clostridium sporogenes* PA 3679**
JEANNE-MARIE MEMBRÉ, UMR1014 SECALIM, INRA, Oniris, Nantes, France

**16.30** **Probabilistic Model for Process Control Measure Evaluation, a Practical Case**
LAURE PUJOL, Novolyze, Daix, France

17.00 Adjourn

## S18 Innovative Nonthermal Technologies for Microbial Biofilm Decontamination on Biotic and Abiotic Surfaces

*The Arc*
**Organizers and Convenors: Maria Baka and Jan Van Impe**

**15.30** **Experimental Design for the Assessment of the Anti-biofilm Effectiveness of Cold Atmospheric Plasma on Abiotic Surfaces**
JAN VAN IMPE, KU Leuven/BioTeC, Ghent, Belgium

**16.00** **Potential of Atmospheric Cold Plasma for Biofilm Control in Food Processing**
PAULA BOURKE, Dublin Institute of Technology, Dublin, Ireland

**16.30** **Scale-up and Optimisation of Large Area Cold Plasma Systems for Rapid Microbial Decontamination**
JAMES WALSH, University of Liverpool, Liverpool, United Kingdom

17.00 Adjourn

## S19 How to Manage Microorganisms with Complex Life Cycles in the Food Industry

*Studio 311–312*
**Organizers: Louis Coroller, Noémie Desriac, Florence Postollec**
**Convenors: Louis Coroller, Frank Devlieghere**
*Sponsored by IAFP Foundation*

**15.30** **Food Spoilage by Fungi or Sporeforming Bacteria: Common Features and Differences**
FRANK DEVLIEGHERE, Ghent University, Ghent, Belgium

**16.00** **Germination and Growth of Spoilage Fungi**
MARIA GOUGOULI, Perrotis College, American Farm School, Thessaloníki, Greece

**16.30** **Growth Limits and Their Uses to Predict the Cycle of Life of Sporeforming Bacteria**
EMILIE GAUVRY, University of Brest - UMT 14.01 SPORE RISK, Brest, France

17.00 Adjourn

## T7 Technical Session 7 – Food Processing Technologies

*Studio 314–316*
**Convenor: George-John Nychas**

**T7-01 15.30** ***Listeria monocytogenes* Control Strategies Applied on Fresh and Cold-smoked Salmon**
EVEN HEIR, Kristian H. Liland, Askild L. Holck, Nofima AS, Ås, Norway

**T7-02 15.45** **Resistance of *Bacillus subtilis* Endospore to Cold Plasma**
CHRISTIAN HERTWIG, Kai Reineke, Oliver Schlüter, Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany

**T7-03 16.00** **Inactivation of MS2 Bacteriophage, Murine Norovirus-1, *Salmonella* and *Enterococcus faecium* on Strawberries by Using Gaseous Ozone**
ZIJIN ZHOU, Frédérique Cantergiani, Frank Devlieghere, Sophie Zuber, Mieke Uyttendaele, Ghent University, Ghent, Belgium

**T7-04 16.15** **A Synergistic Effect of High Pressure and Nisin on the Inactivation of Heat-resistant and Pathogenic Spores in Food Matrices**
CHLOE MODUGNO, Souhir Kmiha, Hélène Simonin, Stéphane Andrè, Chedia Aouadhi, Slah Mejri, Abderrazak Maaroufi, Jean-Marie Perrier-Cornet, Unité Mixte de Recherche - Procédés Alimentaires et Microbiologiques (UMR PAM), Dijon, France

**T7-05 16.30** **Application of UV-C Light Processing on Fresh and Frozen Strawberries, Raspberries and Blueberries to Compare the Inactivation of Viral and Bacterial Pathogens and Their Surrogates**
FREDERIQUE CANTERGIANI, Sophie Butot, Thierry Putallaz, Lise Michot, Mireille Moser, Sophie Zuber, Nestlé© Research Center, Lausanne, Switzerland

**T7-06 16.45** **Effect of a Novel Supercritical $CO_2$ Drying Process on Foodborne Pathogens Inoculated on Coriander and Strawberry**
SIMÉON BOURDOUX, Stijn De Sutter, Sara Spilimbergo, Alessandro Zambon, Filippo Michelino, Mieke Uyttendaele, Frank Devlieghere, Andreja Rajkovic, Ghent University, Ghent, Belgium

17.00 Adjourn

# Day 3 – Friday, 31 March

## 7.30 – 16.00 Registration Open

### 7.30 – 8.30 – Morning Coffee

**RT2** **Globalisation Challenges in Food Safety Management – Emerging Issues in Culture, Systems and Practice**
*Silver Hall*
**Organizer and Convenor: Carol Wallace**

**8.30** **Panelists:**
LONE JESPERSEN, Cultivate, Hauterive, Switzerland

OLIVIER GALARD, Barry-Callebaut, Wieze, Belgium

LIESBETH JACXSENS, Ghent University, Ghent, Belgium

### 10.00 Networking Coffee Break in *Silver Hall Foyer*

**S20** **Application of Bacteriophages as an Antimicrobial Intervention and Detection Strategy in the Food Industry**
*The Arc*
**Organizer and Convenor: Steven Hagens**

**8.30** **Bacteriophage as a Food Safety Tool**
CATH REES, The University of Nottingham, Nottingham, United Kingdom

**9.00** **Combating *Listeria* and *Salmonella* with Bacteriophages from Bench to Factory**
STEVEN HAGENS, Micreos Food Safety B.V., Wageningen, Netherlands

**9.30** **Bacteriophage Endolysins as Promising Tools for Detection and Control of Foodborne Pathogens**
MATHIAS SCHMELCHER, ETH Zurich, Zurich, Switzerland

### 10.00 Networking Coffee Break in *Silver Hall Foyer*

**S21** **Differentiate the Real Culprits from the Presumed Ones: How Emerging Technologies Improve a Typical Day's Work in Routine Testing Labs**
*311–312*
**Organizers: David Tomas Fornes, Danièle Sohier**
**Convenors: Patrice Arbault, Adrianne Klijn**
*Sponsored by IAFP Foundation*

**8.30** **How Do Genome Dissections Reveal the "Right" Identity of Strains?**
MARIE BUGAREL AND ANNE BRISABOIS, Texas Tech University, Lubbock, TX, USA

**9.00** **Food Industry Labs' Expectations: Recent Advances and Open Challenges (30 minutes presentation and open exchanges)**
DAVID TOMAS FORNES, Nestle, Lausanne, Switzerland

**9.30** **How Digital PCR Will Decrease the Number of False Positive Data in STEC Detection (15 minutes presentation and open exchanges)**
JEAN-FRANÇOIS MOUSCADET, Bio-Rad, Marnes-la-Coquette, France

**9:45** **Just Do It with a MALDI! Are Microbiologists Mutating into Chemists? (15 minutes presentation and open exchanges)**
DANIÈLE SOHIER, Bruker Daltonics, Bremen, Germany

### 10.00 Networking Coffee Break in *Silver Hall Foyer*

**T8** **Technical Session 8 – Modeling and Risk Assessment 2**
*Studio 314–316*
**Convenor: Jeanne-Marie Membré**

**T8-01 8.30** **Applicability of Culture Medium-based Predictive Models to Food Scenarios Using *Bacillus cereus* as a Model Organism**
NATHÁLIA B. SILVA, Bruno A. M. Carciofi, Gláucia M. F. Aragão, Jozsef Baranyi, Mariem Ellouze, UFSC - Universidade Federal de Santa Catarina, Florianópolis, Brazil

**T8-02 8.45** **Modelling the Effect of Different Storage Temperatures on the Growth and Toxin Production of *Staphylococcus aureus* in Milk**
VARALAKSHMI SUDAGAR, Liesbeth Jacxsens, Mieke Uyttendaele, Ghent University, Belgium, Ghent, Belgium

**T8-03 9.00** **Quantification of Survival of Pathogenic *E. coli* during Meat Preparation**
LUCAS WIJNANDS, Ellen Delfgou-van Asch, Angelina Kuijpers, Jurgen Chardon, Annemarie Pielaat, Eric Evers, cZ&O/RIVM, Bilthoven, Netherlands

**T8-04 9.15** **A Simple Concept Allowing the Prediction of Microbial Inactivation under Non-isothermal Process, and Taking into Account Non-log-linear Inactivation Kinetics**
NOÉMIE DESRIAC, Mikael Vergos, Ivan Leguerinel, Veronique Huchet, Louis Coroller, Olivier Couvert, LUBEM UBO University - UMT14.01SPORE RISK, Quimper, France

**T8-05 9.30** **Accurate Quantification of *Campylobacter* Contamination on Chicken Carcasses Including Variability and Uncertainty**
Benjamin Duqué, Samuel Daviaud, Sandrine Guillou, NABILA HADDAD, Jeanne-Marie Membré, UMR1014 SECALIM, INRA, Oniris, Nantes, France

**T8-06 9.45** **Integrated Approach to Process Qualification**
BERTRAND COLSON, QuoData GmbH, Dresden, Germany

### 10.00 Networking Coffee Break in *Silver Hall*

***Foyer***

## CLOSING SESSION

*Silver Hall*
**Chair: Lilou van Lieshout**

**10.30** **Food Safety in a Sustainable Production Chain**
WENDIE CLAEYS, FASFC – SciCom, Brussels, Belgium

**11.00** **Minimal Processed Products, Free of Additives, Safe, Tasty, and with Prolonged Shelf Life: The Holy Grail**
FRANK DEVLIEGHERE, Ghent University, Ghent, Belgium

**11.30** **Recent European Commission Initiatives in Food Hygiene and Microbiological Food Safety**
KRIS DE SMET, European Commission, Brussels, Belgium

**12.00** **U.S. FDA Draft Guidance for Industry on Control of *Listeria monocytogenes* in Ready-to-Eat Foods**
MICKEY PARISH, U.S. Food and Drug Administration, College Park, MD, USA

**12.30** **Awards Presentation and Concluding Remarks**
LINDA J. HARRIS, University of California, Davis, CA, USA

**12.45 – 14.00**

**Farewell Refreshements in *Silver Hall Foyer***

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IAFP'S EUROPEAN
SYMPOSIUM ON FOOD SAFETY
29-31 MARCH 2017
BRUSSELS, BELGIUM

# INVITED SPEAKER BIOGRAPHIES

29–31 March 2017 – Brussels, Belgium

# INVITED SPEAKERS

## Ana Afonso

### European Food Safety Authority, Italy

Ana Afonso, DVM MSc, is the Team Leader of the Emerging Risks team at the European Food Safety Authority where she coordinates the activities of the team, including its collaborative networks and various projects on methodology for identification of emerging risks. She is a veterinarian specialized in Aquatic Veterinary studies and joined EFSA in 2006 as a Scientific Officer for the Animal Health and Welfare Unit. Prior to that, she worked as a veterinary official responsible for approval and inspection of food establishments, as a veterinary assistant for hygiene and animal health issues on fish farming and as a Research/ Lecturer assistant at the Veterinary Faculty.

## Roy Betts

### Campden BRI, United Kingdom

Roy Betts is Head of Microbiology at Campden BRI, an independent international food research organisation based in the UK. Roy manages a group of 45 food microbiologists, undertaking a range of industry focused food research and testing projects for a worldwide client base. Roy originally managed a research team at Campden BRI and concentrated on the research, development and validation of microbiological test methods. After becoming Head of Department, his interests moved to the assessment of the microbiological quality and safety of foods, advising industry on techniques and procedures to produce and market high quality safe foods. Roy has published widely in the area and is a member of the ILSI Europe Microbiological Food Safety Task Force, the UK Food and Drink Federation Food Hygiene Sub Committee and the UK Advisory Committee on the Microbiological Safety of Foods as well as British Standards Institute and ISO committees dealing with microbiological test methods.

## Johanna Björkroth

### University of Helsinki, Finland

Johanna Björkroth is a Professor of Food Hygiene at the University of Helsinki since 2002. She has regularly gained competitive research funding and grants for her team. Together with her co-authors, she has over 100 peer-reviewed publications (h-index 33 ISI and 40 Google Scholar) and several book chapters. Björkroth has worked in interaction with food processors and safety authorities but also with different companies providing technology solutions and platforms. She has and has had a wide variety of domestic and international positions of trusts. Her current duties include being the Chair of the Board of Gene Technology and Vice Chair of the Council of Health, Academy of Finland. She is a Scientific Editor of *Applied Environmental Microbiology* journal.

## Karin Blacow

### Commercial Food Sanitation, LLC, Netherlands

Karin Blacow's academic background is in mechanical engineering. Prior to joining Commercial Food Sanitation, LLC, in 2012 as a Food Safety Specialist, Karin spent 12 years working with food manufacturers at Intralox. She has extensive knowledge of the production processes, as well as hygiene and sanitation challenges, in many food segments. Her technical background, combined with her food industry application knowledge and in-field experience working with food processors, affords her a unique perspective when helping customers tackle sanitation challenges. Karin has developed a passion to educate and support food processors in making a positive change in their food safety culture and working on continuous improvement projects. Karin is bilingual in Dutch and English and has a good understanding of the German language. She supports customers throughout the EMEA region in a variety of areas, including cleaning sequencing, sanitation program analysis for

continuous improvement, equipment design reviews, hygienic design improvements, development of SSOPs and associated documentation, and related education and training. Karin is an active member of the European Hygienic Engineering and Design Group (EHEDG), participating in several guideline sub-groups. Karin is a columnist for Food Processing Vakblad.

## Stéphanie Blanquet-Diot

### Université Clermont Auvergne, France

Engineer in Food Science, Dr. Stéphanie Blanquet-Diot has a Ph.D. in Biotechnology, Nutrition and Health (2002). Since 2002, she is Associate Professor at the University of Clermont Auvergne in France. She currently leads the research group PAZ on zoonotic pathogens of UMR 454 MEDIS (Microbiota, Digestive Environment and Health), with research programs on pathogenic *Escherichia coli*, probiotic bacteria and yeasts, gut microbiota dysbiosis and host-pathogen interactions. She has 15 years experiences in artificial digestion, nutrition and microbiology with a specific focus on probiotics and foodborne pathogens. She has authored or co-authored more than 40 research papers and four books.

## Paula Bourke

### Dublin Institute of Technology, Ireland

Paula Bourke is a lecturer in the School of Food Science and Environmental Health, DIT. Her research is currently focused on developing effective strategies and novel technologies for control of microbiological and chemical risks in the food chain and for infection control in healthcare. Her research develops understanding of the mechanisms of action as well as efficacy and safety of technology developments to advance applicability of novel antimicrobial technologies. This is critical for enhanced sustainability in food production but also for regulatory compliance and enduser acceptance.

Dr. Bourke established the Food and Health Research Centre at DIT, and leads a multidisciplinary research group that develops and understands biological interactions with novel technologies. These insights have enabled the group to develop pilot and industrial prototypes supported through FP7, national and internationally funded projects in collaboration with industry. Dr. Bourkes' research profile can be found at https://www.researchgate.net/profile/Paula_Bourke.

## Sara Bover-Cid

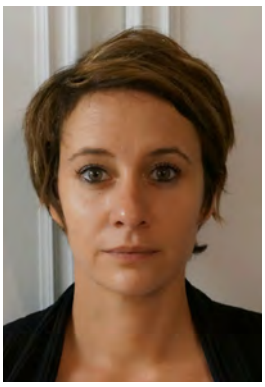
### IRTA, Spain

Dr. Sara Bover-Cid is Head of the Food Safety Programme at the Institute for Food Research and Technology (IRTA) in Monells (Spain). Her research activities deals with microbiological risk assessment and modelling the effect of food preservation technologies (e.g., high pressure processing, biopreservation, hurdle technology) on the behaviour of foodborne microorganisms of technological interests, spoilage and pathogenic bacteria (*Listeria monocytogenes* in particular). She belongs to the Editorial Board of the *International Journal of Food Microbiology* and the Executive Board of the International Committee on Food Microbiology and Hygiene, as secretary. She has participated in numerous national- and EU funded research projects. She has published over 67 papers in international SCI journals and several book chapters.

## Anne Brisabois

### ANSES, France

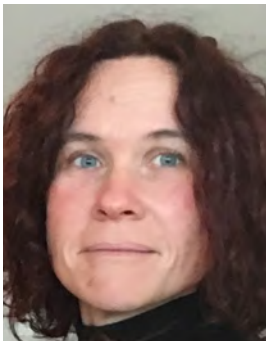
Dr. Anne Brisabois, Senior Microbiologist and Research Director, is currently the Deputy Head of the Laboratory for Food Safety, dedicated to the surveillance and characterization of foodborne pathogens and manages the fishery and aquaculture department at the French Agency for Food, Environmental and Occupational Health Safety (ANSES). This department is dedicated to the microbial and chemical contaminants of the fishery and aquaculture products, it conducts research studies these topics, especially on *Listeria*, *Vibrio*, parasites (Anisakidae), histamine, markers of spoilage, freshness and more recently on emergence of antimicrobial resistance and micro-plastic issues. The department is national reference laboratory for *Vibrio* and histamine in fishery products.



## Marie Bugarel

### Texas Tech University, USA

Dr. Marie Bugarel obtained her Ph.D. in 2012 while working on a contribution to the molecular risk assessment of *Salmonella* and pathogenic *Escherichia coli* associated with severe diseases in humans at Anses, in Paris. After her Ph.D., she moved to the United States for a post-doctoral position at Texas Tech University. Since 2013, she has been a Research Assistant Professor, part of ICFIE research group at Texas Tech University. Dr. Bugarel is part of a collaborative effort dedicated to the better understanding of the invasion and survival characteristics of *Salmonella* in bovine lymph nodes. Her research also focuses on the development of molecular assay to characterize foodborne pathogens.



## Wendie Claeys

### FASFC - SciCom, Belgium

Dr. Wendie Claeys holds a Ph.D. in Applied Bioscience Engineering (K.U. Leuven, Belgium) in the field of food technology. After receiving her Ph.D. in 2003, she continued research as a postdoc with a Scholarship of the Research Foundation Flanders (FWO) at the Centre for Food and Microbial Technology of the Faculty of Bioscience Engineering (K.U. Leuven, Belgium). Currently, she works at the Staff Direction for Risk Assessment of the Belgian Agency for the Safety of the Food Chain (FASFC), where she is responsible for the scientific risk evaluation of chemical hazards in the food chain.

## Pier Sandro Cocconcelli

### Universita Cattolica del Sacro Cuore, Italy

Pier Sandro Cocconcelli is Full Professor of Food Microbiology at the Università Cattolica del Sacro Cuore and he is Rector's delegate for internationalization of the same university. In 1987, he worked at the Institute of Food Research of Reading (UK) on the development of cloning vectors for lactic acid bacteria and in 1994 he was visiting Assistant Professor at the BTPI of the University of Minnesota (USA). Since 2003, he has been a scientific expert of the European Authority of Food Safety (EFSA) as Panel and Working Group member focusing on the microbiological risk assessment. From 2006 to 2010, he has chaired the Standing Working Group on Microorganisms of FEEDAP and now he chairs the Standing Working Group of Genetically Modified Microorganisms. He is also member of the BIOHAZARD Working Group on Qualified Presumption of Safety of Microorganisms. His research activities are focused on food and agricultural microbiology, bacterial molecular biology, bacteria genomics, risk analysis of food pathogenic bacteria, and on the gene exchange of antibiotic resistance and virulence determinants in the food chain. He has been teaching food microbiology and molecular biology over the last 15 years. In the last five years, he has coordinated specific programmes, Ph.D. school and executive courses, on the microbiological risk assessment in the food chain.

## Luca Cocolin

### University of Torino-DISAFA, Italy

Luca Cocolin is Full Professor of Food Microbiology at the University of Torino, Italy. He is also executive board member of the ICFMH. Editor-in-Chief of the *International Journal of Food Microbiology*; an academic editor of *PLOS One*; member of the Editorial Board of *Food Research International*; *Frontiers in Microbiology* and *Current Opinion in Food Science and Food Analytical Methods*. He is co-author of about 300 papers on national and international journals. He is an expert in (i) Molecular methods for the detection, quantification and characterization of foodborne pathogens; (ii) Study of the microbial ecology of foods by using culture independent and dependent methods; (iii) Bioprotection; and (iv) Human microbiome.

## Philippe Dantigny

### University of Western Brittany, France

Dr. Philippe Dantigny is an Engineer in Food Processing and Master's degree in Food Microbiology (1985). He earned his Ph.D. in 1989 in Biochemical Engineering at the National Polytechnics of Lorraine, Nancy, France. After post-doctoral work, he was appointed as a Lecturer in Biochemical Engineering at the University of Bath, UK. Since 1991, he is a Senior Lecturer at the University of Lyon, France in Food Microbiology and Biotechnology. He is the Head of the Predictive Mycology Group at the LUBEM. He is member of the French Food Safety Authority (ANSES) for 8 years – Biohazard group, Expert in Predictive Modelling; Food spoilage; Mycotoxins Contamination; and Fermentations. Dr. Dantigny has coordinated one FP6 project and participated in another EU project. The group of Dr. Dantigny has developed specific modelling tools for fungi, and more generally, a new topic named "Predictive Mycology," which aims at understanding and modelling germination, growth and production of mycotoxins in food and agricultural products. Dantigny has published more than 30 papers (SCI) in this field, and edited a reference book in 2013, *Predictive Mycology*, Nova Science Publisher, with more than 25 contributors from 10 different countries.

## Francesca De Filippis

### University of Naples Federico II, Italy

Francesca De Filippis obtained her Ph.D. in Agri-Food Sciences and Technologies from the University of Naples Federico II in 2015. She specialized in the study of food- and human-associated microbiome by metagenomics and metatranscriptomics approaches. During the Post-Doc, she was involved in several projects aiming to explore the relationships between dietary habits and/or diseases and human microbiome. She is Lecturer in Microbiology at the Department of Agricultural Sciences of the University of Naples Federico II since December 2016.

## Kris de Smet

### European Commission, Belgium

Kris de Smet graduated as Veterinarian Doctor in 1987. From 1988 to 1992, he was researcher at the University of Ghent (Belgium) in the Faculty of Veterinary Science. From 1992 until 2001 he was employed at the private sector. He was mainly involved in veterinary services and quality control of a poultry integration. Since 2001, he has worked as official at the European Commission, Health and Food Safety Directorate-General. He was involved in the management of EU legislation on BSE and zoonoses (mainly *Salmonella*). Since the beginning of 2009, he has been the coordinator of the team dealing with EU legislation on food hygiene and zoonoses control.

## Heidy den Besten

### Wageningen University, Netherlands

Dr. Heidy den Besten obtained a BSc in Food Technology and a BSc in Mathematics and completed her MSc Food Technology cum laude; specialising in Food Safety at Wageningen University. Before starting as Assistant Professor with tenure track in 2011, she completed her Ph.D. project entitled "Quantification of *Bacillus cereus* stress responses" and worked as postdoctoral researcher. Her research domain focuses on pathogen ecology interlinking functional genomics and prediction of microbial behaviour. She acts as an editorial board member for two journals, *Food Research International* and *International Journal of Food Microbiology*. She was invited to join the Singapore Centre for Environmental Life Sciences Engineering as visiting scientist in 2015. Heidy was appointed as Associate Professor in 2016 and became member of the Program Committee of IAFP. Also, she is teaching within the MSc and BSc Food Microbiology programmes of Wageningen University and coordinates a BSc Food Microbiology course.

## Jan Dijksterhuis

### Westerdijk Fungal Biodiversity Institute, Netherlands

Dr. Jan Dijksterhuis is Associate Professor of History of Science at the Centre for Studies of Science and Technology. He studied Applied Mathematics and Science Studies and has worked as a teacher of Mathematics and Social Science at secondary level. In 1999, he finished his dissertation on the history of optics in the seventeenth century, focusing on the work of Christiaan Huygens (1629–1695): *Lenses and Waves. Christiaan Huygens and the Mathematical Science of Optics in the Seventeenth Century* (Kluwer 2004). In 2006, he received an NWO VIDI grant for a five year research project *The Uses of Mathematics in the Dutch Republic*. The project runs from 2007 to 2012 and includes two Ph.D.s.

## Christophe Dufour

### Mérieux NutriSciences France, France

After completing veterinarian studies in Maisons-Alfort France in 1986 and UTC Compiegne University DEA degree, Christophe Dufour joined different food testing laboratories. Entering as Scientific Manager in Silliker in Mérieux NutriSciences France in 1999, Christophe participates in various normalization groups and expert panels in the field of food microbiology, microbiological criteria, food quality, GMO or allergens issues. Christophe contributes to many working groups with professional expertise to develop process criteria for food industry.

## Frank Devlieghere

### Ghent University, Belgium

Dr. Frank Devlieghere is Professor in Food Microbiology and Food Preservation, Fish and Meat Technology at the Faculty of Bioscience Engineering since 2003. He is a Bio-science Engineer, graduated at Ghent University, finished his Ph.D. in 2000 in the field of Predictive Microbiology from Ghent University. From then he became a Post-Doc Researcher at Ghent University and performed research mainly in the areas of predictive microbiology and microbial aspects of food preservation. As a Full Professor he is now responsible for the research in the field of food preservation with the following main research topics: predictive microbiology, microbial spoilage mechanisms, microbial risk assessment, new decontamination techniques, microbial aspects of food packaging, mild preservation, preventive preservation measures towards mycotoxin production, and chemical preservation of food products.

## Stephen Forsythe

### Nottingham Trent University, United Kingdom

Steve Forsythe is Professor of Microbiology at Nottingham Trent University, and also Visiting Professor to the University of Hong Kong. He has authored over 100 peer-reviewed papers and three books on food microbiology. His recent research has been on *Cronobacter* and other emerging pathogens causing infant infections through contaminated feeds. This work ranges from improved primary isolation methods (chromogenic agar) through to whole genome analysis; developing DNA-sequence based profiling procedures based on multilocus-sequence typing (7- loci, 53-ribosomal & core genome MLST), capsule composition and CRISPR-cas arrays. He is curator of the *Cronobacter* PubMLST database.

## Cormac Gahan

### University College Cork, Ireland

Cormac Gahan is a Senior Lecturer in Microbiology in the School of Microbiology and the School of Pharmacy in University College Cork, Ireland. He is also a funded investigator within the APC Microbiome Institute, a centre of excellence devoted to the analysis of the role of the intestinal microbiota in health and disease. Cormac has an interest in how foodborne pathogens (in particular *Listeria monocytogenes*) adapt to environmental and food-processing associated stress and how this influences disease pathogenesis. His recent work has involved analysis of the role of the gut microbiota as a barrier to bacterial infection in the gut. He has published over 100 peer-reviewed papers in the broad areas of microbial stress adaptation, gut pathogenesis and the microbiota.

## Olivier Galard

### Barry-Callebaut, Belgium

Olivier Galard graduated as a Bio-engineer at the University of Ghent and has developed a career as a quality professional committed to quality assurance, customer satisfaction and Food Safety. He started his career in the pharmaceutical industry working for a J&J company in roles that ranged from Quality Control to Supplier Quality Management and continued his passion for quality and consumer satisfaction by joining Coca-Cola Enterprises as a site QA Manager for several years. As the group Quality Director for Alpro, the European leader in plant beverages, he later developed robust quality frameworks in a fast moving environment. Currently, Barry Callebaut's Global Food Safety and Hygiene Manager, Olivier is now primarily focusing on standardization of food safety standards across the global network of the world's leading chocolate company. His leading principle is to find a sustainable balance between food safety, compliance and practical feasibility.

## Emilie Gauvry

### University of Brest, UMT 14.01 SPORE RISK, France

Emilie Gauvry is a third-year Ph.D. student in microbiology at the LUBEM of Quimper. She made her studies at the University of Rennes (France) and obtained her Master's degree in Fundamental and Applied Microbiology. She works on the sporulation abilities of *Bacillus subtilis* according to the environmental factors such as the pH, temperature and water activity in order to predict the sporulation behaviors of sporeformers in the food industry. The LUBEM of Quimper is focused on food safety and food spoilage due to bacterial sporeformers: their biodiversity, their physiology, their metabolism, and the development of control means and tools such as modified atmosphere and predictive microbiology.

## Collette Girvin

### Kellogg Company, Ireland

Colette Girvin is a food safety professional with over 30 years' experience in Quality and Food Safety Systems in the food manufacturing sector. A graduate of the Dublin Institute of Technology, Colette initially studied Food Technology and recently completed a Master's Degree in Food Safety Management. Experience includes Quality Management with Unilever and Mondeléz International. Currently working with Kellogg, based in Dublin, she has responsibility for cleaning and sanitation programs within Europe.

## Elissavet Gkogka

### Arla Strategic Innovation Centre, Denmark

Ms. Elissavet Gkogka is an experienced food microbiologist with approximately 10 years of academic and industrial experience in the areas of food safety, natural antimicrobials, predictive modeling, risk assessment and challenge testing. In her position as a research microbiologist in Arla R&D, she has been involved in numerous new product development projects, giving recommendations on product formulations and processing/packaging conditions to ensure food safety and quality throughout shelf life. Elissavet is also a member of ILSI's Microbiological Food Safety Task Force and has experience in foodborne disease epidemiology, having presented her research as a technical adviser for the World Health Organization.

## Maria Gougouli

### Perrotis College, American Farm School, Greece

Dr. Maria Gougouli is a Lecturer in Food Microbiology at Perrotis College of the American Farm School of Thessaloniki. She holds a B.Sc. in Food Science and Technology from Aristotle University of Thessaloniki, a M.Sc. in Food Science and Technology, and a Ph.D. in Food Mycology from the same university. She has completed three post-docs in the field of Food Microbiology. She has published several referred scientific journal articles and book chapters. Additionally, she has presented her research in national and international conferences where she has received two Research Paper Awards as a Young Scientist from the International Committee on Food Microbiology and Hygiene. She belongs to the Editorial Board of *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. Furthermore, she participated in several research projects funded by the European Union and/or National Resources and industry as well. Recent research efforts have centered on the microbiological quality and safety of food, predictive microbiology, microbial risk assessment, stochastic modeling.

## Kathie Grant

### Public Health England, United Kingdom

Dr. Kathie Grant is an internationally recognised expert in the field of foodborne pathogens with 30-years experience in clinical and public health microbiology and a research interest in exploiting whole genome sequencing (WGS) of bacterial pathogens to improve the understanding and control of foodborne bacterial illness. She is Head of Public Health, England's Gastrointestinal Bacteria Reference Unit in United Kingdom, which is the national reference laboratory for a range of foodborne pathogens including *Salmonella*, *E. coli* VTEC, *Campylobacter*, *Listeria monocytogenes* and *Clostridium botulinum*. Since 2001, she has championed the use of molecular methods leading to improvements in the detection and investigation of bacterial foodborne disease within the UK. Her laboratory is one of the first laboratories in the world to implement the use of WGS for routine bacterial reference service delivery.

## Sandrine Guillou

### Oniris, France

Sandrine Guillou, Ph.D., is a Senior Scientist in the Microbiological Food Safety domain. She has been working in research for 15 years, and more recently in the unit research of Food Safety Secalim, at Nantes. In the past (2002–2008), her research was more focused on the understanding of the microbial behaviour in response to various physicochemical stresses. Currently, her research is more oriented towards quantitative and modelling approaches. She has for instance performed exposure assessment of microbial hazards in the context of food processes including high pressure processing, in which she has developed expertise.

## Steven Hagens

### Micreos Food Safety B.V., Netherlands

Steven Hagens studied Molecular Biology at the University of Groningen, The Netherlands. During his Ph.D. thesis at the Institute of Microbiology and Genetics at Vienna University Austria, he worked on bacteriophage therapy of *Pseudomonas aeruginosa* infections. Steven went on to become a post-doctoral fellow at the Laboratory for Food Microbiology at the Institute of Food Science and Nutrition of the Swiss Federal Institute of Technology (ETH) in Zurich Switzerland where he worked on *Listeria* and *Salmonella* phages and their potential for combating and detecting these food pathogens.

Since 2005, he has worked as Chief Scientific Officer at the interface of Micreos's own research team, public research institutions and industry both in further development of existing products and of new products against other harmful target organisms. The author of several publications on practical application of phages, Steven coordinates research collaborations with universities such as ETH and Ghent University, Belgium as well as other public institutes with a focus on applied solutions for the diverse bacteria problems.

## Linda J. Harris

### Department of Food Science and Technology, University of California-Davis, USA

Dr. Linda Harris is a Specialist in  Cooperative Extension in Microbial Food Safety in the Department of Food Science and Technology at the University of California Davis campus. She oversees a research program on the microbial food safety of fresh fruits and vegetables and tree nuts and provides expertise on food safety microbiology throughout the food chain. She is currently President of the International Association for Food Protection.

## Marc Heyndrickx

### ILVO - Flanders Research Institute for Agriculture, Fisheries and Food, Belgium

Marc Heyndrickx made his Ph.D. in Microbiology in 1991 at the Ghent University (Belgium) and conducted a post-doc in the same lab. Since 1997, he has been a Tenured Researcher at the Institute of Agricultural and Fisheries Research (ILVO, Belgium), where he leads a research team on food safety. In this function, he is responsible for the scientific research on chemical and microbiological food safety, with a special emphasis on detection, identification, typing, virulence, antibiotic resistance transfer and remediation of zoonotic and other foodborne pathogens as well as spoilage organisms in food and antibiotic residues in the environment. He is a member of the Superior Health Council of Belgium and visiting professor of bacterial zoonoses at the Faculty of Veterinary Medicine of the Ghent University. He is author or co-author of more than 200 peer-reviewed publications.

## Gert Jan Hofstede

### Wageningen University, Netherlands

Gert Jan Hofstede (1956) is currently an Associate Professor at Wageningen University, the Netherlands, in Information Technology. He also is a frequent guest lecturer around the world. His book *Exploring Culture: Stories, Exercises and Synthetic Cultures* was translated into several languages; *Cultures and Organizations: Software of the Mind*, 3rd ed 2010, with Geert Hofstede and Michael Minkov, is an international bestseller. He creates and animates group simulation games in areas such as leadership, negotiation and trust. Second, he incorporates believable cross-cultural behaviour into computational models of humans for social simulation. He is the founder of Silico Centre Wageningen.

## Liesbeth Jacxsens

### Ghent University, Belgium

Liesbeth Jacxsens, Ph.D., Bio Science Engineering, is Professor in Food Safety Management and Risk Analysis in Agri-food Chain at Department of Food Safety and Food Quality, Ghent University, Belgium. Her research domain encounters two research lines: food safety management and risk assessment (technical/mathematical compound of the broader framework of risk analysis related to food safety and human health impacts). The research of risk assessment is interacting with food safety management, as outcomes of risk assessment are applied as an input for the food safety management on operational level. Food safety culture is a current research topic in food safety management.

## Kimon Andreas Karatzas

### University of Reading, United Kingdom

Dr. Kimon Andreas Karatzas is a Molecular Food Microbiologist who obtained his degree in Food Science and Technology from Aristotle University of Thessaloniki, Greece in 1997 and his Ph.D. from Wageningen University, the Netherlands in 2002. Subsequently, he held post-doctoral positions in Bristol University, UK and NUIG, Ireland, where he became a PI, SIRG Research Fellow in 2009. Since 2012 he has been Assistant Professor in Food Microbiology, University of Reading, UK. His work investigates bacterial stress-resistance mechanisms presented in 31 papers in peer-reviewed journals while he has supervised more 12 Ph.D. students and 2 post-doctoral researchers. He is also a holder of several grants (more than one million Euros the last 10 years) funded by the BBSRC, SFI-Ireland, Marie Curie and the Royal Society UK.

## Alvin Lee

### Institute for Food Safety and Health, Illinois Institute of Technology, USA

Dr. Alvin Lee is a Microbiologist and Virologist with more than 15-years research experience with a Ph.D. from RMIT University. Dr. Lee currently leads IFSH Center for Processing Innovation and co-leads the joint IFSH/FDA Microbiology Research Platform on food safety and defense-related projects. He leads the Prevention and Control CORE of NoroCORE, a USDA-NIFA Food Virology Collaborative based at North Carolina State University. Current research support includes funding from USDA, US FDA and various industry contracts. Dr. Lee is an instructor for Food Microbiology in the Illinois Institute of Technology's Masters of Science program and has mentored more than 30 graduate students and post-doctoral fellows. He is currently an active member of the International Association for Food Protection – serving on the IAFP Scientific Program Committee, American Society for Microbiology and Institute of Food Technologists.

## Miia Lindström

### University of Helsinki, Finland

Miia Lindström, D.V.M., Ph.D., is Professor of Dairy Processing Hygiene since 2009 at the Department of Food Hygiene and Environmental Health in Faculty of Veterinary Medicine, University of Helsinki (UH). She completed her Ph.D. thesis on diagnostic and food safety aspects of *Clostridium botulinum* in 2003 in UH, and worked as a post-doctoral fellow in research projects focusing on *C. botulinum* in Institute of Food Research, Norwich, UK, in 2003–2004 and back in UH 2004 onwards. She is a principal investigator since 2007, with research interests in the biology and epidemiology of spore-forming food pathogens and *Listeria*. Major research projects focus on the environmental, cellular, and genetic factors regulating *C. botulinum* neurotoxin production and sporulation. The group belonged to the Centre of Excellence in Microbial Food Safety Research in 2008–2013, and found the first repressor of botulinum neurotoxin formation. The group collaborates with the Finnish food industry and with food control authorities. The DFHEH laboratory has 25 years of experience working with *C. botulinum* and runs diagnostics for human and animal botulism. Prof. Lindström has authored or co-authored over 95 international peer-reviewed papers and book chapters in the field of food hygiene.

## Jacques Mahillon

### University Catholic Louvain, Belgium

Jacques Mahillon received his Ph.D. in Bioscience Engineering at the Université Catholique de Louvain (UCL) in 1987. After two years spent as Senior Scientist at Plant Genetic Systems, Ghent (Belgium), where he did most of his Ph.D. research, he moved to Harvard University, Cambridge (USA) as post-doctoral fellow. In 1992, he returned to UCL and was appointed Research Associate of the Belgian National Fund for Scientific Research (FNRS) till 2002. He then became tenured professor and ordinary professor in 2006. He lectures General Microbiology, Food Microbiology, and co-lectures Bioinformatics and Biotechnology. He was vice-dean (2003–2007) and dean (2009–2013) of the Faculty of Bioscience Engineering at UCL. He is head of the laboratory of Food and Environmental Microbiology. His research topics include: molecular and bacterial genetics, with a particular focus on Mobile Genetic Elements and virulence genes in the *Bacillus cereus* group, but also the molecular typing and control of foodborne pathogens.

## Peter McClure

### Mondelez International, United Kingdom

Peter McClure gained his B.Sc. and Ph.D. from Cardiff University and then joined the Institute of Food Research in 1985, in the UK, to work in the areas predictive modelling and microbiological food safety. He worked for Unilever for over 20 years and recently joined Mondelez International as the Section Manager for Microbiology and Food Safety for Europe, Middle East and Africa. Peter is a member of the International Commission on Microbiological Specifications for Foods, and the Advisory Committee on the Microbiological Safety of Food in the UK, is a Co-Editor of *Foodborne Pathogens*, and is a Visiting Professor at Leeds University.

## Jeanne-Marie Membré

### INRA, France

Dr. Jeanne-Marie Membré has a diploma in Food Engineering and a Ph.D. in Food Microbiology. In 1989, she joined the French National Institute for Agricultural Research (INRA) of Villeneuve d'Ascq, Fr, where she was in charge of the predictive microbiology research programme. From 2003 to 2009, she worked at the Safety & Environmental Assurance Centre of Unilever, in Bedford, UK, where she developed predictive models and exposure assessment models for a wide range of food applications. Since 2010, she has been working at SECALIM, UMR1014, of INRA Nantes, France. Dr. Membré has published more than 60 articles in peer-reviewed international journals. She belongs to the Scientific Board of *Journal of Food Protection* and *International Journal of Food Microbiology*.

## Nina McGrath

### EUFIC - European Food Information Council, Belgium

Dr. Nina McGrath joined the European Food Information Council (EUFIC) in May 2015. As Food Safety Projects Manager, she manages the production of science-based communications on food safety and quality related topics. Prior to this, she worked as Science Counsellor for Euro Chlor, the European Chlorine Industry Federation, where she advised on environmental science issues. She holds a Master's degree in Chemistry and a Ph.D. in Inorganic Chemistry and Materials Science from the University of Bristol, UK.

## Jean-François Mouscadet

### Bio-Rad, France

Jean-François Mouscadet holds a master's degree in Agronomy and a Ph.D. in Molecular Pharmacology from Paris XI University. After a post-doctoral period at the California Institute of Technology, (CA, USA), he joined the French Governmental Agency for research (CNRS) where he led a multidisciplinary research group in the field of nucleic acid biochemistry and molecular virology. He contributed more than 100 peer-reviewed articles in this field. In 2007, he was appointed Director of the CNRS UMR8113 Applied Pharmacology Laboratory. He served as Deputy-Director of the Biology Scientific Committee of the French National Agency for Research Funding from 2008 to 2010. He joined the Food Science Division of Bio-Rad in 2011 to lead its R&D department.

## Lapo Mughini-Gras

### National Institute for Public Health and the Environment (RIVM), Netherlands

Lapo Mughini-Gras (DVM Ph.D.) is a Senior Research Epidemiologist at the Dutch National Institute for Public Health and the Environment (RIVM). He also holds a joint appointment at the Faculty of Veterinary Medicine of Utrecht University. After graduating with honors in Veterinary Medicine from Bologna University in 2008, he worked at the Environmental Hygiene Unit of the Municipality of Bologna. This was followed by a Ph.D. (2010–2013) in quantitative epidemiology at the Italian National Institute of Health (ISS). In 2015, he completed a post-graduate specialization in Animal Health, Production and Hygiene at Perugia University. During 2013–2014, he also worked as epidemiologist (distance-work) at the Institute for Animal Health of North-Eastern Italy (IZSVe). His main research interests are the epidemiology of foodborne and zoonotic diseases at the human-animal-environment interface to understand their sources and transmission routes, as well as to assess their burden and set public health priorities. Besides research, he is tutoring Ph.D. students, junior researchers and trainees. As part of his duties, he is often deputed as member of various expert and working groups at the national and international level. Since 2010, he has authored over 60 papers in peer-reviewed scientific journals and books.

## Trond Møretrø

### Nofima, Norwegian Food Research Institute, Norway

Trond Møretrø has a Ph.D. in microbiology from the Norwegian University of Life Sciences, and has worked as a research scientist for the last 18 years at Nofima – The Norwegian Institute of Food, Fishery and Aquaculture Research. He has led and participated in numerous projects within food microbiology, the majority of the projects in cooperation with food industry. He is author of 65 peer-reviewed publications. Main research interests are residential microorganisms in food processing, *Listeria monocytogenes*, biofilms, disinfectant resistance, novel control strategies and kitchen hygiene.

## Theresa Neely
### Unilever, United Kingdom

Theresa Neely started her career in toxicology at a contract research laboratory in the UK, running and reporting studies investigating reproductive toxicity of pharmaceuticals. She then joined Unilever within the Safety and Environmental Assurance Centre (SEAC) based at the Colworth Science Park in Bedfordshire. Her initial role was as a toxicological risk assessor for the Home Care and Personal Care categories, dealing with products such as Persil and Dove. She then moved over to the Foods and Refreshment side of the Unilever business to become the Lead Toxicological Risk Assessor for products such as Lipton tea, Magnum ice cream and Flora ProActive margarine. Tea is a plant-based material and the presence of contaminants during growing, harvesting, processing or packing has been, and continues to be, an issue for the tea industry. As Unilever is the biggest black tea producer globally, consumer safety risk assessments for these contaminants is a key activity for our company.

## John O'Brien
### Nestlé Research, Switzerland

John O'Brien is Deputy Head of the Nestlé Research Centre in Lausanne and Leader of the Nestlé Food Safety & Integrity Research Programme. He was formerly Chief Executive Officer of the Food Safety Authority of Ireland and previously held a number of senior posts with the Danone Group in Paris including Head of Food Safety and Head of Corporate Scientific Affairs. He is currently Chairman of the Board of Directors of the International Life Sciences Institute (ILSI), Europe and a member of the Board of Trustees of ILSI International. He is a member of the Advisory Board, Institute for Food Safety & Health at the Illinois Institute of Technology, a Council Member of Campden BRI and Chair of the Advisory Board of the Northern Ireland Centre for Food & Health at the University of Ulster. He has held academic posts at the University of Surrey and University College Cork and was the founding editor of the leading food journal *Trends in Food Science & Technology*. He is currently a Visiting Professor at the University of Ulster, UK. John O'Brien's training was in toxicology, food chemistry and food science and he is a graduate of University College, Cork, Ireland and the University of Surrey, UK.

## Conor O'Byrne
### National University of Ireland, Galway, Ireland

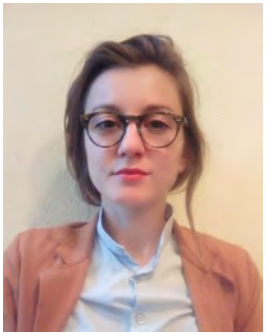
Following his doctoral research at the University of Dundee with Professor Charles Dorman, Conor O'Byrne worked as a post-doctoral researcher at Unilever Plc in Bedfordshire, England, where he became interested in *Listeria* as a foodborne pathogen. He then took up an independent postdoctoral fellowship at the University of Aberdeen to work on stress responses in *Listeria monocytogenes*. In 2002 O'Byrne was granted a lectureship at NUI Galway, where he is now Senior Lecturer and Director of the Bacterial Stress Response Group. He has pursued research and published extensively on the stress physiology and molecular biology of *Listeria monocytogenes*.

## Cian O'Mahony
### Creme Global, Ireland

Cian O'Mahony is Chief Science Officer at Creme Global, a data science company specialising in predictive modelling and software. His background is in pure and applied mathematics, holding a first class honors degree from University College Cork followed by post-graduate studies in Applied Mathematics and Pharmacy. He currently leads a team of analysts developing exposure, intake and risk assessment models in a number of areas including pesticides, food safety, predictive microbiology, nutrition, personal care products and cosmetics. Many of the models and analyses developed by his team at Creme Global are now built into a range of applications used by regulators, industry and academia worldwide.

## Jan Oltmanns
### Forschungs- und Beratungsinstitut Gefahrstoffe GmbH (FoBiG), Germany

Jan Oltmanns, M.Sc., PgDip, has an educational background in Biology, Environmental Toxicology and Pollution Monitoring. He is a company partner and Senior Scientist at FoBiG with more than 25 years of experience in various areas of toxicological risk assessment, including environmental fate modelling and exposure assessment, in different regulatory areas. Mr. Oltmanns has worked both for industry clients and national and international authorities, including several European institutions.



## Eleni Pantiora
### UN World Food Programme, Ethiopia

Eleni Pantiora is a Food Microbiology and Food Safety Expert; holder of an MSc. in Food Technology (by the Agricultural University of Athens) and a M.Sc. in Food Safety (by Wageningen University and Research Center). Since 2011, Eleni is part of the Food Quality Team and the Food Technologist Network of the World Food Programme, the United Nations' leading organization in fighting hunger. She has been working in several developing countries in Africa, Asia and the Middle East to promote the implementation of Food Quality Management Systems and enhance food-handling practices across those supply chains, and also under emergency response contexts. Examples of her more recent works and the collaboration with other institutes and organizations are reflected in the publications of *Aflatoxin Management in the World Food Programme through P4P Local Procurement*; *The Blue Box Initiative: Quality Checks Closer to the Farmer; P4P Training Manual for Improving Grain Post-harvest Handling and Storage*; *Managing the Supply Chain of Specialized Nutritious Foods*. She has previously worked with the European Food Safety Authority's working group for the hazard characterization of isoflavones (2010); the World Health Organization Food Safety and Zoonoses Department for the Response to the H1N1 Pandemic (2009); WUR's Advance Food Microbiology Lab (2008) and AUA's food quality lab (2006). Eleni currently lives in Ethiopia, where she continues the work of building national quality/safety managenent capacities, that prevent food deterioration and ultimately assist to tackle food insecurity globally.

## Mickey Parish
### U.S. Food and Drug Administration, USA

Dr. Mickey Parish is the Senior Science Advisor for the FDA Center for Food Safety and Applied Nutrition where he had previously served as Senior Advisor for Microbiology in the Office of Food Safety. Prior to coming to FDA, Dr. Parish was Professor and Department Chair in the University of Maryland's Department of Nutrition and Food Science, and a Professor of Food Microbiology at the University of Florida. Dr. Parish is the President-Elect of the International Association for Food Protection.

## Trevor Phister
### PepsiCo, United Kingdom

Dr. Trevor Phister received his Ph.D. in Food Microbiology from the University of Minnesota in 2001. He held a number of academic positions in both the U.S. and the UK before joining PepsiCo in 2013. He is currently a Principal Microbiologist in the Global Microbiology team based in Europe and a Co-Chair of the PepsiCo Global Microbiology Council. In his current role, he works with teams to develop and maintain microbiology programs ranging from the assessment of new microbial methods to the development of policies and tools to support risk assessment of materials, products and processes across the PepsiCo portfolio.

## Bert Popping

### FOCOS, Alzenau, Germany

Dr. Bert Popping is Managing Director of the strategic food consulting company FOCOS. He previously worked as Chief Scientific Officer and Director Scientific Development and Regulatory Affairs for multi-national contract laboratories. Dr. Popping has more than 20- years experience in the food industry and authored over 50 publications on topics of food authenticity, food analysis, validation and regulatory assessments. He also edited one book in this field. Dr Popping is member of the editorial board of *J. AOAC*, *J. Food Additives and Contaminants*, *J. Food Analytical Methods* and *Quality Assurance and Safety of Crops & Foods*.

He serves on the Thought Leaders Advisory Committee of AOAC International and on panels of several other international organisations. He also acts as evaluator for the European Commission under the Horizon 2020 (H2020) program. Dr. Popping is an active member of numerous national and international organisations like CEN, ISO, BSI and several German method working groups.

## Laure Pujol

### Novolyze, France

Dr. Laure Pujol studied Food Engineering at the National College of Veterinary Medicine, Food Science and Engineering (Oniris) at Nantes, France. After her graduation, she joined the Research Unit Food Safety and Microbiology INRA-Oniris (UMR1014 Secalim) and obtained her Ph.D. in Quantitative Microbial Risk Assessment and Predictive Microbiology. The main topic of her Ph.D. was to develop a quantitative microbial exposure assessment model applied to aseptic-UHT milk based product, in collaboration with a private industry. Currently, she is working at Novolyze (Dijon), France, as a Project Manager in Validation Studies.

## Andreja Rajkovic

### Department of Food Safety and Food Quality, Ghent University, Belgium

Prof. Dr. Andreja Rajkovic has a main research focus on microbial toxins, foodborne pathogens and host-pathogen interactions. He is involved in projects related to modern food processing and effects on microbial and chemical food safety (e.g., Horizon2020 FutureFood), development of test and risk assessment strategies of mixtures (Horiozn2020 EuroMix), molecular detection and control of microbial water safety (FP7 Aquavalens), control of myxotoxins (Horiozn2020 MycoKey) and many others. He is a Found Editor-in-Chief of *International Journal of Food Contamination* published by Springer Open. He is also a member of Editorial Boards of *International Journal of Food Microbiology*, *Applied and Environmental Microbiology*, and *Food Analytical Methods*.

## Cath Rees

### The University of Nottingham, United Kingdom

Dr. Rees' focus of research is the application of molecular biology to fundamental research on microorganisms of importance to the food industry. Specific research focuses on the foodborne pathogen *Listeria monocytogenes* and the cattle pathogens *Mycobacterium paratuberculosis* (Johne's disease in cattle) and *Mycobacterium bovis* (bovine TB). In addition her research group specializes in the use of bacteriophage to develop rapid methods of detection of bacterial pathogens.

## Gabriele Scholz

### Nestle, Lausanne, Switzerland

Gabriele Scholz is a Food Safety Scientist at the Nestlé Research Center in Lausanne, Switzerland. She is a molecular biologist by training and obtained her PhD at the Freie Universität Berlin. She then moved into the field of the validation of alternatives to animal experimentation and in in vitro toxicological assays at the Federal Institute for Risk Assessment (BfR) in Berlin. Gabriele changed to work in the Pharmaceutical industry where she applied in vitro safety testing using organ cell culture models in early drug development. Since more than 13 years at Nestlé she has built a strong expertise in the health risk assessment of chemical contaminants in foods, particularly those that arise through heat processing. Being active in working groups (e.g. ILSI Europe) with various stakeholders to evaluate the safety relevance of such compounds for human dietary consumption, she has a good record of publications in the area. She is a member of the European Society for Alternatives to Animal Testing (EUSAAT) and the International Maillard Reaction Society (imars).

## Mathias Schmelcher

### ETH Zurich, Switzerland

Mathias Schmelcher studied Biology at TU Munich, Germany, and obtained his Ph.D. from ETH Zurich, Switzerland in 2008, working on engineering of phage endolysins for detection and control of the foodborne pathogen *Listeria monocytogenes* (supervised by Martin Loessner). Between 2009 and 2012, he joined David Donovan's lab at the USDA in Beltsville, MD, USA as a Post-Doctoral Associate, where he focused on the development of endolysin-based therapeutics for treatment of bovine mastitis. After his return to ETH as a Senior Scientist, he has continued research on endolysins and their possible applications against bacterial pathogens in medicine, food safety, and agriculture.

## Samuel Sheppard

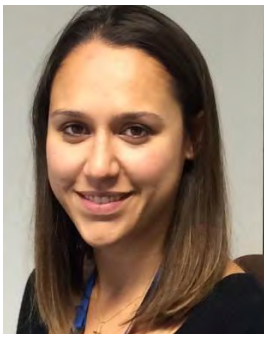
### The Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, United Kingdom

Samuel Sheppard is Professor and Director of Bioinformatics at the Milner Centre for Evolution (University of Bath) and Co-PI of MRC-CLIMB (Cloud Infrastructure for Microbial Bioinformatics). Before joining Bath from Swansea in 2016, he worked for nearly ten years with Prof. Martin Maiden (University of Oxford) as RA and Wellcome Trust Fellow. Research centres on the use of genetics/genomics and phenotypic studies to address complex questions in the ecology, epidemiology and evolution of microbes. His most recent interest focuses upon comparative genome analysis to describe the core and flexible genome of pathogenic bacteria (*Campylobacter*, *Helicobacter* and *Escherichia coli*) and how this is related to population genetic structuring, the maintenance of species, and the evolution of host/niche adaptation and virulence.

## Danièle Sohier

### Bruker Daltonics, Germany

Danièle Sohier has recently moved to Bruker Daltonics (Ge) (www.bruker.com) to coordinate development programs in industrial microbiology. She has spent more than 15 years at Adria Food Technology Institute (Fr), at first to implement molecular microbiology and then to manage the overall activities in food microbiology, combining both R&D and expertise projects for food and diagnostic companies. She is involved in ISO standardization and certification schemes of alternative methods, and has presented over 90 communications on food microbiology in journals and at symposia.



## Pamina-Mika Suzuki

### European Commission, Belgium

Pamina-Mika Suzuki is a Policy Officer dealing with food microbiological risks in the Food Hygiene Unit of the European Commission's Directorate General for Health and Food Safety (DG SANTE). Graduated in Biology Applied to Nutritional Science in Milan in 2012, she joined DG SANTE in 2013. She is currently Desk Officer for microbiological criteria, molecular typing projects, crisis preparedness and coordination of multinational foodborne outbreaks, with experience in EU food law and AMR monitoring. She recently coordinated the extensive 2016 *Salmonella* Enteritidis phage type 8 outbreak investigation, which concerned 18 European and 12 non-European countries.

## Alexandra Tabaran

### University of Agricultural Sciences and Veterinary Medicine, Romania

Alexandra Tabaran is a Veterinarian, graduated in 2008 from the University of Agricultural Sciences and Veterinary Medicine Cluj Napoca. She holds a Ph.D. in Veterinary Science at the same university where she currently works as a Lecturer and is responsible for teaching food hygiene, food technology and food safety. Her main research topics are food microbiology (food contaminants) and the characterisation of antimicrobial resistance of bacteria isolated from various food products by molecular techniques (PCR) and classical methods. She is the author of over 60 scientific publications and over 52 conference papers. She is currently a member in 3 national projects and 2 international projects.

## Bernard Taminiau
### University of Liege, Belgium

Bernard Taminiau has a Ph.D. in Biological Sciences from the University of Namur, Belgium, and has been a Senior Scientist since 2002 at the Department of Food Science of the Faculty of Veterinary Medicine at the University of Liege. He has a sound knowledge in bacterial genomics, metagenomics and amplicon sequencing and he makes use of it to explore two fronts: the characterization of alimentary and digestive flora. He is the author of over 140 scientific publications and conference papers.

## Amandine Thépault
### Anses, France

After earning a master's degree in Genetics and Microbiology at the University of Rennes 1, Amandine Thépault started a Ph.D. in November 2014, at Anses, the French Agency for Food, Environmental and Occupational Health & Safety. Within the unit of Hygiene and Quality of Poultry and Pork Products, her Ph.D. project aims to assess the ability of several genotyping methods to track *Campylobacter jejuni* and identify the origin of human campylobacteriosis. Three methods are compared MLST, CGF40 and Whole-genome Sequencing for source attribution. Thanks to a MedVetNet grant, she has been trained to source attribution using Whole Genome Sequencing data at Swansea University, Wales.

## Pauline Titchener
### Neogen Europe, United Kingdom

Pauline Titchener studied Food Technology at the University of Glasgow. Following graduation, she worked in the food industry in both quality assurance and new product development roles. For the last 10 years, she has been working for Neogen Europe, a leading manufacturer of food safety diagnostic tests. She is currently responsible for the business development of the allergen and speciation diagnostic ranges across Europe.

## David Tomas Fornes
### Nestle, Switzerland

David Tomas Fornes is Lead Scientist in the Microbial and Molecular Analytics group at Nestlé Research Center (Lausanne, Switzerland). He works on the evaluation, development and validation of microbial methods for pathogen detection and sample preparation. He participates in ISO, CEN and IDF technical committees developing reference methods for microbiological food analysis. He has participated as scientist in the development and validation of analytical methods as well as in international cooperation projects supported by the European Committee for Standardisation (CEN) and the Food and Agricultural Organization (FAO).

## Carole Tonello-Samson
### Hiperbaric, Spain

Dr. Carole Tonello-Samson is active Industrial Researcher with emphasis in High Pressure Processing (HPP) for food. She has a Ph.D. in food science on the effects of HPP on destruction of microorganisms. She works as Applications & Process Development Director at Hiperbaric (www.hiperbaric.com), a Spanish company designing, manufacturing and marketing HPP industrial equipment for food. She is a member of the board of directors of the company. Carole is author or co-author of about twenty scientific articles and six book chapters on High Pressure Processing food applications and equipment. She has been Chair of the Nonthermal Processing Division of the Institute of Food Technology (IFT) in 2012–2013.

## Sarah Tozer
### Procter and Gamble, United Kingdom

Dr. Sarah Tozer received her her Ph.D. in Neuropharmacology and Neurotoxicology from King's College London and now has over 16 years' experience in toxicological evaluation and risk assessment in Industry in Procter & Gamble's Beauty and Health Care divisions. Sarah has much experience in the exposure assessment of chemicals, particularly aggregate exposure. She chairs the Cosmetics Europe Exposure Task Force, co-chairs the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Exposure Task Force and works with the Research Institute for Fragrance Materials (RIFM) Task Force to develop tools for modelling aggregate exposure.

## Vasileios Valdramidis
### University of Malta, Malta

Vasileios Valdramidis is Associate Professor of Food Science and Environmental Health at the University of Malta, Malta. He is an executive board member of the ICFMH, part of the IUMS. He is member of the Editorial Board of *Food Research International*, *Current Opinion in Food Science*, *International Journal of Food Microbiology*, *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. He is co-author of about 50 papers on national and international journals, and an expert in Predictive Modelling; Non-thermal Food Technologies; and Applied Microbiology.

## Jan Van Impe
### KU Leuven/BioTeC, Belgium

Jan F.M. Van Impe obtained a master's degree in electrical and mechanical engineering from the University of Ghent in 1988, and a doctorate in applied sciences from the KU Leuven in 1993. Immediately thereafter, he founded the BioTeC (Chemical and Biochemical Process Technology and Control) research group; currently he is a Full Professor at the Department of Chemical Engineering. In the period 2005–2011 he has served as Departmental Head, while during the period 2006–2015 he was a visiting professor at the UAntwerpen.

## Lilou van Lieshout
### ILSI Europe, Belgium

Lilou van Lieshout gained her B.Sc. in Nutrition and Health and her M.Sc. in Molecular Nutrition and Toxicology at Wageningen University, NL, with research experience in nutrient-gene interactions in the pre-diabetic human adipose tissue, and effects of n-3 fatty acids on human platelets and CVD (Rowett Institute of Nutrition and Health, Aberdeen, UK). Currently, she works as Scientific Project Manager at the International Life Sciences Institute, Europe (ILSI Europe) in Brussels, BE. The main aim of ILSI Europe is to foster collaboration among the best scientists to provide evidence-based scientific consensus on the areas of nutrition, food safety, toxicology, risk assessment, and the environment. In this position she is managing task forces and expert groups to create consensus and to write reviews and practical guidance documents on microbiological food safety (Guidance on Next Generation Sequencing, Virus Control Options, Industrial MRA) and other nutrition and food safety related topics.

## An Vermeulen
### Ghent University, Belgium

Dr. An Vermeulen graduated as Bio-Engineer from Ghent University, and performed a Ph.D. at the same university in the field of predictive microbiology. More particularly on the microbial stability and safety of deli salads and sauces. Since then, she has been working as a project manager at the laboratory of food microbiology and food preservation – Ghent University. She coordinates several small or larger projects within predictive microbiology, often in close collaboration with the food industry. All activities regarding predictive microbiology are performed by CPMF², which is a collaboration between BioTeC-KULeuven and LFMFP-UGent. They developed several software packages targeted to a specific industry such as meat industry, salad and sauce producers and the chocolate industry. She is also Co-Director of the service laboratory of LFMFP-UGent which supports more than 200 companies within the food industry and its suppliers.

## James Walsh

### University of Liverpool, United Kingdom

Dr. James Walsh in Associate Professor in the Department of Electrical Engineering & Electronics at the University of Liverpool where he heads the recently established Centre for Plasma Microbiology. His research interests lie in the development of cold plasma technology for use in biomedical applications with activity spanning from the underpinning plasma physics through to the industrial scale implementation. He holds a UK Engineering & Physical Sciences Research Council Healthcare Technologies Challenge Award and is currently developing plasma technologies to combat biofilm formation.

## Marjon Wells-Bennik

### NIZO Food Research, Netherlands

Marjon Wells-Bennik is Principle Scientist Food Safety at NIZO (The Netherlands). The focus of her work is on preventing and solving food safety and quality issues for customers in the food industry. Together with other NIZO experts, multidisciplinary approaches range from (high throughput) challenge testing, troubleshooting activities, process validations, microbial and chemical risk assessments, to in-depth genomics analysis of microbial diversity to detect problem-causing bacteria. She managed large programs on heat-resistant bacterial spores relevant to foods (*Bacillus* and *Clostridium* species) and on microbial contaminants in the dairy chain (spoilers and pathogens). Her broad expertise and background in food safety and quality was shaped by her M.Sc. and Ph.D. research at Wageningen University, Postdoc at Harvard University, and work experience at the Agrotechnological Research Institute (Wageningen), the Institute of Food Research (Norwich, UK), and NIZO. She is an author of more than 60 scientific publications.

## Guido Werner

### Robert Koch Institute, Germany

Dr. Guido Werner works as a Medical Microbiologist in the Department of Infectious Diseases, Robert Koch-Institute, Wernigerode Branch, Germany. He is Head of the Division 'Nosocomial Pathogens and Antimicrobial Resistances' and of the National Reference for Staphylococci and Enterococci. He is also Professor for Medical Microbiology and gives lectures on this subject at the Georg-August-University Goettingen and the Technical University Brunswick. His main research interests are in methods of rapid and molecular diagnostics, molecular strain characterization/typing including NGS of bacterial pathogens and all biological and epidemiological aspects of antimicrobial resistance with the special focus in health-care associated pathogens. He has worked for 18 months as a national expert at the European Commission, General Directorate 'Health and Consumer Protection' in the Unit 'Health Threats' (DG SANCO C3) and is the nominated 'National Microbiology Focal Point' of a microbiological working group at the European CDC, Stockholm, Sweden. He is an active member of several national and international medical societies such as Scientific Officer for Public Health of the ESCMID study group on microbial markers ESGEM. He has gained more than 100 peer-reviewed papers and written several reviews, opinion papers and book chapters.

## Sophie Zuber

### Nestlé Research Center, Switzerland

Sophie Zuber works as Food Safety Microbiologist at the Nestlé Research Centre, based in Lausanne, Switzerland. She received her Ph.D. in Microbiology from the Department of Genetics, University of Melbourne, Australia. In her current position, her principal responsibilities include providing scientific advice and guidance on possible risks of viruses in the food chain and developing risk management strategies in this field. In this context, Sophie has published peer-reviewed publications focusing on the effects of treatments used in food processing on viruses.

## Marcel Zwietering

### Wageningen University, Netherlands

Marcel Zwietering studied Biotechnology at Wageningen University and after earning his Ph.D. in 1993, he worked for the Food Process Engineering group as assistant and associate professor. From 1998-2002 he worked for the research lab of Danone in France. Since January 2003, he has been Professor in Food Microbiology at Wageningen University. Marcel is editor of the *International Journal of Food Microbiology* and member of the International Commission on Microbiological Specifications for Foods (ICMSF).



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29-31 MARCH 2017
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# SYMPOSIUM ABSTRACTS

29–31 March 2017 – Brussels, Belgium

# SYMPOSIUM ABSTRACTS

## Opening Session

### Biocontrol of Foodborne Pathogens: The Pros and Cons

**JACQUES MAHILLON**, University Catholic Louvain, Brussels, Belgium

More than 300,000 human cases of food toxi-infection are reported each year within the European Union. This major health issue is challenged by many factors, such as the globalization of the food market and the increase of susceptible populations, which may increase the risk of food outbreaks. In this context, the preservation of foods and the control of foodborne pathogens by natural, biological methods is an interesting approach that may contribute to tackling these food-related problems. Bacteriocins and bacteriophages have been investigated as innovative, bio-preservation tools. Although these alternative methods display several advantages (e.g., antimicrobial spectrum, heat and/or pH stability, or safety for humans), their development is often hindered by several drawbacks. This presentation will summarize both aspects and will indicate the new trends in the field.

### Global Trends in Food Safety

**LINDA J. HARRIS**, Department of Food Science and Technology, University of California, Davis, CA, USA

There is no question that the food supply has become increasingly dependent on a complex global supply chain significantly impacted by changes in population dynamics, climate, agricultural practices, and conflict. Each of these factors impact food safety risks and risk management is complicated by a constantly changing marketplace influenced by consumer demand and innovations in food processing. Fortunately, technological advances have also led to incremental improvements in foodborne outbreak detection and management. All stakeholders in the global food supply chain share the responsibility to effectively and collaboratively manage food safety.

### The Future of the One Health Approach: From Tracing Foodborne Pathogens and Spoilers to Mobile Genetic Elements and from Farm to Fork via the Environment

**MARC HEYNDRICKX**, ILVO - Flanders Research Institute for Agriculture, Fisheries and Food, Melle, Belgium

Previous research has investigated the flux of the zoonotic pathogens, *Salmonella* and *Campylobacter*, from hatchery to slaughterhouse (for broilers) or to packing stations (for *Salmonella* spp. in eggs) to indicate the transmission from animals to humans. This approach can be regarded as a predecessor of the One Health approach and has enabled food production systems to identify the best interventions for controlling and preventing zoonotic foodborne contaminations. The One Health approach has now emerged as a common platform for effective control and prevention of the increasing antibiotic resistance in pathogens. It is, currently, building on a collaborative, multi-disciplined effort with close cooperation between physicians, veterinarians, and other scientific health and environmental professionals.

Several aspects will be important in the future direction of One Health: (1) The application of whole genome sequencing will reveal a more comprehensive understanding of how pathogens move between different reservoirs. This will be shown for the livestock-associated MRSA CC398 type. (2) The importance of characterizing and tracing mobile genetic elements, which can carry resistance genes (e.g., ESBL- or tetracycline-resistance carrying plasmids or the recently discovered, transferable colistine resistance) and be transferred between different bacteria. (3) The discovery of other possibly transferable traits, such as high heat resistance in sporeformers located on mobile elements, which may become important to safe food production. (4) Finally, it will be important for the One Health approach to incorporate the agricultural environment as an equally important dimension in its scope, where the land application of manure is still largely unregulated in relation to food safety. Recent examples of the effect of application of manure on agricultural soil and of manure treatments on the release of pathogens, antibiotic residues, and resistance determinants will be shown because it is important to demonstrate how this environmental contamination contributes to the infection pressure of humans and animals.

## S1 Employing Whole-genome Sequencing for Successful Traceback Investigation

Recent foodborne outbreaks have demonstrated the importance of accurate subtyping and subsequent clustering of bacterial isolates implicated in outbreak investigations and responses. The underlying methodology used in source traceback links the clinical isolate back to the specific food or environmental source of the outbreak. For over 20 years, PFGE has been the gold standard; however, the genetic resolution it provides is limited and not always able to differentiate between outbreak and non-outbreak related isolates, which is vital for successful traceback analysis.

The application of Whole-Genome Sequencing (WGS) to isolate characterization allows for the elucidation of the complete genetic sequence of a target organism, providing millions of nucleotides of information; thusly, allowing a researcher to more efficiently resolve an outbreak. When this amount of genetic specificity is combined with the ability to provide epidemiological information in real-time, it further underscores the increasing importance of transitioning to WGS (and methodologies ancillary to WGS) to successfully and quickly resolve foodborne outbreaks.The massive amount of sequence infor-

mation generated by WGS needs to be analyzed and stored in a robust, efficient and scientific manner. Therefore, the successful use of WGS in foodborne outbreak investigations requires a large, reference database of sequenced isolates and their associated metadata (e.g., isolate source [e.g., chicken, tomato, environmental swab], date of collection, geographic source) to be able to link a clinical isolate back to an associated food or environmental source.

This symposium will provide an overview of specific, commonly employed methodologies and discuss the importance and success of using WGS with a large, publically available database. Furthermore, this session will provide examples of how this technology is advancing food safety and public health and will highlight some of the technical requirements that are necessary for the successful implementation of WGS as a molecular epidemiological tool.

### Integration of Genomics Technologies in the Management of Food Safety and Outbreaks in Europe

**PAMINA-MIKA SUZUKI**, European Commission, Brussels, Belgium

Molecular typing has developed rapidly in recent years; becoming part of routine strain characterisation in many laboratories in the EU. Two years after the 2011 STEC O104:H4 outbreak, the European Commission asked EFSA and ECDC to provide technical support for the collection of molecular typing data for foodborne pathogens from human and non-human samples and to perform regular joint analyses of these molecular typing data. The molecular typing project is part of the actions envisaged by the Commission to enhance crisis preparedness and management in the food and feed area, in order to ultimately ensure a more effective and rapid containment of future food and feed-related emergencies and crises.

The purpose of the joint ECDC-EFSA molecular typing project is to share comparable typing data in a common repository, so that microbiological data from humans can be linked to similar data from the food chain. This will enable and support early detection and investigation of cross border foodborne outbreaks. At present, the molecular typing data collection covers PFGE for *Salmonella* spp., *Listeria monocytogenes,* and STEC, togetherwith MLVA for *Salmonella* Typhimurium and *Salmonella* Enteritidis.

Given the important role of whole genome sequencing (WGS) analysis in recent, multinational, foodborne outbreak investigations, such as the extended 2016 *S*. Enteritidis outbreak, and the gradually increasing capacity of public health and food laboratories, discussions are ongoing at the European level regarding the possible integration of WGS into food safety management and on the collection of WGS data at the European level. The molecular typing project represents a foundation that will, in the future, enable the collection of other typing data, similar to WGS, at European level.

### Identifying the Source of Foodborne Outbreaks: WGS, the New Sleuth on the Block

**KATHIE GRANT**, Public Health England, Glasgow, United Kingdom

Foodborne infectious outbreaks are a major public health and food safety concern. Their successful detection and investigation depends upon microbiological and epidemiological tools being used in concert with food traceback studies to define and quantify the number of cases, identify the pathogen, detect the source of infection, and determine the route of transmission. This enables effective control measures to be implemented and action to be taken to stop further cases and prevent future outbreaks. Tracing food back to its source is often complicated; many foods go through multiple processing and distribution steps, which may involve more than one country. Once the food source has been established, the point at which and exactly how contamination occurred needs to be determined to enable implementation of effective public health measures.

Whole-genome sequencing (WGS) offers unprecedented resolution for determining the genetic relatedness of bacterial strains and has proven to be a transformational tool for investigating foodborne infectious illness. The application of WGS to foodborne bacterial pathogens is able to provide strong microbiological evidence in identifying clusters and outbreaks previously unidentified by conventional typing and surveillance tools; to link isolates from human illness with those from food and environmental samples; to refine case definitions in outbreaks and, thus, hone epidemiological investigations. In addition, the phylogenetic context derived from WGS can provide enhanced source attribution and evidence for the initial point of contamination in the food chain. This presentation will demonstrate how WGS of bacterial foodborne pathogens is being used by Public Health England to improve the investigation of foodborne bacterial disease.

### Implementing WGS-based Strain Characterization into Pathogen Surveillance: Introducing the Strategy of the Robert Koch Institute

**GUIDO WERNER**, Robert Koch Institute, Wernigerode, Germany

The Robert Koch Institute is the Central Public Health Institute in Germany. The German Infection Protection Act (“Infektionssschutzgesetz”) and the German Antimicrobial Resistance Strategy DART define, amongst other documents, the institute’s legal framework and guide activities in the field of pathogen surveillance of community-associated, health-care associated and food- and water-borne infections, antimicrobial resistance and consumption. The institute hosts a network of reference and conciliar laboratories for more the 80 pathogens (mainly notifiable diseases), some of them directly located at the institute. NGS technology has been introduced about 10 years ago at the institute. WGS-based analyses have been used in recent years in various pilot projects funded by the institute and research activities financed by external funds (viruses, bacteria, metagenomics).

As part of all these diverse, recent activities, various expertise and experiences were gained at different groups, units and departments at the institute. Fast evolving requests for high throughput NGS techniques by an increasing number of groups at the institute embedded in a framework of limited additional resources, led to a common and agreed strategy of consolidation of machines, experiences and resources. Currently, we have (i) consolidated our machine park into a single core sequencing facility; (ii) streamlined our bioinformatics needs in a core

bioinformatic service flanked by external expertise in research groups (not primarily dedicated to service activities) and individual expertise in several divisions and departments and (iii) prioritized our WGS-based requirements in an institute's wide strategy for an "intensified and integrated molecular pathogen surveillance." The talk will provide some detailed information about these aspects and thoughts. Suitability of existing infrastructure, sequencing techniques and bioinformatics pipelines will be demonstrated using a few recent examples.

## S2 Don't Dismiss Clostridia in Food (They are still there!): From Disease Burden to Prevention and Health Promotion

Among of the anaerobes that infect humans, the Clostridia have been the most widely studied. Clostridia are sporeforming bacteria that are ubiquitous in the nature (soils, dust, water) and in the intestines of animals. Consequently, these bacteria are present in many different foods. The Clostridia (including *Clostridium estertheticum* and *Clostridium gasigenes*) are often implicated in the spoilage of a wide range of food products, such as meat and dairy products, fruits, and vegetables, with the production of gas and putrid odours (known as "blown pack"). The most efficient method to control this spoilage is to prevent faecal contamination of foods.

In relation to illness, the *Clostridium* spp. most commonly involved in foodborne disease are *Clostridium botulinum* and *Clostridium perfringens*. *Clostidium perfringens* is commonly present in foods and ingredients, occasionally at hundreds per gram. *Clostridium botulinum* is present less frequently; normally at a few spores per kilogram. However, botulinum toxin is one of the most poisonous biological substances known. Now, even if spores of both species are easily eliminated/inhibited by heating or by the use of preservatives, there is an important concern regarding the risk of botulism in some organic foods.

This session will describe the updates regarding the Clostridia in livestock and foods, including the recent concerns about the role of Clostridia in foodborne illness, food spoilage, and the main "at risk" foods in markets. Other Clostridia updates to be discussed will be: the possibility of *Clostridium difficile* as a new foodborne agent; the spread of multidrug resistant *Clostridium* sp. and its relation with the use/restrictions of antibiotics in livestock; the possible effect of *Clostridium butyricum* as a probiotic; and the role of Clostridia in the gut microbiota and their relation with food allergy or with the development of other foodborne diseases.

### Pathogenic Clostridia in Foods and the Environment

**MIIA LINDSTRÖM**, Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland

Clostridia are a diverse group of anaerobic bacteria that jeopardize food hygiene and public health through production of highly resistant endospores and a multitude of various toxic and/or food spoiling metabolites. Clostridia prevail in soils, aquatic environments, vegetation, and in the animal gastrointestinal tract, and frequently enter various man-made systems, such as the food chains. The spores tolerate food processing and may germinate into active cultures in anaerobically packaged foods during extended storage. Several species include psychrotrophic or psychrophilic strains that grow at low temperatures and thereby challenge the cold food chain. Mesophilic strains, on the other hand, may reach very rapid growth in foods stored at an abused temperature range.

The most well-known foodborne pathogenic clostridial species include *Clostridium botulinum* and *Clostridium perfringens*. The former produces the highly paralytic botulinum neurotoxin (BOT) that causes a life-threatening tetraplegia called botulism, while the latter produces many different toxins with the *C. perfringens* enterotoxin (CPE) being responsible for a common foodborne diarrhea. Moreover, *Clostridium difficile* responsible for a severe nosocomial colitis, may contaminate foods and raw materials. While disease cases of foodborne origin remain to be reported,

Novel aspects and pending research questions on the epidemiology, pathogenicity and control of foodborne pathogenic Clostridia will be discussed, with special emphasis on *C. botulinum*.

### Clostridia Spread in Livestock Animals: Situation and Initiatives

**ALEXANDRA TABARAN**, University of Agricultural Sciences and Veterinary Medicine, Cluj –- Napoca, Romania

*Clostridium* species form a heterogeneous group of environmental bacteria; but, can also be found as pathogens in humans and animals. In this particular bacterial genus there are about 15 pathogenic species which produce the most dangerous toxins known to man. The most important pathogenic species are *Clostridium tetani, Clostridium perfringens, Clostridium botulinum*, and *Clostridium difficile.* Unfortunately, in Europe, the rates of *Clostridium* spp. occurrence are underestimated.

The latest epidemiological study has underlined the need for more research and standardization of surveillance. Most of the studies, to date, have shown an increasing prevalence of *C. difficile* and *C. perfringens* in livestock animals in Europe; but, until now, no data has been published from Romania. These studies are important because knowing the true incidence of Clostridia will allow implementation of appropriate measures for controlling this distressing and sometimes life-threatening infection in animals and humans.

### Clostridia in the Gut Microbiota and Their Implication in Food Allergies and Foodborne Diseases

**BERNARD TAMINIAU**, University of Liege, Leige, Belgium

The gut microbiota is a fundamental component of health in humans and animals and is an important focal point for the diagnosis of several diseases. It has been reported that gut microbes are estimated to contain 100-fold more genetic potential than a human's own genome. Therefore, they can provide many functions; many of them still unknown.

Clostridia are gram positive-rod shape bacteria in the phylum of *Firmicutes,* which play a crucial role in gut homeostasis. For example, commensal Clostridia are associated with the metabolic welfare of colonocytes by releasing butyrate as an end product of fermentation. Another important, recent finding is that elevated levels of *Clostridium* clusters *XIVa* and *IV* in mice lead to resistance to allergens and intestinal inflammation in experimental models. It has, also,

been reported that aging affects the presence of Clostridia in the gastrointestinal tract. Some studies showed a decrease in the number of these strict anaerobes, in favour of an increase in the number of facultative anaerobes; while other studies found an increase of these bacteria in patients over 65 years. Here we discuss the presence of Clostridia in a short cohort study of elderly nursing home residents. We investigated, weekly, the proportions of Clostridia in 23 elderly patients during a 4-month period. We, also, addressed the impact of diarrheal episodes and antibiotic or probiotic treatment on the gut Clostridia structure. Finally, we identified the proportions and the main species of Clostridia present in the gut microbiota of the elderly.

## S3 Foodborne Microbial Toxins, Virulence, and Host-pathogen Interactions

Microbial toxins are primary virulence factors to many foodborne pathogens. Formed toxins can exist independently from their microorganism at the moment of consumption, i.e., the pathogen may be inactive, while the toxin is still biologically active. They can be also expressed in the human gut and play a key role in foodborne infections and host pathogen interactions. In this session three pathogens will be considered, *Bacillus cereus*, Enterohemorrhagic *Escherichia coli*, and *Staphylococcus aureus*. For *B. cereus*, an underlying hypothesis is that production of microbial toxins in food usually occurs in low doses, which do not result in immediate, visible, intoxication symptoms. Exposure to mixtures of toxins (and other contaminates) requires better understanding. There is a major knowledge gap regarding exposure and modes of action.

In this session, we will elaborate on: the effects of *B.cereus* emetic toxin and related depsipeptide mycotoxins, found in the same cereal-based foods, on human intestinal and liver cells; the finding that *B. cereus* and *S. aureus,* often found in the same foods, can simultaneously produce respective toxins when no other background flora is present; an that protective microbe-microbe interactions between *S. aureus* and native lactic acid bacteria result in metabolic shifts, which prevent enterotoxin production. Using an *in vitro* model of the human large intestine, we will, also, look at survival of an EHEC in simulated human colonic conditions and investigate the effect of probiotic treatments under abiotic and biotic parameters of the human gut. For this purpose, three presenters will show multifaceted aspects of microbial toxins, virulence factors, and host-pathogen interactions by sharing data generated by different omics techniques, including transcriptomic, virulomic and functionomic assessments of microbe-microbe-host interactions.

### Mitochondrial Toxicity of *Bacillus cereus* Emetic Toxin with Intestinal and Liver Toxicological Endpoints

**ANDREJA RAJKOVIC**, Department of Food Safety and Food Quality, Ghent University, Ghent, Belgium

Microbial toxins are virulence factors that can exist independently from their microorganism at the moment of consumption, i.e. the pathogen may be inactive, while the toxin is still present and biologically active. Production of microbial toxins usually occurs in low doses which does not result in immediate, visible, intoxication symptoms. Recent findings showed that *Bacillus cereus* emetic toxin cereulide is present in numerous ready-to-serve foods at low doses. Therefore, this presentation will show the latest findings related to the effects of sub-clinical concentrations of *B. cereus* emetic toxin (and related depsipeptide mycotoxins), found in the cereal-based foods, on human intestinal and liver cells, which are considered primary targets for these toxins. The major changes to mitochondrial respiration and glycolysis in Caco2, HepG2 and HepaRG cells, measured by extracellular flux analysis, show that the impact of these toxins was much below acute intoxication doses. This suggests the need for new inputs in risk assessment studies. For this purpose, complementary data generated by different omics techniques will elucidate some of the host-microbe interactions.

### Impact of Abiotic and Biotic Parameters of the Human Gut on Enterohemorrhagic *Escherichia coli* Survival and Virulence

**STÉPHANIE BLANQUET-DIOT**, Université Clermont Auvergne, Clermont-Ferrand, France

Enterohemorrhagic *Escherichia coli* (EHEC) are the major foodborne pathogens responsible for human diseases ranging from uncomplicated diarrhea to life-threatening complications. Even though they play key roles in pathogenesis, the ways EHEC survive and modulate the expression of virulence genes, throughout the human digestive tract, remains poorly described. As no specific treatment is available for EHEC infections and antibiotic therapy has worsened clinical outcomes, alternative strategies using probiotics are under consideration.

The aim of this study was to assess the impact of abiotic (physicochemical factors) and biotic (intestinal microbiota, probiotics) parameters from the human gut on the survival and virulence of EHEC O157:H7 by using complementary *in vitro* (human artificial digestive systems) and *in vivo* (mice ileal loops, human-microbiota associated rats) approaches. We have shown that differences in physicochemical parameters of the human upper gastrointestinal tract may partly explain why children are more susceptible to EHEC infections than adults. In addition, the human gut microbiota was shown to modulate EHEC O157:H7 virulence suggesting that it could influence the clinical outcome of the infections.

Lastly, our data indicate that the probiotic yeast strain *Saccharomyces cerevisiae* CNCM I-3856 may be useful in the fight against EHEC pathogens by limiting the amount of bacteria reaching the colon, beneficially modulating gut microbiota activity, decreasing toxin-encoding genes expression, and inhibiting EHEC tropism to intestinal Peyer's patches. Such data provide a more complete picture of EHEC pathogenesis in the human gut and a better understanding of the mechanisms related to the antagonistic effects of probiotics.

### Microbe-Microbe Interaction between *Staphylococcus aureus* and Lactic Acid Bacteria Resulting in a Reshuffle of the Microbial Metabolisms and Prevention of Staphylococcal Enterotoxin Production

**LUCA COCOLIN**, University of Torino-DISAFA, Grugliasco, Italy

In complex microbial ecosystems, microorganisms interact and communicate resulting in a series

of phenomena, which may lead to dominance, coexistence, and limitation of one species with respect to others. In food microbiology, this aspect assumes specific interest (e.g., when a pathogenic microorganism represents one member of the consortium), as its pathogenic potential may be impaired by the interaction with the food microbiota.

In this study, we investigated the interaction between *Staphylococcus aureus* and *Enterococcus faecalis* in milk using phenotypical and molecular approaches. *Staphylococcus aureus* ATCC 29213 was grown in pure culture or in the presence of a cheese-isolated *E. faecalis* in skimmed milk. Enterotoxin was not produced in co-culture and *S. aureus* population decrease one-log in comparison to single culture. RNA-Seq analysis highlighted deep alterations in the *S. aureus* transcriptome during exponential and stationary growth in co-culture with *E. faecalis*. Several genes were fully repressed in co-culture, including the *agr*, *sar*-family and *mgrA* global regulators; and, as a consequence, several *agr*-regulated exoproteins. In contrast, expression of genes coding for surface proteins (*spa*, *clfB*, *sdrD*, *sdrE*, *sasF*, *sasG*, *sasH*) was increased in co-culture, probably due to the loss of *agr* influence. Interestingly, we observed significant modifications in the transcription of several metabolite-responsive elements, such as *ccpA*, *ccpE* and *codY*, and genes involved in glycolysis and pyruvate metabolism. The present study demonstrates that *S. aureus* virulence and metabolism are profoundly impacted by the presence of *E. faecalis* in a simulated food ecosystem and it provides relevant evidence to better understand the pathogen-lactic acid bacteria interactions in foods.

## S4 Source Attribution of Campylobacteriosis

*Campylobacter* is the most common cause of bacterial foodborne infection in humans in developed countries (WHO, 2012). The ubiquity of *Campylobacter*, as part of the commensal microbiota of various animals, contributes to the threat this organism poses to humans. *Campylobacter jejuni* is commonly isolated from the digestive tract of many mammals and wild and domestic birds. However, factors including the ability to form biofilms (Pascoe et al., 2015) and colonize protozoa (Snelling et al., 2006), mean that *Campylobacter* can be isolated from sources outside of the host gut, such as food and water sources (Sheppard et al., 2009; Guyard-Nicodème et al., 2015). Humans are usually infected by handling, preparation, or consumption of meat, including pork, beef, and especially poultry, contaminated during slaughter (Guyard-Nicodème et al., 2013.

The consumption of raw milk, untreated water, or contact with pets and farm animals have also been identified as potential sources for human infection (Friedman et al., 2004; Olson et al., 2008; Pitkanen, 2013). Quantifying the relative contribution of different infection sources remains an important aim in public health. Bacterial typing methods, including PFGE, MLST, flaSVR, have improved diagnosis and understanding of *Campylobacter* epidemiology. However, questions still remain about the relative contribution of different infection sources and transmission routes to human disease. Foodborne source attribution is the partitioning of the human disease burden of one or more foodborne infections to specific sources, where the term “source” includes animal reservoirs and vehicles (e.g., foods) (Pires et al., 2009).

The current session will point out the source attribution of campylobacteriosis. In introduction, an update on regulatory perspectives on *Campylobacter* in Europe will be covered. Thereafter, the session will combine different approaches of source attribution: Genome-Wide Association Study, Comparative Exposure Assessment and Case-Control study.

### Genomic Signatures of *Campylobacter* Adaptation and Source Attribution Studies

**SAMUEL SHEPPARD**, The Milner Center for Evolution, University of Bath, Bath, United Kingdom

*Campylobacter jejuni* and *Campylobacter coli* are the biggest causes of bacterial gastroenteritis in the developed world, with human infections typically arising from zoonotic transmission associated with infected meat. Because *Campylobacter* is not thought to survive well outside the gut, host-associated populations are genetically isolated to varying degrees. Therefore, the likely origin of most strains can be determined by host-associated variation in the genome. This is instructive for characterizing the source of human infection. However, some common strains, notably isolates belonging to the ST-21, ST-45, and ST-828 clonal complexes, appear to have broad host ranges, hindering source attribution.

Whole-genome sequencing has the potential to reveal fine-scale genetic structure associated with host specificity, but this is dependent upon the extent to which lineages are true generalists. Switching hosts too regularly may prevent the development of host-specific genomic signatures in allopatry. Promising approaches, pioneered in human genetics, include genome-wide association studies where DNA sequence variation across the genome is related to particular phenotypes. This has been challenging to apply to bacteria because of their strong population structure resulting from clonal reproduction.

Recent work, including Sheppard et al. (PNAS, 2013), presents new methods that identify homologous and non-homologous sequence variation in *Campylobacter.* This revealed some of the genetic changes associated with adaptations in birds and mammals that could be used as markers of host association. However, we have found that rates of zoonotic transmission among animal host species in generalist clonal complexes are so high that the signal of host association is all but obliterated; estimating one zoonotic transmission event every 1.6, 1.8 and 12 years in the ST-21, ST-45 and ST828 complexes, respectively. Therefore, the weak signal of host association within these complexes presents a challenge for pinpointing the source of clinical infections and underlines the view that whole-genome sequencing, powerful though it is, cannot substitute for intensive sampling of suspected transmission reservoirs.

### Gene-by-Gene Comparison of *Campylobacter jejuni* Genomes to Identify Host Segregating Epidemiological Markers for Source Attribution

**AMANDINE THEPAULT**, ANSES, Ploufragan, France

*Campylobacter* is a leading cause of bacterial gastroenteritis, worldwide, and is part of the commensal microbiota of numerous host species, which constitute potential sources of human infection. Molecular genotyping approaches, especially multi-

locus sequence typing (MLST), have been used to identify the origin of human campylobacteriosis based on allelic variation at seven MLST loci, among isolates from animal reservoirs and human infections. The increasing availability of bacterial genomes provides data on allelic variation at loci across the genome, providing potential to improve the discriminatory power of data for source attribution.

Here, we present a source attribution approach based on the identification of novel epidemiological markers among a reference pan-genome list of 1,810 genes identified using a gene-by-gene comparison of 884 genomes of *Campylobacter jejuni* isolates from animal reservoirs, the environment, and clinical cases. Fifteen loci were selected as host-segregating markers and used to attribute the source of French and UK, clinical *C. jejuni* isolates. Analyses performed on UK clinical isolates emphasized the importance of the chicken reservoir as an infection source in the UK; while in France, chicken and ruminant reservoirs appeared to be equally involved in clinical cases. The different proportions of French and UK clinical isolates attributed to each host reservoirs illustrate a potential role for local/national variations in *C. jejuni* transmission dynamics, indicating a benefit for further national-scale attribution modelling to account for differences in production, behaviour, and food consumption.

### Combining Case-control and Source Attribution Data: A Way to Reconstruct Campy's Journey Along the Transmission Chain

**LAPO MUGHINI GRAS**, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

Combined case-control and Multilocus Sequence Typing (MLST)-based source attribution analysis allows investigation of risk factors at the point of exposure for human campylobacteriosis attributable to specific reservoirs. Successful applications of this analysis are available for the Netherlands and Luxembourg, where source-specific risk factors for human campylobacteriosis of poultry, ruminant (cattle/sheep), environmental (water/sand/wild birds), pet (dogs/cats) and exotic (travel-related) origin were identified. Nationally representative collections of *Campylobacter jejuni* and *C. coli* isolates from these animals and from human cases included in case-control studies were typed using MLST. The asymmetric island model was the used to estimate the probability for the different sequence types (STs) found in human cases to originate from each of the considered reservoirs. Cases were then split according to their attributed reservoirs. Reservoir-specific risk factors were investigated using logistic regression analysis. Both in the Netherlands and in Luxembourg, most cases (>85%) were attributed to chicken and ruminants. Chicken consumption increased the risk of infection with chicken-attributed *Campylobacter* STs, whereas consuming beef and pork appeared to be protective. Contact with animals, garden soil, barbequing, tripe consumption, and never/seldom chicken consumption were risk factors for ruminant-attributed infections. Game meat consumption and swimming in household pools increased the risk for environment-attributed infections. Dog ownership increased the risk for environment- and pet-attributed infections. Person-to-person contacts around holidays were risk factors for domestic infections with exotic STs, introduced by returning travellers.

Based on these studies, it can be concluded that individuals acquiring campylobacteriosis from different reservoirs have different risk factors, the identification and characterization which allow public health messages to be targeted more effectively. While the outcome of classical case-control studies can be enhanced by incorporating source attribution data, identifying source-specific risk factors also allows us to infer the underlying transmission pathways, from the original reservoir to the level of exposure.

## S5 Use of Molecular Techniques to Understand Mechanisms of Persistence and Removal of Foodborne Pathogens from the Food Supply Chain

Pathogenic *Escherichia coli* and *Listeria monocytogenes* are foodborne pathogens that gained notoriety as two of the deadliest foodborne bacteria since they cause the most deaths associated with foodborne infections. They are able to affect people through persistence in different foods and have caused numerous cases, deaths, and major outbreaks through consumption of contaminated food. Both microorganisms are highly acid resistant and are able to able to survive the acidic conditions in the stomach, pass into the intestine where they can replicate and initiate their pathogenicity cycle. Both microorganisms can achieve that through major persistence in the food chain; from the stages where food is produced and decontaminated to the consumption of contaminated food. To understand this persistence, we can use molecular techniques such as functional genomics through utilization of mutant libraries, or transcriptomics to uncover the molecular mechanisms these microorganisms use in each stage of the food chain.

This session covers, firstly, the use of transcriptomics and functional genomics for the investigation of molecular mechanisms through which *L. monocytogenes* is able to survive different processes and cause disease. Furthermore, it covers the use of an extensive mutant library and functional genomics to understand the molecular targets and mechanisms triggered by antimicrobial compounds in broccoli extracts against *E. coli*. Finally, it looks into the molecular targets and bacterial responses of novel non-thermal applications (e.g., cold atmospheric plasma and gaseous ozone) which through the use of mutants and predictive modelling tools can permit better interpretation of the adaptions of the microbial physiology.

### Understanding the Molecular Targets of Broccoli Extract on *Escherichia coli* through the Use of an Extensive Mutant Library

**KIMON ANDREAS KARATZAS**, University of Reading, Reading, United Kingdom

Understanding of the molecular mechanisms of food processes and antimicrobial compounds is essential for the efficient elimination of pathogens from foods or other environments. Various foods are known to possess antimicrobial properties that can influence microorganisms that might be present on them, on the foods that will be added, or in the gut microbiota. Knowledge of the actual compounds responsible for these effects and their mode of

action are essential for understanding biological processes that affect our health. We could even use these compounds for other applications. One of the foods that possess such properties is broccoli. This food contains sulforaphane, which has antimicrobial activity. To identify the mechanism of sulforaphane, we assessed the growth of a full mutant library of *Escherichia coli* against various concentrations of sulforaphane. Specific mutants that were identified to have a different behavior than the wild type (WT) were selected for further susceptibility tests. The work identified specific genes that play a role in survival against sulforaphane and the molecular targets of this compound.

### Assessing the Antimicrobial Mode of Action of Novel Non-thermal Processes through the Use of *Escherichia coli* Mutants

**VASILEIOS VALDRAMIDIS**, University of Malta, Msida, Malta

The efficiency of food processes have been associated with different stress environments such as thermal shock, oxidative stress, and the presence of antimicrobial compounds. It is known that bacteria have evolved different defense and repair mechanisms to defend themselves against the stresses generated by these technologies.

This work reviews a number of stress environments and the microbiological responses in relation to cold atmospheric plasma and ozone treatments. The regulatory mechanisms of the bacterial responses against reactive oxygen species and thermal stress compounds are showcased. For that purpose, ΔsoxR, ΔsoxS, ΔoxyR, ΔrpoS, ΔdnaK mutants whose deleted genes are mainly transcriptional regulators (soxR, soxS, oxyR and rpoS) capable of responding to, amongst others, hydrogen peroxide and superoxide radicals, were studied as representative markers in microbial stress environments. The macroscopic responses of these mutants where quantified, during treatments, by cold atmospheric plasma (70 kV, 50 Hz for treatments of 15, 30, 60, 90 s) and gaseuous ozone (6 µg/ml for 240 s), in order to interpret the antimicrobial mechanisms of action. The recovery growth rates of the knockout mutants resulted in different responses. For example, ΔsoxS was not recovered following a 90 s plasma treatment indicating the presence of reactive oxygen species, such as nitric oxide and superoxide-generating agents, during this treatment. Overall, functional genomics via mutant libraries can permit better interpretation of the adaptions of the microbial physiology.

### Understanding Stress Adaptation during the Transition of *Listeria monocytogenes* from Environment to Food to Host

**CORMAC GAHAN**, University College Cork, Cork, Ireland

*Listeria monocytogenes* is widely distributed in the environment and a rare, though serious, cause of foodborne disease. The pathogen has the capacity to undergo molecular adaptation to very diverse environmental conditions as it enters the food-chain and ultimately the human host.

We have used a number of approaches to determine how *L. monocytogenes* may adapt to stressful conditions (such as elevated osmolarity, low pH, low iron) encountered during growth in foods and transition to the host. These include whole-genome analyses, generation of gene knock-outs, transcriptional analysis and bioluminescence engineering (to track *L. monocytogenes* in complex environments). We note that the pathogen utilises shared systems to adapt to similar conditions encountered throughout the infectious cycle. The contribution of specific molecular systems to the survival of the pathogen in the food production environment, the food matrix and the host gastrointestinal tract will be discussed.

## S6 How to Exploit Omics Data on Pathogen Behavior in Microbiological Risk Assessment: An Update on the Current Research

New opportunities have become available, in terms of data production and exploitation in the field of microbiological risk assessment (MRA), due to technological advancements in the field of the nucleic acids sequencing, where there is the possibility to obtain a large number of sequences (millions) from the microorganisms present in a single sample without the need for their cultivation. More specifically, the behavior of foodborne pathogens, deciphered with transcriptomic, proteomic, and/or metabolomic techniques, within the whole food chain and in response to specific stresses, can now be studied. The current challenge scientists are facing is the integration of such data into risk assessment schemes.

In 2016, at the IAFP European Symposium in Athens, a workshop on “Next Generation MRA (Microbiological Risk Assessment) Integration of Omics Data into Assessment” was co-organized by ILSI Europe, IAFP, and ICFMH, in which four breakout groups on epidemiology, metagenomics, exposure assessment, and hazard characterization brainstormed and produced a common strategy to go beyond the current knowledge. In this symposium, we will present the main outcomes of the workshop, including the points of view of academia and industry, and we will further discuss to how best use omics data in MRA.

### The Use of Metagenomics in Quantitative Microbiological Risk Assessment

**LUCA COCOLIN**, University of Turin-DISAFA, Turin, Italy

The application of next generation sequencing techniques in food samples offers the potential to investigate the microbial composition and functions in unprecedented depth and in high throughput fashion. Metagenetics, or amplicon sequencing, is an approach that is “taxonomy oriented” that gives a detailed view of the composition of a system. It has been applied to describe the microbial ecology of foods and food-related ecosystems. Metagenomics is a “function” oriented approachm which, as of yet, been less exploited in food microbiology. Both approaches can enrich our understanding regarding interactions between biotic and abiotic factors and also within the microbial community of any given ecosystem.

Understanding such interactions and the behavior of microorganisms is important in the process of assessing biological risks in foods. For example, associations between specific microbial communities and the presence of a foodborne pathogen can be unraveled by the culture independent analysis of large sets of samples. Further, such associations may lead

to the identification of community fingerprints to trace foodborne pathogens at the species or even strain level and their transmission through the food chain.

It is envisioned that risk assessment will gradually integrate information that concerns the behavior of microorganisms, assembled from "omics" data, and shift from the taxonomic definition of the biological hazards. Improvement in the resolution of metagenomics data would allow us to observe and study the foodborne pathogens in their environment even when they are not prevailing components of the microbial community.

### The Use of Omics in Exposure Assessment

**HEIDY DEN BESTEN**, Wageningen University, Wageningen, Netherlands

Exposure assessment plays a central role in microbiological risk assessment. It provides an estimation of both the likelihood and the level of a microbial hazard in a specified consumer portion of food, taking microbial behaviour into account. To date, mostly phenotypic data have been used in exposure assessment.

This presentation will illustrate how mechanistic cellular information obtained through omics techniques could make a difference in: (i) understanding the dynamics of pathogens in a complex food eco-system; and, (ii) predicting pathogen behaviour variability. Advancements in the research activities of these two domains will be presented and discussed, through examples, with a special focus on industrial applications.

### The Use of Omics in Hazard Characterisation

**TREVOR PHISTER**, PepsiCo, Leicester, United Kingdom

Omics technologies, such as Whole-genome Sequencing (WGS), have impacted food safety through its incorporation into epidemiological investigations. While there are still many questions surrounding the use of WGS in source identification, the technology has already been adopted by a number of public health agencies. Academia, industry, and regulatory bodies, however, have only just begun to explore the integration of omics data into microbial risk assessment (MRA).

MRA consists of four stages: Hazard Identification; Exposure assessment; Hazard Characterization; and Risk Characterization. In Hazard Identification, organisms that can potentially confer an adverse health effect are identified and defined. In Exposure Assessment, the dose at the moment of exposure is determined. In Hazard Characterization, the probability and severity of a disease outcome is determined as a function of the dose. Finally, in Risk Characterization the overall probability and severity of the illness is determined, including variability and uncertainty.

In this presentation, we will discuss the issues and challenges in using omics data in hazard characterization. The data may aid in decreasing the variability and uncertainty present in this stage. Current research suggests omics may be integrated in a number of ways from defining the differences in virulence between bacterial strains to the identification of biomarkers that may suggest increased virulence of a pathogen or susceptibility of a host. The use of omics in MRA is just beginning and, as it has with epidemiology, it is sure to have a big impact on how we characterize hazards in our food supply.

## S7 Dietary Exposure to Food Chemicals: Data Needs, Methods, and Case Studies

In chemical food safety, dietary exposure to food chemicals is a key step in assuring that substances present in food are safe for consumers. This is the case regardless of whether the substance is a contaminant or is intentionally added; therefore, everything from flavourings, additives, food packaging migrants, contaminants, pesticides, and even some nutrients require risk assessment in order to assure their safe presence in food. Determining dietary exposure to food chemicals requires data on how foods are consumed and the chemical occurrence in those foods.

A number of approaches to dietary exposure exist, from simple deterministic screening methods to more refined techniques requiring detailed data on food consumption habits and chemical occurrence. Knowing which technique to use depends on the chemical in question and the specific needs in the exposure scenario. Typically, a tiered approach to assessing exposure is followed.

This symposium will present an overview of current approaches that can be used to assess dietary exposure to food chemicals, as well as some case specific studies demonstrating how these techniques have been applied in practice. Exposure models addressed will include screening methods that are used for routine risk assessment, refined high-tier probabilistic models, and methods that can be used for cumulative risk assessment or chemical mixtures. Case studies will include fluoride, as a contaminant in tea, and Vitamin A, which is present in personal care products and cosmetics, but potentially toxic at high levels of exposure.

### Approaches to Dietary Exposure for Chemicals in Food: Data Needs and Modelling Techniques

**CIAN O'MAHONY**, Creme Global, Grand Canal Quay, Ireland

Exposure assessment is a key element of chemical food safety. A plethora of chemicals exist in food as additives, flavourings, food contact materials, pesticides, contaminants, and micronutrients, amongst others. In order to ensure the safety of a chemical present in food, an estimate of the likely exposure to the chemical in consumer populations is needed. This, in turn, can be compared with an appropriate Health-Based Guidance Value (HBGV) or reference dose to assess risk.

A number of techniques exist for estimating consumer dietary exposure to food chemicals; all, generally, centered around estimates of food intake and the level of chemical occurring in food. Techniques range from screening methods based on simple worst-case estimates of exposure to more refined probabilistic models aimed at providing realistic estimates of exposure for consumer populations. For certain chemical groups, like pesticides, methodologies have, also, been developed for cumulative or mixture exposure assessment. This presentation will provide a broad overview of different approaches to food chemical exposure assessment, as well as their data needs, uses, and future directions in the area.

### Aggregate Exposure to Vitamin A from the Diet, Personal Care Products and Cosmetics

**SARAH TOZER**, Procter and Gamble, Egham, United Kingdom

Vitamin A is an essential nutrient in the human diet, and ingredients containing vitamin A and its derivatives are also used in personal care products and cosmetics. Therefore, the consumer can be exposed from multiple products and by multiple exposure routes. Vitamin A deficiencies are associated with a number of health concerns, particularly in children. However, excessive exposures to vitamin A are associated with adverse health effects, such as teratogenicity, and changes in bone mineral density in humans, although the later remains controversial.

In this study, aggregate exposure to vitamin A (considered in the form of retinol equivalents) was assessed in pre-menopausal, menopausal, and post-menopausal women in European and U.S. populations considering sources from foods, dietary supplements, and cosmetic and personal care products. Large data sets measuring consumer habits and practices, including diary data, food surveys and clinical studies, where incorporated at the subject level using probabilistic modelling to calculate population exposure distributions. The model incorporated product occurrence data for vitamin A in 17 cosmetic products, so as not to overestimate exposure. The relative contributions of the different sources of exposure were measured.

In all populations studied the average and P95 exposure was well below the Upper Intake Limit (3000 µg/day). The major source of vitamin A exposure comes from the diet, with cosmetic sources providing only a very small fraction of total exposure (2–3% at P95 in European and American females). In addition to providing a realistic assessment of total vitamin A exposure, this work can be used as a case study on how to approach future complex aggregate exposure questions.

### Contaminants in Tea: Exposure and Risk Assessment Approaches to Ensure Consumer Safety

**THERESA NEELY**, Unilever, London, United Kingdom

Within the Unilever Safety and Environmental Assurance Centre (SEAC), a risk-based approach is used to assure the consumer safety of Unilever's food and beverage products.

Exposure is the starting point and a fundamental part of the risk assessment process. It is therefore key that any exposure estimates made use as much relevant information as possible, using robust assessment tools. Exposure estimations often require a 'weight of evidence' approach which may include: Dietary intake survey data, probabilistic modelling of dietary intake data, consideration of existing consumer exposure to a chemical i.e., how much more are we adding, consideration of bioavailability and consideration of aggregate exposure from multiple sources.

This presentation will describe the risk-based approaches which are used for the safety assessment of chemicals in food and beverages, together with an overview on exposure assessment approaches. A case study on the safety assessment approach for Fluoride contamination of tea, will illustrate the risk-based safety assessment approach and use of 'weight of evidence' exposure estimations as part of this approach.

## S8 Prevalence, Properties, and Control of *Listeria monocytogenes* in the Food Supply Chain

Despite our increasing knowledge about properties and occurrence of *Listeria monocytogenes* in the food chain, this bacterium remains a serious hazard. It can cause a mild non-invasive illness (called listerial gastroenteritis) or a severe, sometimes life-threatening, illness (called invasive listeriosis) with a case-fatality rate ranging from 20 to 30 percent. *Listeria monocytogenes* is ubiquitously found in the environment. Sources of this bacterium in foods include ingredients/raw foods, processing aids, contact surfaces, and plant environments. In the case of food contamination with *L. monocytogenes*, products that pose a high risk of being associated with listeriosis are those that support growth of *L. monocytogenes,* but do not undergo heating before consumption (ready-to-eat, RTE). Foods that do not support growth of *L. monocytogenes*, on the other hand, pose a low risk. Such foods have intrinsic or extrinsic factors to prevent the growth of *L. monocytogenes* (e.g., pH ≤ 4.4, $a_w$ ≤ 0.92, or other factors).

For many RTE foods, contamination of foods with *L. monocytogenes* can be avoided; e.g., through the application of good manufacturing practices (including controls on ingredients, plant hygiene), the use of validated heat treatments, listericidal and listeristatic processes or ingredients, segregation of foods that have been heated from those that have not, sanitation, and, overall, by avoiding processing or production failure. In addition, prevention of outgrowth in a food is an effective strategy to safeguard food safety.

This session covers the occurrence of *L. monocytogenes* in > 50 food production plants, the efficacy of cleaning and sanitation agents to reduce the levels of *L. monocytogenes*, and an example of growth inhibition of *L. monocytogenes* in a RTE food, as demonstrated by challenge studies and evaluation of antimicrobial compounds naturally present.

### A Three-year National Survey of *Listeria monocytogenes* Prevalence in the Irish Food Chain: Implications for Food Safety

**CONOR O'BYRNE**, National University of Ireland, Galway, Galway, Ireland

Numerous studies have examined the prevalence of *Listeria monocytogenes* in different ready-to-eat foods and food processing environments, but they are often confined to a limited range of produce or are limited in time. This talk will present the results of a three-year study on 54 small food businesses spread throughout the island of Ireland that included ready-to-eat foods from four main food categories (seafood, vegetables, meats and dairy produce).

Samples from both foods and the processing environment were collected every two months over the three-year course ofstudy. *Listeria monocytogenes* was isolated using the ISO11290 standard method. Multiplex PCR and Pulse Field Gel Electrophoresis were used to type all isolates obtained in the study, allowing an assessment of the relatedness of isolates from different locations and different food groups. In total, 86 distinct pulso-types were identified in the study. The overall occurrence of

*L. monocytogenes* in foods was 4.2% and in samples from food processing environments, the prevalence was 3.8%. Meat was found to have the highest rate of *L. monocytogenes* positive samples (7.5%), whereas, seafood was the lowest (1.7%). Many of the strains identified were found to be repeatedly isolated from particular environments; suggesting that some strains have a persistent phenotype. The implications of the findings of this study for food safety and public health will be discussed.

### A Novel Targeted Approach in Disinfection and Decontamination through Inhibition of Specific Stress Resistance Mechanisms in *Listeria monocytogenes*

**KIMON ANDREAS KARATZAS**, University of Reading, Reading, United Kingdom

Normally disinfection uses a mix of various compounds that the affect survival of microorganisms. Acidic disinfectants, normally, use compounds that have high pKa to ensure a higher bactericidal effect. Our work demonstrates a novel approach in decontamination and disinfection regimes where specific molecular acid-resistance systems are inhibited, aiming to eliminate microorganisms under acidic conditions. Despite the importance of the Glutamate Decarboxylase (GAD) system for survival of *Listeria monocytogenes* and other pathogens under acidic conditions, its potential inhibition by specific compounds that could lead to their elimination from foods or food preparation premises has not been studied.

This work investigates the effects of maleic acid on the acid resistance of *L. monocytogenes*. Maleic acid was shown to have a higher antimicrobial activity under acidic conditions than other organic acids. It was able to significantly increase the sensitivity of *L. monocytogenes* strains to acidic conditions with more pronounced effects on strains with higher GAD activity. Maleic acid affected the extracellular gamma-aminobutyric acid (GABA) levels, while it did not affect the intracellular GABA levels. All data suggest that there is a major impact by maleic acid; mainly on the GadD2 activity, which is shown in cell lysates. Furthermore, we found that maleic acid is able to remove biofilms of *L. monocytogenes*. Maleic acid is able to inhibit the glutamate decarboxylase of *L. monocytogenes;* and, as such, it can significantly enhance the antibacterial effect of acidic conditions. The above properties, combined with the ability to remove biofilms, make this compound a great candidate for disinfection and decontamination regimes.

### Hurdles to Prevent Outgrowth of *Listeria monocytogenes*: Evaluation of Factors in Gouda Cheese

**MARJON WELLS-BENNIK**, NIZO Food Research, Ede, Netherlands

European Union regulation EC 2073/2005 contains microbiological food safety criteria for *Listeria monocytogenes* in ready-to-eat foods. Product categories are specified based on the potential of this pathogen to grow in a food. Some foods do not support growth of *L. monocytogenes*; for instance, when the pH ≤ 4.4, or the water activity ($a_w$) ≤ 0.92, or when pH ≤ 5.0 and $a_w$ ≤ 0.94. In other cases, evidence that a product does not support growth can be obtained by predictive microbiological modelling, historical data, information from scientific literature, and/or durability or challenge testing.

Knowledge of treatments to eliminate *L. monocytogenes* from foods and of the most important hurdles to control growth in actual food products is critical for defining process, product, and storage conditions that warrant safety. Here, the case of nature-ripened Gouda cheese is presented. This is a ready-to-eat product made from pasteurized milk with a pH just above 5.0 and aw above 0.94. This cheese has not been associated with foodborne outbreaks of listeriosis and various challenge tests have shown that *L. monocytogenes* does not grow in it. To explain the absence of growth, we evaluated the individual factors relevant to Gouda cheese for their potential to inhibit growth of *L. monocytogenes*. Factors included aw, pH, undissociated acetic and lactic acid, diacetyl, free fatty acids, lactoferrin, nitrate, nitrite, and nisin. This revealed that undissociated lactic acid is the main growth inhibitor of *L. monocytogenes* in Gouda cheese. This factor can be included as a criterion in product specifications of this cheese in relation to the risk of outgrowth of *L. monocytogenes*. In addition, this knowledge is applicable in product development of cheeses and other products.

## S9 Predictive Mycology Applied to Spoilage: From Data Collection to User-friendly Tools

The field of predictive microbiology has, historically, focused on foodborne pathogenic bacteria. Models describing spoilage and, specifically, those dedicated to yeast and moulds remain sparse. Spoilage due to yeasts and moulds is, however, an issue of concern for the food industry as it can lead to product recalls, food waste, great economic losses, and it can even ruin a company's reputation.

Prevention of mould and yeast spoilage can be accomplished at the factory by limiting initial contamination and controlling growth of spoilage microorganisms in the products. For products where contamination can be minimized, but not eradicated, control of mould spoilage is traditionally based on formulation and validated by predictive models or challenge tests to define a suitable shelf life. These approaches and challenge test designs are quite different from those currently used for bacteria.

The purpose of this symposium is to give the audience: [1] an introduction to the field of predictive mycology and an overview of challenge test approaches for determining their growth and inactivation in food; [2] a presentation of a selection of available models and their use in industrial applications; and [3] a presentation of a user-friendly software to predict the behaviour of yeasts and moulds in intermediate moisture food.

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### Predictive Mycology: History and Importance of Data Collection

**PHILIPPE DANTIGNY**, Laboratory of Biodiversity and Microbial Ecology, Brest, France

For over 30 years, predictive microbiology focused on food-pathogenic bacteria. The objectives of predictive mycology are to understand and to predict the development of fungi in food and raw materials; the inactivation of fungal spores in the food industry; and the accumulation of secondary metabolites, such as mycotoxins, throughout the food chain.

The number of studies dedicated to food spoilage fungi has increased in recent years. Most of these studies were concerned with the effect of environmental factors on fungal growth, but the major issues were not addressed. Methods are not

unique, and may depend on the objective of the study. Through the examples of fungal starters and food spoilage fungi, the more relevant factors and biological responses were highlighted. Secondary models that describe the effect of some environmental factors on these responses were also detailed.

### Mathematical Models and Probabilistic Approaches Quantifying the Influence of Formulation, Processing, and Environmental Factors on Mould Growth: Application in Shelf-life Assessment of a Food Commodity

**JEANNE-MARIE MEMBRÉ**, INRA, Nantes, France

Mathematically, mould spoilage can be expressed as a combination of probabilities: probability to be contaminated and probability to grow (germination and mycelium proliferation) up to a visible mycelium before product consumption (Membré and Dagnas, 2016). Each probability is described by a statistical model with a response (the probability) and several factors of variation, which are linked, not only to formulation, processing, environment, but also, to shelf life. Moreover, with cooked products, the contamination, generally, occurs after the cooking step, meaning that the contamination level is low: only one or few spores per product.

The objective of this presentation is to give the general framework built when quantifying mould spoilage, followed by an overview of the most commonly used statistical models (primary and secondary models). The differences between models built at the population or at the single spore level will be pinpointed. These framework and models will be illustrated through an example of shelf-life assessment using recent studies obtained on bakery product (Dagnas et al., 2014, 2015, and 2017).

### Sweetshelf: A User-friendly Tool to Predict the Growth of Yeasts and Moulds in Intermediate Moisture Food

**AN VERMEULEN**, Ghent University, Ghent, Belgium

In collaboration with more than 30 Belgian chocolate producers a software packages was developed predicting the microbial stability of intermediate moisture foods at different temperatures. SWEETSHELF is based on growth/no growth models for osmophilic yeast (*Zygosaccharomyces rouxii*) and xerophilic moulds (*Eurotium herbariorum*) developed in laboratory growth media (Vermeulen et al., 2012; Deschuyffeleer et al., 2015; Vermeulen et al., 2015). The models were validated by performing challenge tests on industrial recipes of sweet fillings.

Growth/no growth models were developed based on optical density data gathered in Sabouraud broth during 90 days. The medium was modified mimicking intermediate moisture food by adding high amounts of sugar (50% (w/w), glucose and fructose in a 1:1 ratio). The media were varying in: *(i)* $a_w$ (0.76 – 0.88, by adding glycerol); *(ii)* pH (5.0 – 6.2, by adding HCl); *(iii)* ethanol (0 - 4.5 % [w/w] on total medium basis); and *(iv)* temperature (8 - 25°C)

For osmophilic yeast, three different models were included: (i) without organic acids, (ii) with acetic acid (1% on product basis), or (iii) with sorbic acid (1500 ppm on product basis) added as potassium sorbate. The model predicted the growth probability for yeast and molds at discrete time points: 30, 60, and 90 days of incubation.

## S10 The Race to Zero: Everybody Loses

Analytical methods for contaminants in food have evolved over the last few decades to push the limits of detection from parts per million (ppm) down to parts per billion (ppb), and in some cases even lower. While methods “race towards zero” in terms of the ability to detect chemical contaminants, risk managers need to adapt to this new environment by creating new processes for evaluating whether the detection of trace amounts of these compounds pose a risk that requires management. These challenges impact individuals across the risk assessment process: from the analytical chemists that are challenged to develop methods that are fit for the purpose of risk assessment; to hazard assessors who are looking to methods such as application of the Threshold of Toxicological Concern (TTC) to help evaluate trace chemicals; to risk managers that need to incorporate the information from the other areas when they build risk management programs. Only through a cooperative effort and the use of new ideas will these different functional areas be able to build effective programs that ensure the safety of consumers without committing undue resources towards chasing zero.

### Chasing Zero: Holy Grail, Marketing or Necessity?

**BERT PÖPPING**, FOCOS,Alzenau, Germany

Limits of detection for methods targeting food contaminants and adulterants have quickly decreased from pp thousands in the 1950ies, to ppm and ppb in the 70ies and 80ies, to ppt and ppq in the 90 and 2000. The question to be asked is if a very low detection limit is always helpful, or if it can create problems. The presentation will be looking at some key examples, including chloramphenicol, glyphosate, acrylamide, perchlorate, as well as food allergens and gluten. It will put the new and lower detection levels in perspective with customers expectations and industry efforts to obtain goods below the LoD of the target analyte. It will look at the constantly increasing cost for laboratories due to the disproportional increase in equipment cost.

The presentation will also look at the way media report, and how governments present results and findings of toxicological studies. It will discuss consumer perception and the idea of zero-risk products.

### Approaches for Prioritizing and Evaluating Trace Contaminants

**GABRIELE SCHOLZ,** Nestle, Lausanne, Switzerland

See online doe programme for abstract.

### Communicating with Consumers: How to Talk about Food Risk

**NINA MCGRATH**, EUFIC – European Food Information Council, Brussels, Belgium

In Europe today, our food is arguably safer and more accessible than ever before. Despite this, there appears to be an increasing lack of public confidence in the food supply. A proactive approach to communicating about food would help to reassure the public about its safety, restore consumers’ trust in the authorities charged with regulating it, and help people understand how to eat safely and healthily.

This presentation will introduce a recent publication from the European Food Information Council “How to talk about food risk,” a practical

handbook that aims to guide communicators through a sequential step-by-step process for developing and implementing a proper risk communication strategy. This includes a systematic evaluation of the risk, the environment, and a self-analysis of the communicator; tools for understanding audiences and developing targeted messages and content; communication channel selection; and the importance of monitoring public response.

## S11 Progress in Food Safety Education and Training: Learnings from Tailored Small Group Offerings to Running Massive Open On-line Courses

Training is crucial both for young students and professionals. Both in university teaching and in professional education, new developments have occurred in the last decades. Apart from live lecturing and reading material, group and on-line work form a part of many courses.

This symposium is an overview of knowledge and skills needed for professionals in the food industry. The challenge in all education is finding the right balance between basic knowledge and needed skills. Experiences using teaching methods involving real problem solving and active learning will be presented. Materials that are developed for distance learning can be used in many settings, such as MOOC's (Massive Open On-line Courses), SPOC's (Small Private On-line Courses), professional courses, and on-line courses, as part of other courses, background material, etc. All together, these materials and experiences can be used to develop blended learning programs, in which optimal use is made of the various building blocks, which are fine-tuned for a specific situation.

### What Food Safety Knowledge and Skills Will Employers Expect When Recruiting Professionals to Work in the Food Industry?

**PIER SANDRO COCCONCELLI**, Univesita Cattolica del Sacro Cuore, Cremona, Italy

The implementation of the food Risk Analysis (RA) concept, in the different sectors of the food system, research, institutional bodies, and food enterprises, requires specific education and training. Thus, to handle the three components of RA (Risk Assessment, Risk Management, and Risk Communication), transdisciplinary and multidisciplinary competences are required.

Although food safety is a fundamental discipline in several university programmes aimed to educate experts for the food sector, master programmes are, mostly, targeted on food science and technologies and Ph.D. programmes on specific research topics. Consequently, more flexible forms of higher education should be developed, which specifically focus on food safety risk analysis and taking into account recent technological advances that can be used to assess and manage the risk in the entire food chain. The main challenge for any of these programmes will be to train students and professionals, who possess a high level of scienctific knowledge, on transdisciplinary topics such as emerging risks, crisis management, European Union food law, consumer behavior, and risk communication.

The experience of an international summer school for PhD students and young Postdoctoral fellows, organized in collaboration with the European Food Safety Authority (EFSA), will be presented and discussed. This summer programme has been designed to focus on specific topics (Biological risk assessment in 2016; *In silico* methods for risk analysis in 2017). In addition to the core knowledge on the selected topic, the programme uses simulation games and problem-solving approaches aimed at training young experts.

### Examination Challenges for Teachers of Food Safety: Methods vs. Knowledge and Skills Gained

**STEPHEN FORSYTHE**, Nottingham Trent University, Nottingham, United Kingdom

Food microbiology encompasses three aspects of food production, food stability (shelf life) and food poisoning (infection and intoxication). It is both laboratory-based and managerial with numerous, in-house and legal requirements to be met. For the purposes of this presentation, my focus will be on the teaching aspects of laboratory-based, food poisoning control; and not HACCP, etc.

Advances in Next Generation Sequencing (NGS) have given a greater depth to understanding foodborne pathogens; and, as with other aspects of our lives, access to technical information, which has drastically changed since many lecturers were students. In addition, information/teaching resources (e.g., smart phones!) have changed. In fact, "NGS" should stand for "Next Generation Students." Despite all of these advances, we still have issues with the control of foodborne pathogens, including *Salmonella*, *Campylobacter*, and *E. coli* pathovars. So as lecturers/mentors of the next generation in food safety, do we examine according to knowledge, or according to understandstanding?

In our experience, problem-solving scenarios can be a useful approach for individual, final year undergraduate projects and we have trialled a modified SCALE UP approach with teams of final year students. SCALE UP stands for "Student-Centered Active Learning Environment for Undergraduate Programs" and "Student-Centered Active Learning Environment with Upside-down Pedagogies." Initially, the students are given a two to three sentence description of an outbreak and a budget to work within. From the description, they draw up a short list of plausible causative organisms and decide on the next step of their investigation. This is submitted to the lecturer for approval.

In this session, I will share my experiences with individual and modified SCALE UP, group-oriented, problem-solving teaching and its relevance to assessing students, as well as preparing students for food microbiology in the real world.

### On-line Courses in Food Safety – SPOCs and MOOC

**MARCEL ZWIETERING**, Wageningen University, Wageningen, Netherlands

In our modern society, food safety is a growing concern for many consumers. People want their food to be safe and are worried by the numerous, sometimes conflicting messages thrown at them. The Wageningen departments of Toxicology and Food Microbiology have teamed up in building an open course that aims to make food safety education freely available, worldwide. Elements of this MOOC are,

also, used as a SPOC (small private online course); a refresher for new students, especially if they do not have a background in food sciences. Apart from these open course materials, the Laboratory of Food Microbiology at Wageningen University has built on-line course materials for the BSc level that have been used, successfully, in Singapore and Wageningen, during introductory Food Microbiology courses.

Finally, a distance learning programme (www.dl-fsm.nl) has been developed on selected aspects of food safety management, targeting both students and food professionals around the world, at the BSc or MSc level, who are interested in strengthening their knowledge on, for example, sampling and monitoring, hygienic design, or preservation. On-line course materials, adapted for various target groups, are useful tools both in curriculum education and for open access for small or large groups, alike. Digital, interactive, variable learning materials can be an effective, efficient, and a persistent part of an education program. Furthermore, they can be stimulating for staff, students, professionals, and the general public.

## S12 Novel Insights into the Microbial Ecology of Food Processing Using Next Generation Sequencing Methods

Although the food industry, authorities, and legislation tend to focus on surveillance and control of pathogenic bacteria, the majority of bacteria in the food processing environment are, in fact, non-pathogenic. These bacteria, which can be isolated on non-selective growth media are often referred to as the natural flora, total bacterial count, heterotrophic plate count, aerobic plate count, etc.; and, for the majority of food industries, the identity of these microorganisms is unknown. There is a number of reasons for increased attention to the residential, non-pathogenic bacteria in the food production environment. Obviously, they may pose a threat for the food quality.

Secondly, several studies imply that the fate of pathogens introduced into the processing environment may be affected by the non-pathogenic bacteria present. Also, for other ecological niches, it has been speculated that residential bacteria could play a role in the persistence and spread of antimicrobial-resistance genes. Recently, the introduction of sequence based microbiota analyses have given new insight into the identity of microorganisms on surfaces in the food industry. This methodology provides high throughput and in depth identification compared to cultivation based techniques; and, its use provides new insights into the microbial ecology of processing environments, as well as contamination routes for spoilage bacteria.

### Residential Bacteria in the Food Industry: Why? Who? So What?

**TROND MØRETRØ**, Nofima, Norwegian Food Research Institute, Aas, Norway

Bacteria are frequently present on surfaces in food processing environments. The majority of these bacteria are non-pathogenic. Pathogenic bacteria are, usually, present sporadically and in low numbers. Most food processors do not know the identity of the non-pathogenic, residential bacteria found in their production plant. Increased knowledge is important to understanding how the bacteria in the food production environment can affect food quality and food safety.

The introduction of sequence-based microbiota microbiota analyses has given new insight into the identity of microorganisms on surfaces in the food findustry. Gram negative bacteria dominate in most processing environments, with *Pseudomonas* as the dominating genus followed by *Acinetobacter* and *Enterobacteriaceae*. In some environments, especially dairies, Gram positive bacteria are more frequently isolated. Characteristics such as growth at low temperatures, low growth requirements, biofilm formation, tolerance to drying, heat, and sanitizers are believed to be important for the growth and survival of residential bacteria in food processing environments. If the bacteria on machines and equipment are transferred to food during processing and are able to grow in that food, they may have a role in spoilage. Several types of important spoilage bacteria are common in food processing environments, such as *Pseudomonas*, *Enterobacteriaceae* and lactic acid bacteria. Also, it has been described that bacteria dominating the food industry can influence the fate of pathogens in the processing environment and, thus, affect food safety.

### High-resolution Exploration of Microbial Consortia in Food Processing Environments

**FRANCESCA DE FILIPPIS**, University of Naples Federico II, Portici (NA), Italy

Microorganisms inhabiting food-processing environments play an important role in defining the initial contamination pattern of food and, therefore, influence the shelf life and quality of the final product. Monitoring the presence of spoilage microorganisms in the food-processing environment and mapping the possible contamination routes is necessary to prevent microbial spread along the processing chain and, consequently, the transition to the finished product. However, facility-resident microbiota is sometimes addressed as a source of microbes that may be beneficially involved in the manufacturing process.

Different food processing and manufacturing environments, including beef, dairy, and ready-to-eat meals processing plants, were analysed by culture-independent, high-throughput sequencing. Swabs were collected from plant surfaces, tools, and operators' hands. Moreover, food products from the same manufacturers were evaluated.

The presence of a resident microbiota was highlighted, consisting of a few taxa that were well adapted to the considered environment, where food residues and exudates can act as substrates. This resident microbiota can be the source of food contamination and proliferate during storage to unacceptable levels, compromising food quality and safety. Nevertheless, depending on the type of manufacturing considered, food processing microbiota may sometimes play a positive role. Indeed, cheese manufacturing plants often harbour lactic acid bacteria, which are beneficially involved in the fermentative and ripening processes. Therefore, depending on the nature of the microorganisms and on the type of food manufacturing process, the environmental microbiota can exert positive functional activities or be a hazard for product quality and safety. Food-environment relationships deserve to be further explored, since they have the potential to affect the food processing dynamics and the quality of final products.

### Elucidating Contamination Routes of Meat Spoilage Bacteria with Next Generation Sequencing Methods

**JOHANNA BJÖRKROTH**, University of Helsinki, Helsinki, Finland

Food processing chains create specific man-made niches for bacteria. Lately, by implementing culture-independent technologies, we have carried out studies aiming at understanding the complexity of microbial ecology associated specifically with food chains. Yet we are still quite far from comprehensive views on how microbes contaminate our foods during primary production, harvesting and processing even though more holistic research approaches are available.

To prevent perishable foods from spoiling, the modern food industry is applying two major hurdles, i.e., refrigerated temperatures and carbon dioxide. They are the main selective pressures having an effect on food spoilage organisms growing in perishable foods packaged under modified atmospheres and cold stored throughout the distribution chains from manufacturers to consumers. Food processing facilities are also refrigerated to maintain food temperatures during manufacture within the limits set by the authorities. Meat processing provides one example of a strictly regulated food chain. Although some variation exists in regulatory temperature limits globally, the temperature of meat during cutting should not generally rise to above 7°C. Therefore meat processing facilities are maintained at 4°C to 12°C. In addition to chilling, strict washing and hygiene practices shape the microbial communities within these premises.

Perishable food items are increasingly manufactured “case ready” allowing retail distributors to place readymade packages on shelves. This omits handling of the food in shops and has been an increasing trend in many countries especially what comes to meat products. From the microbial ecology perspective, microbiomes in these packages are developing through time-dependent succession which is also associated with development of spoilage changes.

Food processing facilities and minimally-processed packaged foods are specific niches for bacterial growth. The selective pressures affecting bacteria in these niches are man-made rather than nature driven. We do not fully understand how cold-tolerant bacteria contaminate food processing facilities and persist in them. Neither do we know the main factors behind microbial succession even though we have been able to recognize the prevailing species present in foods for decades. In my talk I will be walking you through of one of our comprehensive spoilage problem analysis carried out using 16S rRNA gene amplicon analysis. We showed how different harmful bacteria (both pathogens and spoilage bacteria) had their own “behavioral patterns” what comes to the contamination within the processing facility and the subsequent growth in food. According to our study 16S rRNA gene amplicon analyses provide useful information of microbial ecology of a processing site and how this data can help manufacturers solving problems associated with their products.

## S13 Cleaning and Disinfection Methods for Low-water Activity Foods

Low-moisture foods are often associated with allergen recalls (chemical contamination) and there has been an increased number of recalls associated with microbial contamination of ready-to-eat low moisture foods. While many of the allergen recalls are labeling related, using verified cleaning methods to prevent cross-contamination continues to be a preoccupation for the industry. In addition, we will discuss whether the recalls associated with the presence of *Listeria monocytogenes* in low moisture foods could also affect Europe.

During this symposium, the first presenter will discuss some of the challenges, faced by the industry, with biological and chemical contamination and commonly used technologies to mitigate them. The second presenter will focus on lesser known technologies or alternative approaches using available technologies, such as dry steam, dry ice, etc., to clean and disinfection manufacturing equipment and provide examples that industry can relate to. Finally, the last presenter will focus on verification and validation of alternative technologies. And, for processor exporting to the USA, we will attempt to clarify the need to verify and/or validate cleaning and disinfection methods in the FSMA era and to reconcile what needs to be done to meet different global audit schemes. We will provide examples and solutions that will bring new ideas and generate discussions with the attendees.

### Technologies and Associated Challenges Related to Cleaning and Disinfection in Dry Environments

**KARIN BLACOW**, Commercial Food Sanitation, Amsterdam, Netherlands

Amongst the many challenges with microbial and allergen contaminations in low-moisture environments, finding the right cleaning methods to prevent cross-contamination within the production process continues to be a one that sticks out. The industry produces food for our families and friends to consume and that food needs to be 100% safe all the time to protect those consumers.

This presentation explains the challenges of getting 100% clean time after time and some of the available methods and technologies for cleaning in low-moisture environments to mitigate contamination risks.

### Cleaning Methods for Low-water Activity Food, the Successful and Less Successful

**COLLETTE GIRVIN**, Kellogg Company, Dublin, Ireland

There are three basic cleaning techniques relative to food manufacturing production-line equipment in a dry environment: dry cleaning, controlled wet cleaning and wet cleaning. Limiting the introduction and use of water is one of the primary means of controlling pathogens in low moisture food establishments.

The way to effectively clean is driven by the appropriate target level of clean: there are different zones in a plant and different foods that present more or less risk. Based upon these considerations, there are different areas of a facility that require different “target levels of clean”: the target level of clean will determine the method of cleaning required.

In addition to discussing "target levels of clean," this presentation will discuss cleaning technologies or alternative approaches using available technologies, such as dry steam, dry ice, etc. to clean and disinfection manufacturing equipment, and provide examples that industry can relate to.

### Verification and Validation of Sanitation Controls – What Should We Do?

**PAULINE TITCHENER**, Neogen Europe, Auchincruive, United Kingdom

The definition of validation, as given by the *CODEX Alimentarius*, "Guidelines for the Validation of Food Safety Control Measure" (2008) is: "Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome." In the case of sanitation, the process refers to the materials and procedures used by food processors for cleaning after a production run. The goal for validation of the cleaning process is to prove that it effectively removes particulates, residues, and microorganisms to a safe and satisfactory level. Per the *Codex Alimentarius*, verification is the application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.

This presentation aims to cover the practical processes that food manufacturers should consider during validation and verification with regards to sanitation and provide an overview of methods that can be used to monitor hygiene and minimize allergen cross-contact in the facility environment.

## S14 Foodborne Viruses: Detection, Risk Assessment, and Control Options in Food Processing

Foodborne viruses were recognized among the top rated food safety priorities in a very recent report by risk assessment experts on the identification of food safety priorities using the Delphi technique (Rowe and Bolger, 2016) and have become, over the past few years, a greater concern to the food industry. All parties agree that control measures for viruses are required throughout the food chain. However, much still needs to be understood with regard to the effectiveness of these controls and how to properly validate their performance, whether it is the personal hygiene of food handlers, the effects of processing on foods at risk, or the interpretation and action on a positive test result in a virus testing program (EFSA, 2011; FSA, 2015).

In this session, we will present the current work of an ILSI Europe expert group that provides a description of foodborne viruses, their characteristics, and responses to stress. We will, also, discuss the technologies developed for their detection and control and the way forward on the applications for science and industry. The recommendations in this review will allow industry to perform effective control options for viruses in food processing. We present the current state of the science on epidemiology, public health burden, risk assessment, and management options for viruses in food processing environments and draw practical conclusions.

### Pros and Cons of Methods of Detection for Viruses in Foods

**ALVIN LEE**, Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, USA

Enteric viruses, particularly human noroviruses (NoV), are the most common cause of foodborne disease and a significant contributor to global disease burden. Viruses enter the food supply across the farm-to-fork chain by exposure to contaminated waters, surfaces, and/or human hands. Unlike bacterial pathogens, for which there are widely used validated detection technologies, virus detection methods are less developed. There are many reasons for this, which, when taken together, necessitate processing food or analyzing environmental samples for virus concentration and purification prior to application of detection methods. These detection methods can be cumbersome, expensive, inefficient, and fraught with complications, including the inability to discriminate infectious from non-infectious viruses.

The purpose of this presentation is to discuss the outcomes arising from the ILSI expert working group's recent work; recent advances in viral detection for human NoV, Hepatitis A, and emerging viruses, such as Hepatitis E; the pros and cons of various detection methods and their applicability to foods and environmental samples; and recent developments in food virology arena and how these methods can be used to address real-world issues.

### Translating Risk Assessment of Viruses in Foods into Practice

**ELISSAVET GKOGKA**, Arla Strategic Innovation Centre, Brabrand, Denmark

Viruses are the most frequent cause of foodborne illness, worldwide, and a major contributor to the global foodborne disease burden (Havelaar et al., 2015). To assess risks associated with viruses and other hazards in the food chain and set appropriate control measures, the use of risk assessment techniques has been suggested by international bodies (FAAO/WHO, 1995; WTO, 1995) and increasingly accepted by governments around the world as a basis for national legislation in relation to food safety (European Community, 2002; Dong, Q.L. et al., 2015).

There are two main approaches in performing a microbiological risk assessment (MRA): (i) an epidemiological approach (top-down approach) starting from data on illness and moving towards the hazard in the product and (ii) a food chain approach (bottom-up approach) starting from the hazard in the product and moving towards an estimate of the probability of illness (Zweitering and van Gerwen, 2000).

This presentation aims to give a general introduction into the use of MRA, by both industry and governments, as a tool for quantifying the risk of foodborne illness due to viruses and to discuss bottlenecks and differences in available methodologies (top-down and bottom-up), providing examples from recent literature. A special focus will be given into translating the results of MRA into practical interventions for the protection of public health.

### Effect of Processing Technologies to Control Viruses in Foods

**SOPHIE ZUBER**, Nestlé Research Center, Lausanne, Switzerland

Traditionally, processing technologies rely on the control of bacterial contaminants as a measure of their effectiveness. However, various studies have shown that some foodborne viruses are more resistant than vegetative bacteria to certain control mechanisms and, thus, may not be inactivated at the same rate as

bacteria. In addition, as the food industry increasingly moves towards milder thermal processes, as well as the use of nonthermal technologies, the likelihood of viruses surviving such treatments increases. Therefore, validations of control strategies need documented scientific evidence to demonstrate effectiveness of control measures for reducing or eliminating viruses from foods.

Validation approaches are hampered by the difficulty in cultivating enteric viruses. The replication assay recently developed for certain human NoV strains should, in the future, allow evaluation of control strategies for these viruses. However, at present, the most common approach has been to use cultivable surrogate viruses such as FCV, MNV, TuV and bacteriophages, such as MS2. This presentation will give an overview on the efficiency of intrinsic and extrinsic factors of foods and various processing technologies, both traditional thermal and nonthermal technologies, and chemical based technologies to inactivate enteric viruses in foods. There are difficulties in comparing inactivation studies as numerous factors, such as surrogate choice and its preparation, treatment time, inoculation methods, and time allowed for inoculum to attach to product, could have a significant impact on the reduction observed. A standardised method for evaluating decontamination strategies for foods would be very useful to the food industry; future research needs to develop such guidance will be discussed.

## S15 Identification of Emerging Risks in Food: Different Approaches to Achieve a Common Goal

The successful identification of risks at their early inception is at the heart of public health protection and becoming increasingly important in food safety management. Early recognition of emerging risks has the potential to become a significant preventive instrument at the disposal of the food safety community. This symposium will provide an overview of some of the current approaches to identification of emerging risks being developed and applied by International and European institutions, industry, and academic researchers. Three presentations will be followed by a panel discussion aimed at addressing the strengths and weaknesses of such approaches, the challenges in identification of emerging issues, and the importance of collaboration in optimising such approaches.

At the regional and international level the approachs to identification of emerging issues, to date, have focussed mainly on the exploitation of knowledge networks and individual and institutional expertise.

The session will look at the how different organisations (EFSA, FAO, WHO) have approached this and used the resulting information to guide their work.

Industry well recognizes the importance of being forewarned of potential risks and this session will present some of the tools which are being used in the food industry, including webscouting tools, based on text mining principles, to identify emerging risks with the support of a network of experts. It will, also, look at how industry is exploring the opportunity to extract additional information from raw data generated for other purposes (e.g., analytical data), trend analysis, and vulnerability maps.

Other more systematic approaches are being developed by research and academic institutions. To illustrate this, the session will include an approach to assess chemicals currently registered under the REACH legislation for the identification of potential emerging chemical risks in the food and feed chain by evaluating parameters related to exposure (tonnage, release, biodegradation, and potential accumulation) and toxicity.

### Application of Food Safety Early Warning Systems: Industry Perspective

**JOHN O'BRIEN**, Nestlé Research, Lausanne, Switzerland

The emergence of new chemical and microbial risks in food, over the past twenty years, has led to profound changes in the way food products are manufactured and regulated. Major food fraud incidents, such as melamine, horse meat substitution for beef, and use of illegal food colorants, have led to urgent responses by regulatory authorities and manufacturers to protect consumers and the integrity of supply chains. While every single risk is difficult to foresee, it is possible to define a finite number of drivers associated with emerging risks. Understanding such drivers can help identify systemic risks in the food chain and can help to target action in the event of such risks being expressed. Factors influencing the emergence of new food risks include the growing complexity of the global food supply; differences in regulatory controls throughout the global supply chain; longer supply chains and more rapid distribution; fluctuations in commodity prices; developments in analytical science; consumer demands; differences in consumer vulnerability to foodborne hazards; growth in use of agricultural land for other uses (e.g., crops for biofuels, pharmaceuticals); climate change; and differences between risk perception by groups in society and scientists informed by risk assessment.

Early warning and rapid alert tools are now used in both the private and public sectors to detect and manage emerging issues. Tools include cross-functional communication technical networks, webscouting, vulnerability assessment of food chains, and rapid analytical screening technologies that can rapidly assess risk of the presence of undesirable agents. Such approaches have been shown to assist, greatly, in early management of issues; thereby, affording greater consumer protection. However, gaps remain in global coverage and in consistency of use of such tools.

### Networks of Knowledge – Sharing of Information and Expertise

**ANA AFONSO**, European Food Safety Authority (EFSA), Parma, Italy

Identification of emerging risks related to food safety provides the possibility to develop measures aimed at the prevention or mitigation of public health risks, as well as tools for an overall risk/benefit assessment of new food supply chains and technological developments. The definition of an emerging risk encompasses the context of novelty, a new hazard, or a known hazards emerging in new conditions. The challenges arise from the uncertainty, lack of data, and the difficulty to quantify the risk. Risk assessment frameworks, such as the one adapted by *CODEX Alimentarius* and the European Union food law, are not directly applicable.

The growing complexity of globalised food chains requires that different information be considered. The European Food Safety Authority has developed a process for the identification of emerging risks in food and feed, which relies on networks of knowledge with multidisciplinary expertise. The Food and Agriculture Organization has worked to develop a pragmatic approach that can be coherently applied by the different divisions/units dealing with Food Safety/Animal and Plant Health. The International Food Safety Authorities Network is a joint FAO/WHO voluntary global network of food safety authorities and provides an important platform for the rapid exchange of information in the case of food safety crises and for sharing data on both routine and emerging food safety issues. The availability of global information is a unique opportunity to improve our capacity of identifying risks; but, collaboration between all partners is even more necessary. Further efforts are needed to create a common mechanism to share experiences on the different methodologies applied, as well as to pool the intelligence gathered.

### Testing New Methodologies for Identification of Emerging Chemical Risks in Food

**JAN OLTMANNS**, Forschungs- und Beratungsinstitut Gefahrstoffe GmbH (FoBiG), Freiburg, Germany

The objective of this study was to develop and test a procedure for the identification of chemicals registered under the REACH Regulation that are of potential health concern and are likely to occur in the food chain. For this purpose, 100 data-rich substances registered under REACH, together with four positive controls, were evaluated. The evaluation of the 104 substances took into account parameters related to exposure (tonnage, release, biodegradation, and potential bioaccumulation) and toxicity (repeated dose toxicity, genotoxicity, and reproductive toxicity) organised in six blocks. All substances were scored for each block. ACC-HUMAN steady software was used to evaluate the potential for bioaccumulation in eleven different food items using input data derived from QSAR predictions. Several weighting scenarios were tested to aggregate scores for the six blocks into a total score, which enabled ranking the 104 substances. In addition, a Pivot table selection was implemented that can be used without weighting.

Further analyses compared the scores derived from experimental data with those derived from predicted data. These analyses found a good agreement of scores for biodegradability, but considerable disagreement of scores for toxicity endpoints. In conclusion, a scoring and ranking procedure was developed for the identification of chemicals of potential concern in the food chain (potential emerging risks) that showed a good level of differentiation. The focus on (semi-)automated processes ensures that this procedure can be applied to all chemicals registered under the REACH Regulation.

## S16 Ensuring Food Safety of Meat Products by Use of High Pressure Processing (HPP): From Recent Research Initiatives to Commercial Developments

In these recent years, there has been an increasing demand by consumers for healthier and fresh-like food products. High Pressure Processing (HPP) is a nonthermal technology for inactivation of vegetative microorganisms, which enables food preservation with limited effects on the organoleptic and nutritional quality. Although commercial implementation of HPP for food preservation has been fast growing since the late 1990s, ensuring the food safety of minimally processed products is still challenging, since both vegetative and sporeforming pathogenic bacteria have to be inactivated or inhibited until the end of the shelf-life.

This symposium will explore recent advances in the use of predictive modelling and challenge-testing in HPP process validation. The first presentation will be focused on the impact of high pressure on pathogen inactivation and the influence of product characteristics. The High Pressure Process Predictor (HP3), a user-friendly simulator, will be presented. In addition, the contribution will, also, deal with the behaviour of surviving cells during the subsequent storage of pressurised meat products.

The second topic will highlight an innovative initiative combining HPP and biopreservation to enable inactivation of sporeformers in a meat product. In this study, three lactic acid bacteria were first selected regarding their innocuousness, inhibition against sporeformers, and susceptibility to antibiotics. Once selected, their inactivation and growth following HPP was determined.

The last presentation will give an overview of the commercial developments of HPP, worldwide, with a special focus on the current, main interests of HPP for meat product preservation. One of the main interests resides in the reduction or removal of chemical preservatives, while maintaining commercial shelf-life without compromising food safety.

### Understanding the Behavior of *Listeria monocytogenes* in High Pressure Processed Meat Products: Resources for Process Validation

**SARA BOVER-CID**, IRTA, Monells, Spain

High pressure processing (HPP) is recognised as an interesting, nonthermal technology used to inactivate vegetative bacterial cells, enabling the in-package, cold-pasteurisation of food with limited negative effects on the organoleptic and nutritional traits. Commercial implementation of HPP has grown exponentially in the last two decades; with the meat industry being one of the food sectors taking the greatest advantage of this emerging technology within the hurdle technology approach. As a killing step (i.e., post-lethality treatment for ready-to-eat products) aiming to assure food safety, it should be considered a control measure and be included in the HACCP plan. The effect of HPP depends on the microorganism (i.e., species and strain, as well as the physiological state), the process parameters (pressure, holding time, and temperature) and product characteristics (including piezoprotective and sensitising agents). Therefore, the specific high pressure treatment needs to be designed, assessed, and validated for each particular product.

Besides the quantification of the lethality of the treatment, the behaviour of surviving cells during the subsequent storage has to be taken into consideration. In this presentation, different approaches to carry out validation studies will be presented, particularly focused on *Listeria monocytogenes* in meat products. Besides scientific literature, product-oriented challenge testing and

available predictive models are the main resources providing scientific evidences about the validity of the technology. The High Pressure Process Predictor ($HP^3$), a user-friendly, on-line simulator, will be presented as a useful tool to assess the effects of HPP on pathogens in meat products.

### Combined Use of High Pressure Processing (HPP) and Biopreservation to Preserve Meat Products: Screening, HPP-Inactivation and Regrowth of Three Lactic Acid Bacteria

**SANDRINE GUILLOU**, Oniris, Nantes, France

Ensuring the safety and the quality of food products during the entire shelf life, while minimizing the use of food additives, is an issue that food manufacturers have to address to comply with both regulations and consumer demands. High Pressure Processing (HPP) represents an innovative alternative technology, likely to be used to offset reduction of preservatives. However, the application of such mild preservation techniques may give selective advantage to development of sporeforming bacteria such as *Bacillus* spp. and *Clostridium* spp. In the BlacHP project (*ANR*-14-CE20-0004), the combined use of HPP and biopreservation using lactic acid bacteria (LAB) was challenged to stabilize refrigerated processed meat products.

In this presentation, the main steps involved in the selection procedure of the bioprotective LAB strains will be described. They comprise assessment of innocuousness, inhibition against sporeformers, and susceptibility to antibiotics. The three LAB strains selected, were then assessed regarding their resistance to and regrowth following HPP in a model medium (ham). A reparameterized Weibull model was used to determine HPP-induced LAB inactivation from challenge-test experiments. Their regrowth following HPP during chilled storage at 8°C was then modelled using the Rosso model. The strains were ultimately selected on the basis of either their high resistance following HPP or ability to regrow after HPP.

### High Pressure Processing Comercial Developments: Global Market, Equipment and Applications in the Meat Industry

**CAROLE TONELLO-SAMSON**, Hiperbaric, Burgos, Spain

It can be estimated that around 300 million of Kg of meat products processed under high pressure have been commercialized during 2016, thanks to more than 80 HPP machines installed in meat companies in the world.

The technology ensures the post-packaging destruction of spoilage microorganisms (coliforms, lactic acid bacteria) and pathogens (especially *Listeria monocytogenes*) of raw minced meats, sliced and diced dry cured or cooked hams, ready-to-eat meat meals, poultry cuts and sausages.

A majority of HPP meats are regular products (with chemical preservatives) which are processed under pressure in their final commercial package to achieve:

- a shelf-life extension (multiplied by two or three, compared to the same product non-pressure processed which is kept at the same temperature of storage)
- a better sensorial quality up to the end of the shelf life
- a reduction recall risks due to possible pathogen adulteration

Nevertheless, one of the main interests of the technology is that it allows chemical preservative/salt reduction or removal, keeping an acceptable commercial shelf life (one to two months) without compromising food safety. This has open a premium market for HPP in the field chemical preservative-free meat products, especially organic ones, with a “clean label.”

Several approvals have been given by the French Food safety authorities for HPP meat products commercialization, so pressure processed meats (and HPP food in general) are no more considered as novel foods in Europe.

## S17 Use of Predictive Microbiology for Process Validation Encompassing Biological Variability

Based on the CODEX Alimentarius, the food industry should provide “evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specific outcome.” Scientific evidences can be obtained by performing challenge tests during food processing. However, the introduction of pathogens in food production environments is, of course, not sensible. Consequently, the use of surrogate microorganisms (such as *Enterococcus faecium* for *Salmonella* spp. or *Clostridium sporogenes* for *Clostridium botulinum*) for in-plant control measures validation is recommended. One major issue when using this methodology is to assess the behavior of the pathogen in-situ with the data from surrogate experiments.

The objective of this symposium is to illustrate and discuss the use of probabilistic approaches to quantitatively assess the log reduction of the targeted pathogen with the use of surrogate microorganisms. Deterministic values, with conservative assumptions, have been traditionally used. However, the use of probabilistic approaches offers many advantages, such as encompassing the variability and uncertainty inherent to modelling biological phenomena. On one hand, uncertainty reflects not only the lack of information available, but also the approximation by a functional form of a real phenomenon.

On the other hand, variability refers to natural or non-controlled heterogeneity between individuals. This presentation will (i) present quantitative tools and data helpful for process validation and (ii) present practical examples of the use of such probabilistic approaches to predict pathogen behavior with the use of data from surrogate microorganisms.

### How to Validate in a Variable World: Use Data, Lots of Data, Both from Experiments and from Literature

**MARCEL ZWIETERING**, Wageningen University, Wageningen, Netherlands

For food safety we want to be absolutely sure! But absolute does not exist; food products, micro-organsims, and humans are biological entities showing large variability. And above that, technical process parameters, also, show variability. In order to validate processes in our variable world, many pieces of data are needed. Data sources include literature

data, databases, historical data, experimental data, storage tests, challenge tests, predictive models, basic knowledge, and logic. All these data sources have their weak and their strong points; and therefore, it is useful to draw on of all kinds of sources of data. On one side, due to the large variability in data it is more diffuse and difficult to judge; but on the other side, also more realistic, since in reality, variability does exist.

### Comparison of Thermal Resistance of *Clostridium botulinum* and Its Surrogate Strain *Clostridium sporogenes* PA 3679

**JEANNE-MARIE MEMBRÉ**, INRA, Oniris, Nantes, France

In a previous study, the thermal resistance of proteolytic *Clostridium botulinum* and its surrogate strain *Clostridium sporogenes* PA 3679 was compared by meta-analysis (Diao et al. 2014). A total of 911 D-values (time required to have a 10-fold decrease of the spore count) were collected from 38 scientific studies. It was shown that the heat resistance of proteolytic *C. botulinum* was significantly lower than the *C. sporogenes* PA 3679. For instance, at 121.1°C, the mean D121.1°C of *C. botulinum* was estimated to 0.19 min, while *C. sporogenes* mean D121.1°C was estimated to 1.28 min, in liquid media of neutral pH. Since data were collected over a range of heat-treatments from 100 to 145°C, z-values (temperature increase to have a 10-fold decrease of D-values) were also calculated.

Finally, *C. botulinum* strain variability and residual error (i.e. uncertainty) were quantified. The difference in thermal resistance of *C. botulinum* and its surrogate strain, *C. sporogenes*, makes challenge tests performed for food processing validation difficult to interpret. The objective of this presentation is to tackle this issue by showing how pathogen behaviour could be predicted from the surrogate strain by taking variability and uncertainty into account.

### Probabilistic Model for Process Control Measure Evaluation, a Practical Case

**LAURE PUJOL,** Novolyze, Daix, France

The objective of this study was to compare the thermal inactivation of *Salmonella* spp. and its associated surrogate microorganism, *Enterococcus faecium*, during the drying step of an extrusion process. The log reduction was calculated using two different methods: deterministic and probabilistic. The main stress factor evaluated was the effect of temperature on the two microorganisms.

Cat kibbles, independently inoculated with *E. faecium* and a cocktail of *Salmonella* strains, were heat-treated at three different temperatures, which allowed calculation of model parameters: D-values and z-values. The time/temperature profile of the drying process was evaluated and recorded for three different production batches. These time/temperature profiles, combined with the data obtained from the Thermal Death Time (TDT) study, allowed estimation of the log reduction in *Salmonella* spp. and *E. faecium* for the three batches, using both the deterministic and probabilistic models.

Finally, model estimations for *E. faecium* were compared with the results of an in-plant process challenge test where the surrogate microorganism had been used. The log reduction of *Salmonella* spp. was deduced from the log reduction of the surrogate, taking uncertainty into account. The deterministic approach has the advantage of rapidly being understood by non-model-users, but includes variability and uncertainty only through an empirical safety margin (generally retained as 1-log). The probabilistic method provides the probability of a process to achieve the targeted log reduction, including a quantitative evaluation of variability and uncertainty.

## S18 Innovative Nonthermal Technologies for Microbial Biofilm Decontamination on Biotic and Abiotic Surfaces

During the last decades, food safety became a major concern for producers, consumers, and governments. Food safety implies that food products need to be safe in a chemical, physical, and microbiological way. With respect to microbiological safety, the occurrence of foodborne pathogens in food products can be a real threat for human health. Contamination of food products can occur at different stages of the production process; e.g., during cultivation/breeding, harvesting/slaughtering, processing, and storage. In the processing plant, food contact surfaces are a primary area of concern as contamination sources.

Currently, disinfection processes involve rinsing with (hot) water and antimicrobial agents, together with a mechanical action. However, this has some important drawbacks. First of all, it has become clear that human pathogens, i.e., *Salmonella* spp., and *Listeria monocytogenes*, grow predominantly as biofilms on a variety of biotic and abiotic surfaces, rather than in planktonic forms or as colonies. Biofilm-associated cells exhibit higher resistance to antimicrobial agents due to different defence mechanisms. One of those mechanisms is the production of extracellular polymeric substances (EPS), forming a physical barrier that limits the diffusion of antimicrobial agents. Since most of the implemented disinfection processes are based upon their potential to inactivate planktonic cells, they can be highly ineffective when applied to inactivate biofilms. Secondly, the residues of antimicrobial agents can be toxic and alter the sensory values of food products.

Since the current inactivation methods are not efficient, new methods need to be investigated for the inactivation of biofilms grown on biotic and abiotic surfaces. Cold atmospheric plasma (CAP) and light at different intensities and wavelengths are nonthermal, emerging antimicrobial decontamination technologies. These technologies have a great potential for application in the food industry; for decontamination of surfaces and pieces of equipment, as well as for fresh vegetables and fruits, juices, and several processed foods.

### Experimental Design for the Assessment of the Anti-biofilm Effectiveness of Cold Atmospheric Plasma on Abiotic Surfaces

**JAN VAN IMPE**, KU Leuven/BioTeC, Ghent, Belgium

As a non-thermal decontamination technology, cold atmospheric plasma (CAP) offers great potential for treatment of heat-sensitive food products, as well as food contact surfaces for reducing energy consumption. CAP is created by applying a high voltage to a gas stream, resulting in microbial inactivation. CAP research is case specific, due to

its focus on specific target microorganisms, food products or surfaces. As the efficacy of CAP depends on multiple factors, most existing studies have a limited applicability. Additionally, little is known about the effect of CAP on biofilm inactivation. In this study, factors affecting the CAP efficiency on colonial and planktonic growth morphologies are assessed: CAP set-up, type of microorganism, sample (model system properties) and experimental protocol. By using (food) model systems, the influence of the properties of the understudy products is accurately investigated.

Considering all influencing factors, this work provides guidelines and critical points that need to be included to ensure successful treatment in the experimental design for studies with CAP. Additionally, taking into account the existing knowledge on the CAP efficiency for inactivation of colonies and planktonic cells, guidelines for effectual experimental design are provided for using CAP as a novel non-thermal technology for biofilm inactivation present on abiotic surfaces. Moreover, biofilm inactivation kinetics are provided and the results are compared with inactivation kinetics of *Listeria monocytogenes* and *Salmonella* Typhimurium in planktonic and colonial growth morphology.

Inactivation curves and estimation of inactivation parameters, allowing comparison, were obtained by fitting the model of Geeraerd et al. (2000) on the experimental data. The findings of this study provide promising results for using CAP as a novel non-thermal technology for biofilm inactivation formed on abiotic surfaces.

### Potential of Atmospheric Cold Plasma for Biofilm Control in Food Processing

**PAULA BOURKE**, Dublin Institute of Technology, Dublin, Ireland

The microbiological challenges presenting on foods and related contact surfaces include microbial spores and biofilms. Biofilms are three-dimensional communities of microorganisms attached to a surface, shielded inside an extracellular matrix that represent a common mode of microbial growth in industrial settings. Biofilms are problematic in a wide range of food industries. Biofilms formed on food contact surfaces are resistant to many disinfectants; particularly in biofilm formations comprised of mixed species rather than a single culture.

Spores are another common and highly resistant form of bacterial contamination, which can form part of a biofilm community. The elimination of these inherently resistant microbial forms is a major challenge. Cold plasma has rapidly evolved as a technology for biological applications, such as microbial decontamination, wound healing, and cancer treatment, owing to the chemical and bio-active radicals generated, known as reactive oxygen species (ROS) and reactive nitrogen species (RNS). The action of ROS/RNS can inactivate microorganisms present within a biofilm, degrade the biochemical structure of the biofilm EPS construct, inactivate virulence factors, and can interfere with quorum sensing. Reactive species generated in liquids subjected to plasma exposure act as mediators for reactions with biological or chemical targets. These solutions retain their activity after plasma exposure and are of interest as novel decontamination agents in their own right. Boehm et al. proved that plasma activated water had a higher potency than the corresponding $H_2O_2$ control in both mammalian and microbial systems. This suggests that other plasma generated species are also important for the observed effects. The potential for enhanced mediation of effect through water and the containable nature of plasma species provides opportunities to apply this technology, widely, in the food environment.

This presentation will outline some means of cold plasma decontamination, the key mechanisms of action, and interactions with biofilms pertinent to food safety and processing.

### Scale-up and Optimisation of Large Area Cold Plasma Systems for Rapid Microbial Decontamination

**JAMES WALSH**, University of Liverpool, Liverpool, United Kingdom

Atmospheric pressure plasma is an extremely potent antimicrobial agent due to the synergistic production of a wide variety of highly-oxidising chemical species, UV photons, and transient electric fields that are created directly at the point of need. Such plasmas offer a convenient, consumable-free, and environmentally friendly means of efficient and large area microbial decontamination. This contribution will provide an overview of the current state of the art in atmospheric pressure plasma technology for large scale microbial decontamination applications. Of all plasma systems currently under investigation, the Surface Barrier Discharge (SBD) has shown the greatest promise, as it operates in ambient air and can be scaled to cover many meters. Recent efforts at the University of Liverpool to develop a pilot scale plasma device that is suitable for the in-situ decontamination of food and food-processing equipment will be detailed.

## S19 How to Manage Microorganisms with Complex Life Cycles in the Food Industry

Sporeforming bacteria and fungi share the ability to form spores. These biological structures are ubiquitous and highly resistant to stresses. Despite the fact that the types of spores may differ in their ecological functions, spore formation offers original strategies for contaminating foods and to have new nutritional resources. Spore production and dispersion are crucial steps for the survival of these microorganisms. This symposium will discuss the common features and the main differences between these two types of microorganisms, which lead them to contaminate, to colonise, and to spoil food. Special attention will be paid to explaining how their complex cycle of life contributes to their survival in industrial food environments.

The session will also address the impact of environmental conditions on the germination efficiency of fungal spores. Some examples will be provided to illustrate why heterogeneity in the germination and subsequent growth of spores has to be taken into account to predict the responses of spoilage fungi in foodstuff.

In the last part of the symposium, the impact of industrial leverage on the cycle of life of *Bacillus* spp. will be presented. Indeed, if the cardinal values are commonly accepted to predict the vegetative bacterial growth, then new research may be able to use these values to predict the efficiency of the sporulation process, i.e. resistance of spores and yield of sporulation.

This symposium will deliver knowledge on sporeforming bacteria and fungi, in order to better understand the diversity and physiology of these microorganisms, which have complex life cycles; and in order to better manage their development in foods.

### Food Spoilage by Fungi or Sporeforming Bacteria: Common Features and Differences

**FRANK DEVLIEGHERE**, Ghent University, Ghent, Belgium

Microbial spores are omnipresent in nature and in a large portion of food products. Due to the demand for less and less intensively heat-treated food products, the role of microbial spores in food spoilage and food safety has significantly increased. In contrast to the demand, the amount of quantitative and qualitative information about the behavior of spores during food preservation processes is relatively scarce. In this talk, an overview will be given about the different types of microbial spores, i.e., bacterial spores, fungal conidiospores, and fungal ascospores. The sensitivity of the different types of spores towards food preservation stresses will be, generally, discussed. Special attention will be given to quantifying the combined effect of pasteurization and other stresses present in the stored food, such as pH and water activity. Finally, areas where we are still lacking data to allow us to predict the shelf life and safety of specific types of food products will be discussed.

### Germination and Growth of Spoilage Fungi

**MARIA GOUGOULI,** Perrotis College, American Farm School, Thessaloníki, Greece

Fungi are ubiquitous in nature and have evolved, over time, to colonize a wide range of ecosystems, including foods, due to their limited requirements for nutrients. Airborne transfer of fungal spores is now seen as a significant route for contamination in many sectors of the food industry. If growth is permitted by environmental conditions, the colonization of foods results in spoiled products (visible mycelium and/or off-flavor development) and subsequent, significant economic losses.

Within the domain of quantitative mycology, a series of research studies had been conducted on empirical descriptions of population kinetics (deterministic approaches), which did not take into account realistic events of contamination of foods with low numbers of fungal spores. However, in order to improve the predictive efficiency of the kinetic growth models, it is important to account for the heterogeneity that characterizes germination and growth kinetics of individual spores; since that would potentially provide important information with regard to the ability of a single spore to germinate, grow, and spoil a food product. Thus, studies which provide a quantitative description of the variability of single spore behavior, as affected by the environmental conditions, can be used as valuable tools for the prediction of the shelf life of foods susceptible to fungal spoilage or, additionally, they can constitute the basis for risk assessment of mould spoilage.

### Growth Limits and Their Uses to Predict the Cycle of Life of Sporeforming Bacteria

**EMILIE GAUVRY**, University of Brest – UMT 14.01 SPORE RISK, Brest, France

Sporeforming bacteria are responsible of food poisoning (37% in France, 2014) and involved in the spoilage of various processed foods. Bacterial spores are commonly found in the environment, making bacterial spores the natural contaminant of raw materials. The spore properties (heat resistance, germination ability) are impacted by the environment of their formation. During food processing, spores can encounter favourable conditions and then germinate. The new vegetative cell can grow, producing spoilage or toxic enzymes making food unfit for human consumption. Predictive microbiology has proved its efficiency to predict bacterial growth in foods with the growth limits (or cardinal values) according to the environmental factors like pH, temperature or water activity.

In this talk, we propose to go further by showing that the growth limits of spore-forming bacteria can also be used to predict the different steps of their cycle of life: growth, sporulation, resistance, germination, and outgrowth. For example, it was shown that the temperature boundaries of *Bacillus licheniformis* and *Bacillus weihenstephanensis* were similar for growth, recovery after a heat treatment and sporulation. The heat resistance of their spores was also tightly linked to the sporulation conditions for temperature and pH.

Another striking example concerns the behaviour of the thermophilic bacteria *Geobacillus stearothermophilus* (i.e., its growth, sporulation and recovery after a heat treatment) that could be explained by the growth limits for pH and temperature only.

## S20 Application of Bacteriophages as an Antimicrobial Intervention and Detection Strategy in the Food Industry

Bacteriophages are the natural enemy of bacteria that have evolved to specifically bind and infect their host cells and then rapidly replicate, leading to lysis of the bacterial cell. Also, bacteriophages are safe to use on food surfaces, supported by the fact that several commercial bacteriophage products received GRAS status from the FDA. These properties make them promising agents for both detecting bacteria in factories and food products (biosensors) and eradicating them from the food processing environment (biocontrol).

The purpose of this session is to discuss the possibilities and limitations of bacteriophage-based technology in biosensors and biocontrol in the food industry. Therefore, the first part of the session reviews the different applications that have been proposed for biocontrol, both pre- and post-harvest, including a consideration of the effects of the food matrix and the potential for the development of phage resistance. In addition, the pros and cons of routine application of phage-based detection methods in the food industry will be discussed. The second part of this session provides insight into the current commercial application of bacteriophages in food processing environments. Application data of bacteriophages on several types of food products is presented together with the challenges of transferring the technology from the laboratory to processing plants.

The last part of this session discusses endolysins, which are bacteriophage-encoded cell wall-lytic enzymes (peptidoglycan hydrolases) that have recently gained attention as potential antimicrobial agents. These enzymes rapidly and specifically kill foodborne pathogens, such as *Listeria monocytogenes* and *Staphylococcus aureus*, and are refractory to resistance. Proof of concept data for endolysin applications as biosensor and biocontrol, both in suspension and biofilm treatment, are presented.

All in all, this session presents an up-to-date view on bacteriophage technology, the newest antimicrobial intervention and detection strategy in food industry.

### Bacteriophage as a Food Safety Tool

**CATH REES**, The University of Nottingham, Nottingham, United Kingdom

Bacteriophage are viruses that have evolved to specifically bind and infect their host cells and then rapidly replicate, leading to cell lysis. These properties make them promising agents for both detecting bacteria in factories and food products (biosensors) and eradicating them from the food processing environment (biocontrol). Initially the development of biosensors was seen as the most promising application of phage, but in fact the most number of commercial products have been launched in the area of biocontrol.

In this presentation I will review the different applications that have been proposed for biocontrol, including a consideration of the effects of the food matrix and the potential for the development of phage resistance both pre- and post-slaughter. The commercial development of phage-based biosensors has been slower, but commercial products are now beginning to appear. Phage-based detection methods provide an advantage over methods that detect either DNA (e.g., PCR) or proteins (e.g., ELISA assays) alone, in that phage will only detect viable cells. This is important when analysing food or environmental samples, as the manufacturer does not want to detect cells that have been inactivated by either a processing or cleaning step.

In this talk, I will describe the two major approaches that have been taken to developing phage-based biosensors (GM and non-GM), and discuss the pros and cons of each route for routine application in the food industry, including the need for enrichments to reach the desired level of sensitivity (1 cell per 25 g food) and the problem of introducing GM reagents in to food microbiology labs for routine analysis. However, I will also show how new approaches have started to address these problems an there is now potential that phage can be employed to solve specific problems and improve food safety.

### Combating *Listeria* and *Salmonella* with Bacteriophages from Bench to Factory

**STEVEN HAGENS**, Micreos Food Safety B.V., Wageningen, Netherlands

Bacteriophages are a novel tool in food-safety. Not all phages are suitable for bio-control and criteria that need to be met will be discussed on the basis of two examples, a single phage product against *Listeria* and a two phage cocktail effective against *Salmonella*. Laboratory data demonstrating the efficacy of phages will be discussed. Food manufacturers need to comply with rules governing the presence of these pathogens in food and the possibilities of integrating phages into these frameworks will be shown. The issue of resistance will be addressed. It will be shown that phages cannot mask bad hygiene and that phage use certainly cannot replace hygiene in any way.

Lastly, the transition from laboratory bench and the challenges thereof will be discussed, from a simple application such as in smeared cheeses to treatment of composite foodstuffs. This will show both possibilities as well as limitations of using phages as bio-control agents in food manufacture.

### Bacteriophage Endolysins as Promising Tools for Detection and Control of Foodborne Pathogens

**MATHIAS SCHMELCHER**, ETH Zurich, Zurich, Switzerland

Endolyins are bacteriophage-encoded cell wall-lytic enzymes (peptidoglycan hydrolases) that have recently gained attention as potential antimicrobial agents. These enzymes rapidly and specifically kill Gram-positive bacteria and are refractory to resistance development. Endolysins feature a modular architecture, consisting of enzymatically active domains (EAD), which cleave certain bonds within the peptidogylcan and cell wall binding domains (CBD), which direct the enzyme to its cell wall substrate with high affinity and specificity. These properties make CBDs ideal tools for diagnostics. CBDs, from *Listeria* phages, fused to fluorescent proteins allowed simultaneous detection and differentiation of *Listeria* cells from different serovars in mixed bacterial cultures. Furthermore, *L. monocytogenes* cells could be recovered from contaminated food samples via paramagnetic beads coated with these high-affinity binding molecules. This CBD-based magnetic separation procedure was demonstrated to be superior to established, standard detection protocols in terms of sensitivity and time requirement.

Besides diagnostic applications, endolysins hold promise for control of foodborne pathogens. A collection of unique staphylococcal peptidoglycan hydrolases exhibited lytic activity against *S. aureus* planktonic cells and biofilms, as demonstrated in static and dynamic models. In addition to these parental enzymes, we have compiled a large library of engineered endolysin constructs (> 400 constructs) featuring versatile enzymatic and antimicrobial properties. Using a newly developed screening protocol, we identified enzymes with high activity in cow milk from this library. The most promising candidates eradicated *S. aureus* in milk, within minutes, at nanomolar concentrations, acted synergistically when applied in combination, and retained their activity in raw milk. Overall, our results demonstrate the high potential of bacteriophage endolysins as tools for detection and control of bacterial pathogens in food production.

## S21 Differentiate the Real Culprits from the Presumed Ones: How Emerging Technologies Improve a Typical Day's Work in Routine Testing Labs

New routine testing methods in food microbiology are currently emerging, combining the results of microbial sequence dissection and advanced technologies. These new methods are, currently, modifying daily work in routine labs, as well as the analysts' profiles, hopefully offering more serenity at the end of the day. Food microbiology is, most likely, at the forefront of a technological revolution. As always, the main goal is not only to reduce the time to results or the handling time; but also, to offer better specificity to enable easier differentiation of the real culprits from the presumed ones. Selecting the right method is often tricky, particularly when this impacts laboratory workflow.

This symposium will start with a reminder of the true goal in routine testing: short time to result and specific go/no go data, which can be easily used by decision makers in food industries and by food safety authorities. Illustrations will follow, showing

the expected unlocking adaptation of analytical testing under the so called “FoodOmics” evolutionary pressure, with a focus on two applications.

### How Do Genome Dissections Reveal the “Right” Identity of Strains?

**MARIE BUGAREL, ANNE BRISABOIS,** Texas Tech University, Lubbock, TX, USA

In the field of food safety, whole genome sequencing is becoming a modern, powerful tool used to identify, characterize, and differentiate foodborne pathogens at the deepest level. This presentation will illustrate the utilization of this new technology using two examples: (1) the discovery and characterization of a new serovar of *Salmonella*: *S. enterica* serovar Lubbock isolated from bovine lymph nodes and harboring interesting genetic features; and (2) the identification of pathogenic clones of *Listeria monocytogenes* that cause distinct diseases, as well as the virulence factors associated with each outcome.

### Food Industry Labs’ Expectations: Recent Advances and Open Challenges

**DAVID TOMAS FORNES**, Nestle, Lausanne, Switzerland

The challenge for the food industry is to conduct analytical testing as rapid and as close as possible to the site of sampling whilst maximizing the analytical efficiency and keeping the cost down. Innovative technologies allow reduction in the time to results, improve sensitivity, facilitate automation, and in some cases, simplify interpretation of the results. However, they are still complementary to cultural methods and must be aligned with international and local regulations.

Two basic aspects should be considered as the minimum baseline for method implementation in food industry: (1) Validation against reference method(s) following internationally recognized standards (e.g., ISO 16140). (2) Protocols that are fit-for-purpose, considering aspects like laboratory layout, method workflow and biosafety requirements. Examples of recent technologies for the detection and characterization of microorganisms in food industry will be highlighted. The link to cultural principles, such as sample preparation and isolation of microorganisms, will also be discussed.

### How Digital PCR Will Decrease the Number of False Positive Data in STEC Detection

**JEAN-FRANÇOIS MOUSCADET**, Bio-Rad, Marnes-la-Coquette, France

The last decade has seen many advances in the area of molecular diagnostics. Amplification methods such as PCR are now widely applied for detection of food pathogens. Yet, designing specific assays requires identification of unique molecular signatures, an often challenging task due to genetic relatedness. Recent advances in sequencing technology and genome comparison methods alleviated this problem as exemplified by the possibility to single out *Salmonella* serovars. However, for routine testing, molecular methods remain complicated when pathogen identification involves detecting several markers. This is the case for pathogenic STEC that are still defined by the concomitant presence of *stx* and *eae* virulence genes.

Although significant efforts were devoted to the search of a STEC unique signature, reference methods rely on co-detection of these genes. Positive PCR results for both genes obtained in one sample do not allow concluding that one bacterium displaying both markers is present rather than two nonpathogenic bacteria bearing one marker each. Such a result is, therefore, deemed presumptive positive; and, when both genes are present, the subsequent confirmation leads to a significant rate of positives. Development in digital PCR (dPCR) may solve this problem. With this method, a single genome can be probed and determinations as to whether markers are collinear or not can be made. Digital PCR may, therefore, be a straightforward method that detects, in one PCR run, the presence of a multi-marker pathogen and alleviates the need for hazardous confirmatory steps; thus, warranting its implementation in routine testing.

### Just Do It with a MALDI! Are Microbiologists Mutating into Chemists?

**DANIÈLE SOHIER**, Bruker Daltonics, Bremen, Germany

The challenges for food microbiology are as varied and numerous as the requirements for bacterial identification methods. One requirement, among many, is that food spoilers, foodborne pathogens, starter cultures, and probiotics must be accurately identified. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has revolutionized microbial identification, and is increasingly recognized as an established tool and alternative for identification of isolates in the complex food chain.

MALDI-TOF MS identification is based on the acquisition of a protein fingerprint of the microorganism. In contrast to conventional biochemical or molecular methods, same workflows can be used whatever the microorganisms. This facilitates the implementation into routine analysis. The measured MS profiles are compared to a database for identification. A critical factor, influencing the accuracy of the identification, is the quality of the database. Results are available within minutes. After a short summary of the principle and workflow, the use of this high-throughput technology in food microbiology will be illustrated with recent applications.

At the end of the 20th century, microbiologists underwent an initial transfer by becoming molecular biologists and producing genotypes. Are they now moving toward becoming chemists, by just doing it witha MALDI? It looks like this emerging technology improves workflow during a typical day in routine testing labs.

## CLOSING SESSION

### Food Safety in a Sustainable Production Chain

**WENDIE CLAEYS**, FASFC - SciCom, Brussels, Belgium

There are many different views as to what falls within the scope of “sustainability,” and what constitutes a “sustainable” food production chain. A sustainable food production chain encompasses all steps from farm to fork, and might be translated as ensuring sufficient and healthy food, at an acceptable price, that is not only determined by the total production cost, but also by all external social and environmental costs. It, thus, encompasses a range of issues, including security of supply, health, affordability, quality, a strong food industry in terms of jobs, and growth, as well as respect for natural resources, biodiversity, animal welfare, water-soil-air quality, etc.

When all this is taken into consideration, one must not lose sight of "food safety." A sustainable production chain, where food safety requirements need to be balanced with broader societal concerns, poses a challenge to regulators/legislators and food operators. Often a risk evaluation is required to obtain an acceptable solution for all parties concerned.

Based on an open dialogue between scientists, food operators, policy makers, surveyors, inspectors of the food chain, consumers, consultants, and representatives of NGOs, an inventory was made of possible bottlenecks or concerns arising when initiatives are taken to increase sustainability in the food chain. This inventory illustrates the area of tension between food security and a sustainable food chain. It offers, at the same time, a listing of potential opportunities and could be a useful information source for further actions that can be taken at various levels.

## Minimal Processed Products, Free of Additives, Safe, Tasty, and with Prolonged Shelf Life: The Holy Grail

**FRANK DEVLIEGHERE**, Ghent University, Ghent, Belgium

The increasing demand on European retail for food products that have a fresh-like image places important challenges before the food industry. To cope with these demands, their products should be produced with milder processing techniques and without the addition of food additives. At the same time, safety, as well as an acceptable shelf life with natural organoleptical properties, has to be guaranteed. Food scientists, therefore, are continuously looking for mild processes and new bio-active compounds and are exploring the boundaries of microbial development.

In this talk, different strategies to meet the above described demand, with their advantages and disadvantages, will be given. Several examples regarding processing of fresh fruit products will be discussed and an example of a quantitative microbial exposure assessment approach to identify the key steps in guaranteeing the microbial safety of mildly heat-treated food products, more specifically for *Bacillus cereus* in REFPEDS, will be presented.

## Recent European Commission Initiatives in Food Hygiene and Microbiological Food Safety

**KRIS DE SMET**, European Commission, Brussels, Belgium

The Health and Food Safety Directorate General (SANTE) of the European Commission is the food safety management body, laying down all food safety requirements, directly applicable in all Member States of the EU and to all imports into the EU. These EU rules are aimed at equally protecting all EU consumers, in Member States, at a high level. They also ensure fair competition and trade by requiring the same standards for all food business operators. When laying down requirements, a risk analysis approach is used with the involvement of European risk assessment bodies, such as the European Food Safety Authority. Information on public health is provided by the European Centre for Disease Prevention and Control. The practical collaboration between these European institutes and with Member States and Stakeholders will be explained.

Apart from proposing and managing requirements for food business operators, SANTE provides itself guidance on implementation, encourages and evaluates sectorial guidance developed by stakeholders' organisations, and sets up a harmonised framework for controls by competent authorities. Part of these controls is the network of European and national reference laboratories, which ensures the high quality and reliability of laboratory testing in the EU. SANTE is, also, focusing on preparedness and management of foodborne outbreaks with the development of new tools and procedures. The purpose is to protect the consumer and to prevent or minimise the economic impact of outbreaks. Concrete, recent initiatives in the area of food hygiene and microbiological food safety will be provided during the presentation to illustrate the European food safety policy approach.

## U.S. FDA Draft Guidance for Industry on Control of *Listeria monocytogenes* in Ready-to-Eat Foods

**MICKEY PARISH**, U.S. Food and Drug Administration, College Park, MD, USA

On January 17, 2017, the United States Food and Drug Administration published a draft guidance document regarding control of *Listeria monocytogenes* in ready-to-eat (RTE) foods. This new draft replaces the previous draft guidance published in 2008. In the new draft document, FDA maintains the current "non-detect" standard for all RTE foods, but provides new incentives for industry to adopt robust environmental monitoring and corrective action procedures that provide control of listeriae in food processing and manufacturing facilities.

This presentation will describe new incentives and will provide the rationale for changes made in the new document. Comments to the new draft document are encouraged and can be made to the official docket through July 17, 2017.

# ROUNDTABLE ABSTRACTS

## RT1 Putting the Limelight on Non-pathogenic Microbes

Whilst there is a lot of attention and debate devoted to the societal burden that pathogenic microbes pose in terms of food safety, the problems associated with non-pathogens are far from well regognized. This situation is not desirable, since non-pathogens are responsible for a major economic burden and may be a bottleneck to solving current and future issues associated to food security and waste. Notably, there is not always consistency in differentiating pathogens from non-pathogens, whilst the regulatory approaches to the management of non-pathogens is even less harmonized than that of pathogens, and at times the priority balance between the importance of food security and of food safety is difficult to make. This session will provide a platform to debate the underlight position of non-pathogens, consider potential undersirable consequences thereof and reflect on directions towards resolution.

Panelists:

ROY BETTS, Campden BRI, United Kingdom

CHRISTOPHE DUFOUR, Merieux NutriSciences, Cergy Pontoise Cedex, France

JAN DIJKSTERHUIS, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

PETER MCCLURE, Mondelez International, Birmingham, United Kingdom

ELENI PANTIORA, UN World Food Programme, Addis Ababa, Ethiopia

## RT2 Globalisation Challenges in Food Safety Management – Emerging Issues in Culture, Systems and Practice

Global trade challenges food safety and integrity. Modern food businesses deal with complex food supply chains, often with some elements of uncertainty of origin when sourcing globally, and frequently with multiple legal frameworks and private standards. People and communication within and between businesses focus our attention on how different organisational and national cultural backgrounds impact food safety management in the global food supply chain.

In order to assure food safety and integrity, the reaction of public and private standard makers tends toward increasing QA requirements, making their standards and requirements more stringent. This impacts food businesses, requiring a heavy focus on system design and compliance, even when many stakeholders are calling for risk-based approaches.

Recently there has been much discussion of culture and people factors and how these impact food safety management system effectiveness. This links back to global trade issues and the pressures of dealing with multiple cultures in multiple countries within the global marketplace. Cultures also exist within different hierarchical levels of food companies and this suggests questions about how to deal with all of these cultures – clearly there is not one approach that fits all.

This complex picture requires businesses to operate their food safety management systems with consistency and adaptability and raises important questions for us all. Using a panel of expert speakers from industry and research domains, this roundtable will aim to answer the following:

- How do we get everyone working in the same direction and to the same food safety and integrity goals?
- What lessons can we learn from recent scientific research on food safety culture?
- How can food companies adapt to their dynamic environments and inherent food safety risks?
- How can we build positive food safety cultures that integrate effectively with our food safety management systems to deliver product safety and integrity?

Panelists:

OLIVIER GALARD, Barry-Callebaut, Wieze, Belgium

LIESBETH JACXSENS, Ghent University, Ghent Belgium

LONE JESPERSEN, Cultivate, Hauterive, Switzerland

IAFP'S EUROPEAN
SYMPOSIUM ON FOOD SAFETY
29-31 MARCH 2017
BRUSSELS, BELGIUM

# TECHNICAL ABSTRACTS

29–31 March 2017 – Brussels, Belgium

# TECHNICAL ABSTRACTS

***Denotes Developing Scientist Awards Competition**

## T1 Technical Session 1 – Intervention Strategies

**Wednesday, 29 March –11.00 – 12.30**

### T1-01* Cold Plasma Treatment for the Inactivation of *Salmonella* Enteritidis PT 30 on the Surface of Unpeeled Almonds

**Christian Hertwig**[1], Kai Reineke[1], Nicolas Meneses[2] and Oliver Schlüter[1], [1]Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany, [2]Buehler AG, Uzwil, Switzerland

***Introduction:*** The contamination of nut products with human pathogens is a reoccurring concern in food industry. Common decontamination technologies, like oil roasting or steam processes, can alter the color and flavor of nuts. Cold plasma enables a microbial inactivation at moderate conditions.

***Purpose:*** In this study, the inactivation of the 2001 outbreak strain *Salmonella* Enteritidis PT 30 (ATCC BAA-1045) on the surface of unpeeled almonds by cold plasma was investigated.

***Methods:*** Inoculum preparation and inoculation of almonds was done according to the guidelines recommended by the Almond Board of California. For plasma treatment, a diffuse coplanar surface barrier discharge (DCSBD) plasma system was used. Almond samples (15g) were inoculated in a treatment chamber with a static gas atmosphere between two DSCBD plates. The chamber was flushed with the process gas (air, $N_2$, $O_2$) and the almonds were treated up to 15 min. Generated plasma was characterized using optical emission spectroscopy, by the quantification of ozone, and by temperature measurement in the plasma. After treatment, the almond color was measured.

***Results:*** Plasma treatment successfully inactivated *S.* Enteritidis. The inactivation and, also, the color of the almonds were affected by the process gas. Air-plasma emitted different reactive oxygen and nitrogen species and showed the highest inactivation, with up to 6 $\log_{10}$ after 15 min treatment with a peak treatment temperature of 69°C. The $O_2$-plasma had a peak temperature of 71°C and generated the highest ozone concentration of about 20,000 ppm in the treatment chamber, which might be responsible for the achieved 5 $\log_{10}$ inactivation. The lowest inactivation of 2 $\log_{10}$ was obtained using $N_2$, whereby $N_2$-plasma emitted the amount of UV-C photons with a peak temperature of 72°C. Plasma treatment with air and $N_2$ resulted in a browning of the almonds.

***Significance:*** The results showed that plasma treatment can effectively inactivate *S.* Enteritidis PT 30 on the surface of almonds, while maintaining the color.

### T1-02* The Impact of Drying on Foodborne Pathogens *Salmonella enterica* and *Cronobacter sakazakii*

**Emilie Lang**[1], Stéphane Guyot[1], Pablo Alvarez-Martin[2], Jean-Marie Perrier-Cornet[1] and Patrick Gervais[1], [1]Unité Mixte de Recherche - Procédés Alimentaires et Microbiologiques (UMR PAM), France, Dijon, France, [2]Novolyze, Dijon, France

***Introduction:*** *Salmonella enterica* and *Cronobacter sakazakii* are foodborne pathogens responsible for severe infant illness. Their ability to survive in harsh environmental conditions make these species a matter of concern for the low moisture food industry.

***Purpose:*** This study aimed to evaluate and understand the impact of drying conditions on survival, physiology, and invasion capacity of *Salmonella* Typhimurium and *C. sakazakii*.

***Methods:*** *Salmonella* Typhimurium and *C. sakazakii* were mixed into whole milk powder and dried at different water activity ($a_w$) levels (0.25, 0.58 and 0.80). For each strain, the impact of each drying condition was evaluated by estimating the loss of cultivability, membrane permeabilization, and the loss of a respiratory enzymatic activity by flow cytometry. The invasion capacity in Caco-2 cells was, also, evaluated after drying for each bacterium by the gentamicin test.

***Results:*** Our results showed that intermediary initial drying kinetics increased bacterial inactivation. No significant differences were observed between bacterial cultivability at $a_w$ 0.25 and 0.58. Nevertheless, the bacterial cultivability was significantly higher ($P < 0.05$) at $a_w$ 0.80 than at 0.25 or 0.58. An increase in percentages of uncultivable cells correlated with percentages of permeabilized cells. Furthermore, our results showed that drying (at 0.80, 0.58, and 0.25) significantly increased ($P < 0.05$) the invasion capacity of *S.* Typhimurium and *C. sakazakii*.

***Significance:*** These results indicate that drying parameters have a high impact on *S. enterica* and *C. sakazakii* and could be managed to promote foodborne pathogen inactivation. Drying could, also, be improved to avoid virulence pathway activation and ensure the safety of dried food products.

### T1-03 Evaluation of the Hygienic Design of an Industrial Device for Drying Food Using Supercritical Fluids

**Ilija Djekic**[1], Simeon Bourdoux[2], Cynthia Akkermans[3], Gerard Hofland[3], Frank Devlieghere[2], Nikola Tomic[4] and Andreja Rajkovic[2], [1]University of Belgrade-Faculty of Agriculture, Belgrade, Serbia, [2]Ghent University, Ghent, Belgium, [3]Feyecon, Weesp, Netherlands, [4]University of Belgrade – Faculty of Agriculture, Belgrade – Zemun, Serbia

***Introduction:*** Poor hygienic design may cause difficulties in both cleaning and maintenance of food processing equipment affecting food safety and

food quality. This problem is underestimated in food industry, especially during the design period. There is still little awareness of the possible consequences when equipment is not hygienically designed. Last, but not least, hygienic design of equipment with supercritical fluids is not the focus of many researchers.

***Purpose:*** The purpose of this study was to develop a tool that enables quantification of fulfillment of hygienic design criteria during the prototype development of an industrial device for drying food using supercritical fluids.

***Methods:*** The prototype of an industrial device for drying food using supercritical fluids was evaluated against 50 requirements, based on published hygienic design, scientific manuscripts, and standards. The requirements have been deployed in three dimensions: types of materials and their compatibility in food industry; hygienic designs and construction of the new industrial device; and functional requirements for the new food processing equipment. Finally, a hygienic design index was developed as a scoring method, covering three possible outcomes for each of the 50 requirements: satisfactory and minor and major nonconformity.

***Results:*** In this phase of the project, the overall hygienic design index for the prototype was 46%. For the group of requirements covering types of materials and their compatibility, the prototype achieved 40%. Within the second group comprising hygienic designs and construction requirements, a scored of 60% was achieved. The score for the last group, covering functional requirements, reached 35%.

***Significance:*** Results confirmed that development of specific hygienic design criteria for new food processing equipment may aid during the design phase, as well as during future development of similar drying technologies based on supercritical fluids. This type of tool may, also, help in evolving technology readiness levels.

## T1-04 Reduced Contamination of Pork Carcasses with Hygiene Indicator Bacteria, ESBL/AmpC-producing *Escherichia coli*, *Salmonella* spp. and *Yersinia enterocolitica* by Alternative Removal of the Pluck Set during Slaughter

**Wauter Biasino**, Lieven De Zutter, Tanuja K.G.M. Gowda and Inge Van Damme, Ghent University, Merelbeke, Belgium

***Introduction:*** Pigs are well-known, asymptomatic carriers of pathogenic and antibiotic-resistant bacteria, which may contaminate pork carcasses during slaughter. In particular, opening the oral cavity during the removal of the pluck set (i.e., lungs, heart, liver, and tongue) is a potential risk for spreading bacteria over the pork carcass.

***Purpose:*** This research aimed to compare carcass contamination between pigs from which the pluck set was removed following standard procedures and pigs froom which the pluck set was, alternatively, removed (leaving the tongue and highly contaminated tonsils inside the unopened oral cavity).

***Methods:*** In two Belgian slaughterhouses, 20 carcasses (10 slaughtered normally and 10 using the alternative method) from 12 pig batches, were sampled, after removal of the pluck, by swabbing (100 cm²) the elbow, throat, and sternum. Samples were analyzed using direct plating to quantify the total aerobic count, *Enterobacteriaceae* and *Escherichia coli,* and to determine the presence of ESBL/AmpC-producing *E. coli*, *Salmonella* spp., and *Yersinia enterocolitica*.

***Results:*** Average total aerobic counts for throat samples ranged, between batches, from 2.1 to 3.8 $\log_{10}$ CFU/cm² , with mean reductions up to 0.6 $\log_{10}$ CFU/cm² when using the alternative method compared to standard procedures. Median throat *Enterobacteriaceae* and *E. coli* numbers varied, between batches, from 0.6 to 2.8 $\log_{10}$ CFU/cm² and 0.4 to 2.3 $\log_{10}$ CFU/cm², respectively, with maximal mean reductions of 1.0 $\log_{10}$ when applying the alternative method. The proportions of *Salmonella* spp. and *Y. enterocolitica* positive throat samples were equal for both slaughtering methods and pathogens (1.7%). The presence of ESBL/AmpC-producing *E. coli* in throat samples ranged from 3–16%, with up to three-fold reductions for the alternative method. Similar results were seen for other carcass areas.

***Significance:*** This alternative slaughter method requires only minimal adaptations in the slaughter-house, but improved the microbial quality and safety of pork carcasses.

## T1-05 The Glutamate Decarboxylase System in Bacterial Food Pathogens and Its Inhibition by Dicarboxylic Acids

**Ruth Barnes** and Kimon Andreas Karatzas, University of Reading, Reading, United Kingdom

***Introduction:*** The glutamate decarboxylase system (GAD) is the major mechanism for acid resistance in a number of key bacterial pathogens.

***Purpose:*** This research was conducted to gain a better understanding of the GAD system, which would allow more effective control of pathogens. By interfering with the GAD system, it may be possible to decrease acid resistance in several food pathogens, which would allow a more targeted approach to the reduction of specific foodborne pathogens.

***Methods:*** A number of dicarboxylic acids and their salts were tested for their antimicrobial properties, in terms of survival, with two organisms *Escherichia coli* K12 and *Listeria monocytogenes* 10403S. In addition, mutants of the GAD system were used to assess the role of selected acids on the GAD system under acidic conditions. The effectiveness of the acids was determined by examining levels of gamma aminobutyric acid (GABA), the byproduct of the GAD system, using enzymatic assay methods in combination with gas chromatography.

***Results:*** Of the compounds tested, sodium fumarate (SF), was shown to be the most active. Under acid conditions, its presence resulted in *L. monocytogenes* reductions up to four logs (8.6 mM). For *E. coli*, FS achieved a reduction of up to two logs (10 mM). Analysis of GAD system byproducts indicated that the presence of SF had a significant impact on the GAD system. A significant difference was noted in the extracellular levels of GABA produced by both organisms. A significant decrease in GABA was found in *E. coli* and a significant increase in *L. monocytogenes* (paired student *t*-test; *P*-value $< 0.05$).

***Significance:*** Developing more targeted methods for disrupting the acid protection mechanisms in foodborne organisms could result in their efficient elimination from food.

## T1-06* Reverting Multidrug-resistant Phenotypes of *Escherichia coli* Isolated from Cattle Using 1-(1-Naphthylmethyl)-Piperazine

**João Anes**, Séamus Fanning, Daniel Hurley and Shabarinath Srikumar, University College Dublin, Dublin, Ireland

***Introduction:*** The extensive use of antimicrobial agents, in both the health and food sectors, has led to the emergence of multidrug resistant (MDR) bacteria; a development of importance to public health. Efflux pumps extrude antimicrobial compounds from cells contributing to the development of resistance. Chemosensitisers, with the capacity to modulate efflux pump activity, are being studied as adjuvants, in efforts to reverse resistant phenotypes. However, little is known about their efficacy and mechanism of action.

***Purpose:*** The purpose of this study was to systematically analyse the MDR reversal activity of the chemosensitiser 1-(1-naphthylmethyl)-piperazine (NMP) when applied as an adjuvant with antibiotics onto both planktonic and sessile *Escherichia coli* isolates.

***Methods:*** Bovine *E. coli* isolates from the UCD Veterinary Hospital, were screened for their MDR phenotype. A panel of 12 isolates, resistant to different classes of antibiotics including fluoroquinolones, tetracyclines, and chloramphenicol, were studied. All isolates were characterised by whole genome sequencing. The ability to form biofilms and fimbriae was, also, determined. Minimum inhibitory concentration (MIC) for each antibiotic, alone or in combination with NMP at sub-MIC levels, was determined by broth microdilution using planktonic and sessile-grown cells. Transmission electron microscopy (TEM), using NMP was performed.

***Results:*** Isolates had diverse antimicrobial resistance (AMR) and virulence gene profiles. Based on these data the *gsp* operon appeared to be associated with strong biofilm formers. In planktonic cells, using NMP as adjuvant, the MIC of ciprofloxacin, chloramphenicol and tetracycline exhibited a 2-, 6- and 10-fold reduction in comparison to the antibiotic, alone. In the case of sessile cells, half showed reductions in biofilm biomass when tetracycline was combined with NMP. TEM imaging demonstrated cell wall damage with NMP.

***Significance:*** These findings showed that NMP damaged the cell walls, thus, increasing drug permeabilisation. The use of NMP and NMP-like structures has the potential to reverse MDR in bacteria.

# T2 Technical Session 2 – Contamination Source Tracking, Epidemiology and Regulation

**Wednesday, 29 March –14.00 – 15.30**

## T2-01 Whole-genome Comparisons of *Listeria monocytogenes* Isolates: A Two-step Analysis Combining Whole-genome Multilocus Sequence Typing (wgMLST) and Whole-genome Single-Nucleotide Polymorphism (wgSNP)

Katleen Vranckx, **Katrien De Bruyne** and Hannes Pouseele, Applied Maths NV, Sint-Martens-Latem, Belgium

***Introduction:*** *Listeria monocytogenes* (Lmo), although an uncommon cause of illness, is an important pathogen in pregnant patients, neonates, elderly individuals, and immunocompromised individuals. Following considerable costs reductions, Lmo genome sequencing dramatically increased the number of publically available genomes on NCBI/SRA. The key challenge is to rapidly compute and interpret the relevant information from this vast amount of data. Rapid and automated processing of WGS data ensures a reliable and easy to follow workflow in routine molecular surveillance, reducing the time to detect and contain an outbreak. WGS has the potential to provide information on traditional typing technologies such as MLST, virulence, and resistance determination.

***Purpose:*** This study compared two subsequent pipelines for high resolution WGS-based molecular typing.

***Methods:*** First, whole genome multilocus sequence typing (wgMLST) was applied to WGS data from all 10,000+ isolates available on NCBI, with the purpose to detect clusters of highly related strains. Clusters, defined by wgMLST, were then characterized by whole genome single-nucleotide polymorphism (wgSNP) analysis. SNP variants detected by mapping the WGS reads to a reference chosen from within the cluster were used to maximize the resolution. As working examples, we identified clusters containing isolates originating from different food sources. Both analysis pipelines were run on the BioNumerics® Calculation Engine, which is fully integrated with the BioNumerics®7.6 software.

***Results:*** We demonstrated that wgMLST was suitable for the analysis of very large (growing) datasets; making it a suitable technique for outbreak surveillance. The added resolution of wgSNP against an internal reference sequence increased the confidence in the detected clusters. This supports epidemiologists in their source tracking efforts, opening many perspectives for cost efficient food safety and public health monitoring programs.

***Significance:*** BioNumerics® 7.6 software offers a powerful platform where both wgMLST and wgSNP analysis can be performed at high-throughput rates, and in combination with traditional typing data (MLST, PFGE, etc.) to rapidly provide a robust, portable, and high resolution picture of molecular typing data.

## T2-02 Practical Application of Whole-genome Sequencing for *Listeria monocytogenes* Source Tracking in the Food Industry

**Katia Rouzeau-Szynalski**[1], Caroline Barretto[1], Catherine Ngom-Bru[1], Coralie Fournier[2], Johan Gimonet[1] and Leen Baert[1], [1]Nestec Ltd- Nestle Research Center, Lausanne, Switzerland, [2]Nestle Institute of Health Sciences SA, Lausanne, Switzerland

***Introduction:*** The utility of whole genome sequencing (WGS) for source tracking was assessed by investigating an environmental factory contamination.

***Purpose:*** The study was conducted in order to analyze 41 *Listeria monocytogenes* factory isolates by WGS.

***Methods:*** DNA was extracted and sequenced on an Illumina MiSeq platform. In parallel, selected isolates were sequenced with PacBio to create reference genomes. Single Nucleotide Polymorphism (SNP) calling, based on raw read mapping on a closely

related reference, using the CFSAN-FDA pipeline, was performed to obtain the pairwise SNP distance matrix. The closest public available sequence data was included in the analysis to understand the biological context.

***Results:*** From the 41 isolates, 30 isolates were grouped in 4 clusters (< 20 SNPs within one cluster) and 11 isolates could not be grouped to any of the defined clusters. One cluster consisted of 22 isolates where the other 3 clusters consisted of 2 to 3 isolates. The largest cluster consisted of isolates from and around a drain in one area of the factory. The drain was suspected of being the source of contamination by the factory quality team. The WGS results confirmed the suspicion of a drain as a source of contamination. Within this cluster, three isolates were found in other places in the factory, identifying a cross-contamination from the drain. The results showed that no raw material was linked to the drain contamination.

***Significance:*** WGS was able to confirm the cause of contamination in the factory, which was suspected to be due to a "resident" strain with no links to recent raw materials. The application of WGS for source tracking showed to be promising; although, today, it is still a research tool, since bioinformatics pipelines evolve very quickly and the knowledge of the biological interpretation from SNP differences, in function of time and place, between isolates is scarce.

## T2-03 The Global Importance of a Publically Available, Genomic Database for Environmental and Food Isolates

**Eric Stevens**, FDA/CFSAN/ORS/DM, College Park, MD

***Introduction*:** In 2012, the United States Food and Drug Administration (FDA) launched GenomeTrakr, a pilot project that aimed to use whole-genome sequence (WGS) technology to respond to foodborne disease outbreaks. This freely available repository is currently supported by a network of about 60 federal, state, international, and public health laboratories, which collect and share WGS data in real time.

***Purpose:*** The GenomeTrakr project provides an example for how public health agencies can use genomic data (with its associated metadata) alongside traditional epidemiologic methods to resolve foodborne outbreaks. As the cost of WGS decreases, its emerging rollout among foodborne disease surveillance systems emphasizes its increasing importance as a public health tool.

***Methods:*** The GenomeTrakr network continued to expand in 2016 by adding new labs and over 50,000 foodborne isolates. The data analysis pipeline implemented within GenomeTrakr, and our partners at NCBI, allowed us to compare and cluster pathogens across four public surveillance efforts: *Salmonella enterica*, *Listeria monocytogenes*, *E. coli*, and *Campylobacter*. The network also provides daily phylogenetic updates that are both freely and publically available to allow greater transparency between public health agencies, our industry partners, academia, and international partners.

***Results:*** WGS has fundamentally altered the way we approach and respond to foodborne diseases by combining multiple microbiological tests into one (e.g., organism identification, antimicrobial resistance, subtyping). Here we provide two examples for how WGS can provide critical insight into resolving both domestic and global foodborne outbreaks.

***Significance:*** GenomeTrakr serves as a resource for generating possible matches to inform outbreak investigations within the United States and this function only becomes more pronounced as the database grows. These genomic sequences – and the associated metadata (e.g., year of collection, geographic location, food source) – need to be made available for its successful use as a global public health resource to make food safer globally.

## T2-04 FSMA (USA) Versus Hygiene Package (EU): Differences and Opportunities

**Claudio Gallottini**[1], Franco Rapetti[2] and Sara Trombetti[3], [1]ITA Corporation, Miami, FL, [2]ESI - Euroservizi Impresa Srl, Torgiano, Italy, [3]CISRAD Srls, Roma, Italy

***Introduction:*** The EU REG. CE N. 178/2002 and US FSMA are two rules that have revolutionized food safety in Europe and America. In Europe, 15 years after the publication of the Hygiene Package, many small businesses are struggling to adapt to hygienic standards-based preventive procedures. As a result, the member states, characterized by SME, are projected towards forms of simplified application of HACCP. What will happen in the U.S.? Currently, the Food Safety Plan and the HARPC, seem to require management-rich procedures that are very redundant, but necessary to ensure the chain of accountability.

***Purpose:*** We proposed to analyze the differences between the two regulations and their implementation by analyzing the path that a manufacturing company must follow to comply with the respective regulations in the EU and U.S.

***Methods:*** Data was gathered from 180 Italian manufacturing companies, subject to European legislation, and registered with FDA for export to the U.S., which by June 2016 to December 2016, had qualified a PCQI for the design of their Food Safety Plan according to PCHF.

***Results:*** Different approaches in the design of HACCP (per phase in EU, for single ingredient in U.S.), different concept of critical limits (points in the EU, average values in the U.S.), introduction of the "correction," zoning in sanitation procedures, weekly frequencies document review, and introduction of the concept of "food safety culture" on FSMA, are some of the major differences found.

***Significance:*** Not only American companies but, also, European companies will confront a great challenge. We illustrate our solution and our consultancy approach for making this integration for European SME possible.

## T2-05* Third-party Auditors in the Food Protection System: Comparing the Role of Third-party Auditors to Government Regulatory Agents

**Elizabeth Driscoll**, Ryerson University, Toronto, ON, Canada

***Introduction:*** Food safety is an important and well-recognized component of public health. To promote the health of its citizens, governments have implemented food protection systems in the form of food safety legislation, which is enforced by regulatory agents who are recognized public health professionals. In comparison, private regulatory systems, such as the Global Food Safety Initiative, use a different regulatory agent, the third-party auditor,

to ensure compliance with the private standard. As a result, these auditors are directly involved in the food protection system, but their role in public health systems has not, yet, been investigated.

***Purpose:*** This study investigates the role of the third-party auditor in the public health system to determine if they can be considered analogous to a recognized public health professional, the government regulatory agent, commonly known as an inspector.

***Methods:*** A literature review and document analysis was conducted to compare the role of third-party auditors to government inspectors. Areas of comparison include auditor/regulatory agent education and training requirements, enforcement activities, interactions with policy, level of discretion, and place in the food protection system.

***Results:*** Based on their role characteristics and food protection activities, the third-party auditor has an important, but largely unrecognized, role in the public health system.

***Significance:*** Given the globalized food supply, as well as the prominence of private regulatory systems, third-party auditors have the potential to dramatically affect public health through their assessment of the food safety management systems of the facilities they visit. Understanding their role is important in ensuring the proper functioning of the food protection system and the general safety of the food supply.

## T3 Technical Session 3 – Laboratory and Detection Methods

**Wednesday, 29 March –16.00 – 17.30**

### T3-01 Enabling Accurate Measurements of Staphylococcal Enterotoxin A (SEA) in Food by Use of a Comprehensively Characterised Calibrant Solution

**Reinhard Zeleny**[1], Sébastien Boulo[1], Amalia Muñoz[1], Heinz Schimmel[1], Dominique Baiwir[2], Gabriel Mazzucchelli[2], Isabelle Mutel[3] and Yacine Nia[4], [1]European Commission, Geel, Belgium, [2]University of Liège, Liège, Belgium, [3]Université Paris-Est, ANSES, Laboratory for Food Safety, Maisons-Alfort, France, [4]Université Paris-Est, ANSES, Maisons-Alfort, France

***Introduction:*** Staphylococcal enterotoxins account for a substantial number of food poisoning outbreaks in the EU and elsewhere. Besides the current methods, which are mainly focussed on presence/absence testing (toxin detected/not-detected), more and more quantitative methods are being developed (e.g., immunochemical assays, mass spectrometry-based methods). For accurate quantification of toxin levels in complex food matrices, however, suitable calibration solutions are required.

***Purpose:*** The scope of the study was an in-depth purity and identity characterisation of commercially available Staphylococcal enterotoxin A (SEA) and the accurate determination of the protein concentration of a solution prepared from the lyophilised toxin, with the aim to produce a suitable calibration solution for quantitative SEA measurements.

***Methods:*** The set of methods applied to assess the purity and identity of the toxin comprised of SDS-PAGE (initial mass determination), Q-TOF (accurate mass determination), multi-enzyme LC-MS/MS (sequencing of the protein for identity), and ELISA (cross-reactivity check and indicative concentration). The prepared SEA stock solution was value assigned for its protein concentration by amino acid analysis (AAA), using an in-house isotope dilution LC-MS/MS method.

***Results:*** SDS-PAGE indicated one major band, confirming the >95% purity statement issued by the material provider. Sequencing confirmed the published SEA sequence, and ELISA for Staphylococcal enterotoxins A-D confirmed the absence of those toxins in the preparation. However, a strong cross-reaction was obtained with a SEE-specific ELISA (sequence similarity of SEA and SEE). AAA provided a reliable means to establish the protein concentration of the SEA solution. Moreover, AAA measurements spread over a period of 3 years confirmed excellent sample stability for a storage at -20°C.

***Significance:*** The comprehensively characterised and thoroughly value-assigned SEA solution provides the basis for accurate quantitative SEA analyses in complex food matrices; and thus, will contribute to establish and maintain reliable measurement results for effective consumer protection.

### T3-02* Development and Application of Peptide Nucleic Acid Fluorescence *In Situ* Hybridization for the Specific Detection of *Listeria monocytogenes*

**Rui Rocha**[1], José Mário Sousa[2], Laura Cerqueira[2], Maria João Vieira[3], Carina Almeida[3] and Nuno Filipe Azevedo[1], [1]LEPABE, Department of Chemical Engineering, Faculty of Engineering of the University of Porto, Porto, Portugal, [2]BIOMODE - Biomolecular Determination S.A., Braga, Portugal, [3]CEB - Centre of Biological Engineering, LIBRO - Laboratory of Research in Biofilms Rosário Oliveira, University of Minho, Braga, Portugal

***Introduction:*** *Listeria monocytogenes* is one of the most important foodborne pathogens, with high hospitalization and mortality rates. Molecular methods that accelerate identification of *L. monocytogene* are continuously being developed; however, conventional culture-based methods remain the gold standard.

***Purpose:*** This study aimed to develop a Peptide Nucleic Acid Fluorescence *In Situ* Hybridization (PNA-FISH) method for the detection of *L. monocytogenes* in food matrices.

***Methods:*** Available, online rRNA databases were analyzed for probe design. The selected probe was synthetized and tested using 67 representative strains, for determination of sensitivity and specificity. Detection in food matrices was optimized, testing rich and selective broths, individually and in combination. A validation assay was performed in ground beef, ground pork, milk, lettuce, and cooked shrimp using two ranges of contamination; low (0.2 – 2 CFU/25 g or mL) and high level (2 – 10 CFU/25 g or mL). Samples were analyzed by PNA-FISH and ISO 11290.

***Results:*** The designed probe, when coupled with a blocker probe (1:2 ratio), was highly sensitive and specific; 100% (88.6 – 100, 95% CI) and 93.1% (75,8 – 98.8, 95% CI) respectively. None of the tested universal and selective broths were able to increase *L. monocytogenes* to detectable levels by PNA-FISH at 24h, with the exception of One Broth *Listeria* (OBL) and University of Vermont Medium (UVM); however, the fluorescence signal obtained for those enrichments was weak. For the double enrichment

steps, the best outcome was reached using OBL for 24h followed by OBL for 18h, even at low inoculum levels. This combined step was able to successfully detect *L. monocytogenes* in a variety of food matrices, with a detection limit of 0.5 CFU/25g (0.2 – 0.8, 95% CI), which was in line with ISO 11290.

***Significance:*** The procedure, here described, is specific, sensitive, and able to detect *L. monocytogenes*, similarly to ISO 11290, while reducing the time of analysis to two days.

## T3-03 *Campylobacter* spp. Strain Choice and Food Matrix Strongly Affect $LOD_{50}$ Results

**Wilma Hazeleger**[1], Ida Jongenburger[1], Wilma Jacobs-Reitsma[2] and Heidy den Besten[1], [1]Wageningen University and Research, Wageningen, Netherlands, [2]RIVM National Institute for Public Health and the Environment, Bilthoven, Netherlands

***Introduction:*** Campylobacteriosis is the most commonly reported zoonosis in the EU and the occurrence of *Campylobacter* in broiler meat remains high. The detection in food may be hampered due to abundant growth of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* during enrichment, resulting in false-negative samples. Therefore, the ISO protocol (ISO-DIS 10272-1; 2015) was revised to include, next to Bolton Broth (BB), Preston Broth (PB) as a prescribed enrichment broth to inhibit competitive flora in samples with suspected high levels of ESBLs. An Inter-Laboratory Study (ILS) was performed to validate this protocol, using four food matrices and chicken caecal material.

***Purpose:*** The ILS validation included one different strain per food matrix; therefore, in the current study, enrichment procedures were carried out with all strains used in the ILS in each food matrix.

***Methods:*** Enrichment procedures according to the ISO protocol were conducted using spinach, minced meat, raw milk, and chicken skin. Each matrix was inoculated with a different strain of *Campylobacter jejuni* (3 strains) or *Campylobacter coli* (2 stains). Results were expressed as $LOD_{50}$ (Level of Detection), which is the concentration at which the probability of detection is 50%.

***Results:*** The $LOD_{50}$ for all strains tested in spinach was approximately 0.7 CFU/sample, which complies with the ILS results. Results for the other food products, however, showed a large variation in the $LOD_{50}$, with statistically significant differences between food products and between strains in raw milk and minced meat.

***Significance:*** When a laboratory is validating the ISO method, care should be taken to extrapolate the ILS results to other *Campylobacter* spp. strains. One of the strains used in the ILS (*C. jejuni* WDCM 00156) is not the best choice to use as the reference strain.

## T3-04 Monitoring the Quality of the Foods with Real-time Sensors for the Detection of Pathogenic Bacteria

Grazia Lupoli[1], Danila Mosconi[2], Stanislao Maria Di Amato[1], Giancarlo Barraco[3] and **Claudio Gallottini**[4], [1]REBORN - Recover and Engineering with Biotechnology Optimisation and Robotic Nanotechnology, Latina, Italy, [2]University of Rome Tor Vergata, Roma, Italy, [3]University of Perugia, Perugia, Italy, [4]ITA Corporation, Miami, FL

***Introduction:*** Bacterial contamination is a major problem in food processing and food residues in process lines. New chemical technologies and microbiological analyses with biosensors are innovative, reliable choice for quality food control. New biomolecular techniques for food pathogen detection are being developed to improve the biosensor characteristics, such as sensitivity and selectivity.

***Purpose:*** This technique is rapid, economic, effective, and suitable for *in situ* analysis. Biosensors act as analytical devices employing a biological material or biomimic, as a recognition molecules integrated within a physicochemical transducer or transducing microsystems.

***Methods:*** Agents or bacterial toxins or fragments from microbial infections that can assure the presence of specific pathogenic bacteria were evaluated: *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Listeria monocytogenes*. The analysis was performed in real time using biosensors with the specific reaction to specific antibodies, which bind analyte on the reactive surface. The selectivity of graphite-based, amperometric detectors for significant substrates was selected for the development of low-cost disposable sensors.

***Results:*** We have developed a microbial based biosensor to determine the presence of specific pathogenic bacteria. This immunosensor was able to detect 80–100 CFU specified bacteria/ml in a water solution. The assays were specific and produced a signal in the presence of all microorganisms tested, such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Listeria monocytogenes*.

***Significance:*** We have the advantages of the use of sensors, which are rapid, reliable, specific, and cost effective and which do not require trained workers and use of minimaml equipment. Biosensors provide biochemical, analytical, alternatives to classical methods, with advantages like easy handling, portable, quick, and user-friendly.

## T3-05* Impact of Chronic Exposure of Low Concentrations of Microbial Depsipeptide Cereulide on Mitochondrial Disruptions in Caco-2 Cells

**Marlies Decleer**, Sarah De Saeger and Andreja Rajkovic, Ghent University, Ghent, Belgium

***Introduction:*** Cereulide (CER) is a lipophilic cyclododecadepsipeptide produced by certain strains of *Bacillus cereus*. This toxin is known to induce an acute and even fatal emetic syndrome, food poisoning at doses of 8 µg/kg body weight. In contrast with acute doses associated with food poisoning, recent prevalence data demonstrated relatively low concentrations of cereulide in rice and pasta dishes. The effects of repeated exposure to low levels of cereulide is largely unknown.

***Purpose:*** This investigation was conducted to determine the impact of continuous exposure to low doses of cereulide on the behavior of intestinal cells. Caco-2 cells were used as model of the intestinal mucosa.

***Methods:*** First, the limit of CER toxicity in undifferentiated Caco-2 cells was evaluated after a three-day exposure to low concentrations. Next,

cells were exposed to varying concentrations around the predicted limit of CER toxicity for 18 days, to investigate the effect of longer exposure. To explore the mechanisms involved in cytotoxic response and mitochondrial function, the Seahorse Bioscience XFe24 analyzer (Massachusetts, USA) was used, in combination with well-established assays for mitochondrial activity (MTT), to observe changes in protein content (SRB). The effects of cereulide on the mitochondrial oxygen consumption rate (OCR) in Caco-2 were assessed using the Seahorse Bioscience XF Cell Mito Stress Test kit. In this assay, modulators of cellular respiration (oligomycin, FCCP, and mix of rotenone and antimycin A) were serially injected, providing insight into different aspects of mitochondrial function.

***Results:*** Both MTT and SRB assays showed toxicity on undifferentiated cells at 0.125 ng CER/mL, after three days exposure. The three-day low CER concentration treatment on mitochondrial respiration in intact cells showed perturbations in mitochondrial respiration at a concentration of 0.125 ng/ml.

***Significance:*** These *in vitro* data suggest that repeated exposure to CER might injure intestinal cells, even at relatively low doses. Cereulide appears to be more toxic than other cyclodepsipeptide toxins with ionophoretic properties, like valinomycin and beauvericin.

### T3-06 Quantitative Detection of Deoxynivalenol in Multiple Grain Commodities Using Envirologix DON-Flex, a Rapid Lateral Flow Assay

**Anna Rice**, Serguisz Polakowski, Cheryl Bailey, Keith Tanguay, Jamie Welch, Brendan Gow and Terry Goddard, EnviroLogix, Inc., Portland, ME

***Introduction:*** Deoxynivalenol (DON), a mycotoxin produced by several species of *Fusarium* molds, is a common contaminant of grain commodities. Varied adverse health effects are caused by ingestion of DON-contaminated food and feed products. Levels of the mycotoxin are regulated and monitored in products entering the food chain, at both the raw and processed materials points, necessitating rapid, accurate, and precise test methods for detection at the regulated levels.

***Purpose:*** The purpose of this study was to evaluate the performance characteristics of the DON-Flex test protocol, across several matrices commonly tested for DON contamination.

***Methods:*** Various corn and wheat derived matrices, containing multiple levels of DON contamination, were extracted and tested across three lots of the DON Lateral Flow Assay. Quantitated results were compared to other reference detection methods and to the USDA defined criteria for accuracy.

***Results:*** Linearity was achieved across the detection range, spanning multiple dilution protocols, from 0.1-30 ppm, with $R^2$ values of > 0.99% for all matrices tested. The test exhibited precision across the dynamic range, with %CVs between 4-9%. Results for all contamination levels, of each matrix tested, fell within the USDA DON standard criteria for accuracy.

***Significance:*** Study results illustrate that the rapid lateral flow test enables accurate and reproducible deoxynivalenol detection, in multiple commodities, within appropriate regulated ranges of contamination.

## T4 Technical Session 4 – Molecular Characterisation of Microorganisms

**Thursday, 30 March – 8.30 – 10.00**

### T4-01* Molecular Characterization of Shiga-toxin Producing *Escherichia coli* in Food Products Marketed in Romania

**Sorin Daniel Dan**[1], Alexandra Tabaran[2], Marian Mihaiu[3], Oana Lucia Reget Reget[3] and Alina Dana Magdas[4], [1]University of Agricultural Sciences and Veterinary Medicine, Cluj Napoca, Romania, [2]University of Agricultural Sciences and Veterinary Medicine, Cluj - Napoca, Romania, [3]University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, Cluj Napoca, Romania, [4]National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj Napoca, Romania

***Introduction:*** Shiga toxin-producing strains of *Escherichia coli* (STEC) are known as major pathogens contaminating various food products. Ever since the discovery of *E. coli* O157 in 1982, these strains are considered one of the major emerging pathogens, which need thorough surveillance. Since then, many STEC strains of *E. coli* have been identified, but not all associated with human disease.

***Purpose:*** Our study aimed to characterize the virulence factors and antimicrobial resistance genes of STEC strains isolated from Romanian meat, milk, and product thereof, so as to reveal the risk posed by these products to human health.

***Significance:*** STEC can be found in raw food products marketed in Romania. These strains show a high resistance to antimicrobials and may pose a serious risk to human health.

### T4-02* *Listeria monocytogenes SigB*-Induced Hypersensitivity against Oxidative Stress is Mediated by a Down Regulation of Catalase Expression

**Marcia Boura** and Kimon Andreas Karatzas, University of Reading, Reading, United Kingdom

***Introduction:*** *Listeria monocytogenes* is a foodborne pathogen that causes listeriosis, a life-threatening disease. *Listeria monocytogenes* needs to cope with hostile conditions, such as oxidizing environments, in order to survive and cause disease. Oxidative stress (OS) occurs during aerobic respiration, disinfection process (oxidative disinfectants), and during the infectious process. *sigB* is a well-known transcriptional regulator involved in regulating the expression of numerous stress- and virulence-related genes, whose role in OS is still controversial.

***Purpose:*** This research was conducted to clarify the role of *sigB* against OS during different stages of the cell cycle.

***Methods:*** *Listeria monocytogene*s 10403S, wild type (WT) and *sigB* mutant (Δ*sigB*), were challenged with $H_2O_2$ in mid-exponential and stationary phases of growth. Bacterial viability was assessed by quantifying the CFU during the 60 min of challenge. In paralle,l the catalase activity was determined, using a novel visual method. Cell proliferation assays were performed by infecting Caco-2 (MOI=50) cells and following intracellular growth for 12 hours.

Transcription of the catalase gene (*kat)* in different stages of growth was determine by RT-qPCR, using LightCycler 480 software to calculate the relative expression in comparison to the 16S rRNA gene.

***Results:*** During stationary phase, the *sigB* mutant was significantly more resistant to $H_2O_2$ than the WT strain (~5 logs difference, $P < 0.05$). However, during mid-exponential phase both strains showed similar resistance to OS (~4 logs reduction). During mid-exponential phase, the catalase activity was very low, both in WT and Δ*sigB* strains; however, after 12/14 hours of growth, the Δ*sigB* presented stronger catalase activity than the WT. The catalase activity assays showed a good correspondence with *kat* transcription, which was constantly up-regulated in Δ*sigB* ($P < 0.05$), and demonstrated peak expression at ~12 hours of growth. The proliferation assays showed that both strains have similar intracellular growth.

***Significance:*** These findings will help us understand, in depth, the OS resistance mechanisms of this pathogen and reduce the occurrence of disease.

## T4-03* Phenotypic and Pan-genomic Characterisation of *Salmonella enterica* Serovar Uganda, an Uncommon Foodborne Pathogen

**Daniel Hurley**[1], Maria Hoffmann[2], Ellen Wall[1], Eric Brown[2], Marc Allard[2], Salim Mattar[3] and Séamus Fanning[1], [1]University College Dublin, Dublin, Ireland, [2]U.S. Food and Drug Administration, College Park, MD, [3]Universidad de Córdoba, Córdoba, Colombia.

***Introduction***: *Salmonella* Uganda is an uncommon serovar rarely isolated from humans. It has been implicated in only three foodborne outbreaks reported in the United States between 1993 and 2012, and little is known about its genetic diversity or pathogenicity.

***Purpose***: The purpose of this study was to phenotypically characterise 14 isolates and study the pan-genome of *S*. Uganda.

***Methods***: Intracellular survival of select isolates in human THP-1 macrophages was comparatively assessed using *Salmonella* Typhimurium. Macrophage proinflammatory cytokine and chemokine markers were quantified, post-infection. Whole-genome sequencing was performed using the Illumina MiSeq platform. A high-quality reference genome for *S*. Uganda CFSAN006159 was generated on the Pacific Biosciences RS II platform.

***Results***: In THP-1 macrophages, *S*. Uganda CFSAN006159 recorded a < 2 $\log_{10}$ reduction between 2 and 168 hours, post infection; whereas, *S*. Uganda CFSAN006173 recorded a < 1 $\log_{10}$ reduction over the same time course. Both *S*. Uganda isolates persisted for seven days within human macrophages, unlike the *S*. Typhimurium references, which were unrecoverable. Infection with *S*. Uganda stimulated increased cytokine (CXCL8, IL1B and TNF) and chemokine (CCL2, CCL3 and CCL22) release, compared to the reference strain.

SPI-13, containing the *lgl-ripABC* operon, in addition to three uncharacterised genes, has currently, only been reported to be highly up-regulated within macrophages. *Salmonella* Uganda CFSAN006159, which has been shown, in this study, to readily survive in THP-1 macrophages, in addition to eliciting a large proinflammatory response, uniquely harbours two complete chromosomal copies of SPI-13, located approximately 460-kbp apart. Bioinformatic analyses suggests that these loci appear to have been acquired from distinct genetic lineages. This finding may contribute to the extreme pathogenicity of this isolate during infection resulting from a gene dosage effect.

***Significance*:** Characterising the phenotypic virulence and genomic diversity of this serovar further extends our understanding of its ability to illicit a host-cell response during infection.

## T4-04 Estimated Infectivity of Human Norovirus in Environmental Water Samples by an *In Situ* Capture RT-qPCR Method

**Peng Tian**[1], David Yang[1], Qianqian Li[1] and Dapeng Wang[2], [1]ARS, USDA, Albany, CA, [2]Shanghai Jiao Tong University, Shanghai, China

***Introduction:*** Human Norovirus (HuNoV) genomic signals have been detected in environmental samples using RT-PCR. However, it does not necessarily suggest the presence of infectious viruses. Histo-blood group antigens (HBGAs) have been recognized as receptors for HuNoVs. We have, previously, demonstrated that porcine gastric mucin (PGM) contains human HBGAs, and could be bound by multiple strains of HuNoVs. Refinement of prior viral binding/sequestration techniques have led to our current version, which utilizes PGM-coated hybrid binding/amplification containers. These hybrid binding/amplification containers serve as the medium for the binding/sequestration of HBGA-binding viruses, which is, immediately, followed by *in situ* amplification of the captured viral genomes by RT-qPCR (ISC-RT-qPCR).

***Purpose:*** This study was conducted to estimate the potential infectivity of HuNoV in environmental water, using the ISC-RT-qPCR method.

***Methods:*** Tulane virus (TV) was incorporated into samples as an internal control for the indication and quantitation of RT-qPCR inhibition. The ISC-RT-qPCR method was, first, validated using heat-released viral RNA. Samples were tested for both TV and HuNoV, using ISC-RT-qPCR, and compared against the results of RT-qPCR and RNAse protection assays.

***Results:*** We demonstrated that heat-released TV and HuNoV viral RNAs could not be captured and amplified using the ISC-RT-qPCR method. From 72 samples, positive for GI HuNoV by RT-qPCR, 20 samples (27.8%) tested positive by ISC-RT-qPC; suggesting that 72.2% of RT-qPCR-positive samples were unlikely to be infectious. Similar results were observed with the RNase protection assay, as only 14 (19.4%) of RT-qPCR-positive samples were resistant to RNase. Of 16 samples, positive for GII HuNoV by RT-qPCR, only one tested positive by ISC-RT-qPCR; suggesting that 93% of RT-qPCR-positive samples were unlikely to be infectious. However, five samples that had initially tested negative by RT-qPCR, tested positive for GII HuNoV by ISC-RT-qPCR.

***Significance:*** Overall, the ISC-RT-qPCR assay is a promising alternative for the estimation of HuNoV infectivity in environmental samples.

## T5 Technical Session 5 – Food Safety and Microbiology

**Thursday, 30 March – 10.30 – 12.00**

### T5-01 Insight into the Variables Affecting Bacterial Transference during the Washing Process of Fresh-cut Lettuce and Spinach in Simulated Reused Fresh-cut Produce Wash Water

**Cristina Pablos**, Aitor Romero and Javier Marugán, Rey Juan Carlos University, Mostoles, Spain

***Introduction:*** Inadequate quality of water used in the washing of fresh-cut produce has the potential to be a direct source of contamination and a vehicle for spreading bacteria.

***Purpose:*** This study was conducted as an evaluation of the influence of washing methods (time, temperature, NaClO concentration, water/produce ratio); wash water quality (organic matter, turbidity, conductivity, pH); and type of fresh-cut produce in bacterial cross-contamination during the washing step.

***Methods:*** Physical-chemical characterization of fresh-cut produce wash water was carried out from effluents of a local fresh-cut processing industry. Fresh iceberg lettuce pieces (25 g) were inoculated with gentamicin-resistant *Salmonella enterica* (LFMFP 687) and washed in one liter of simulated wash water with stirring at 260 rpm for 2 min at 4ºC; this was repeated twice with two subsequent washing cycles in reused water. *Salmonella* concentration was quantified in water and in the produce before and after the washing treatment. Similar experiments were conducted with fresh spinach.

***Results:*** The main chemical properties of the prepared, simulated wash water can be summarized as 150 mg/L TOC, 100 NTU, 1000 µS/cm, pH of 6.2. *Salmonella* was transferred from the inoculated lettuce to the wash water and remained in the reused wash water, accumulating after each, subsequent, cycle of washing (reaching concentration values up to $10^5$ CFU/mL). The produce to water transfer ratio was quantified for fresh-cut lettuce and spinach. The wash water became contaminated; with 99% of *Salmonella* present on the inoculated lettuce. For spinach, only 20–50% of the *Salmonella* inoculated was transferred to the water. Spinach washing led to a notable increase in the concentration of suspended solids in the reused wash water.

***Significance:*** Development of a solid understanding of the washing process and its effects on microbial growth is required to prevent cross-contamination, enhancing produce quality and safety.

### T5-02 The Lack of Tools to Track *Bacillus thuringiensis*-(Bt) based Insecticide Isolates from Farm to Fork

Anne-Gabrielle Mathot[1], Emeline Cozien[2], Pierre Gehannin[2], Rodolphe Vidal[3], Nadine Henaff[2] and **Florence Postollec**[2], [1]LUBEM UBO University - UMT14.01 SPORE RISK, Quimper, France, [2]ADRIA Food Technology Institute - UMT14.01 SPORE RISK, Quimper, France, [3]ITAB French Research Institute for Organic Farming, Paris, France

***Introduction***: *Bacillus thuringiensis* (Bt) is a widespread, sporeforming bacteria with a complex life cycle. Due to its ability to produce parasporal crystalline inclusions that show insecticidal properties, it has become the main microorganism used for pest control in organic farming. Today, it is successfully used as a bioinsecticide against caterpillars, beetles, and flies, including mosquitoes and blackflies. Yet from a farm-to-fork prospective, the major issue relies on the lack of tools to distinguish Bt-based bioinsecticide isolates from other closely related strains of the *Bacillus cereus* group that may be involved in food poisoning or food spoilage.

***Purpose:*** The aim of this study was to collect isolates from Bt-based commercial products and develop a PFGE method to track *B. cereus* contaminants.

***Methods:*** Commercial Bt-based products were used to isolate Bt strains that were further characterized by the presence of toxin encoding genes, Guinebretiere et al. phylogenetic classification, as well as the observation of parasporal crystalline inclusions using a phase contrast microscope. The Pulsed-Field Gel Electrophoresis (PFGE) subtyping protocol was adapted from Liu, et al. (1997) and Zhong, et al. (2006).

***Results:*** For most prevalent Bt strains used in Europe, all tested isolates showed the production of huge parasporal crystals, belonging to group IV phylogenetic classification, during the sporulation phase. PFGE is the gold standard used by the CDC and European public health laboratory network for foodborne disease surveillance. Molecular fingerprints generated clearly enabled the clustering of strains belonging to the subspecies *Bt aizawai*, *Bt kurstaki* and *Bt israelensis.*

***Significance:*** The promising results obtained in this study will be extended to Bt strains used in the composition of Bt-based products used outside Europe and, also, to a selection of strains to be representative of *B. cereus* diversity. When validated, this tool could be applied to track Bt contamination from farm to fork.

### T5-03* Characterization of Microorganisms Isolated from Biofilms in Food Companies: Identification and Biofilm-forming Properties

**Sharon Maes**[1], Son Nguyen Huu[2], Thijs Vackier[3], Marc Heyndrickx[1], Hans Steenackers[4], Alex Verplaetse[3], Katleen Raes[2] and Koen De Reu[1], [1]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium, [2]UGent, Kortrijk, Belgium, [3]KU Leuven, Gent, Belgium, [4]KU Leuven, Leuven, Belgium

***Introduction:*** The importance and role of biofilms in persistent infections with spoilage organisms and pathogenic bacteria is still insufficiently known. Research about sampling, detection, and characterization of biofilms in the food industry can help to provide new insights into this issue.

***Purpose:*** The aim of this study was to sample biofilms in different food companies and to characterize the microbial population and matrix components of these presumptive biofilms.

***Methods:*** Surfaces in eight food companies were sampled after cleaning and disinfection. Different microbiological enumerations were performed on the samples and the dominant bacteria were identified using $(GTG)_5$ clustering, followed by 16S rRNA gene sequencing. Also, the biofilm matrix components proteins, carbohydrates, and uronic acids were

determined. The possibility of the collected dominant bacteria to form a biofilm, under lab conditions, was evaluated in microtiter plates.

***Results:*** The proportion of microbiologically contaminated surfaces varied from 0-64% across the different food companies, with values varying from 0.00 to 7.23 log CFU/100 cm². For 0-33% of the sampled surfaces from the food companies, microorganisms were found in combination with biofilm matrix components. Identification of the collected isolates showed wide diversity; but, the most common identified species were *Pseudomonas* spp. (26.3%), *Stenotrophomonas* spp. (8.3%) and *Microbacterium* spp. (8.1%). Regarding the biofilm-forming properties, microorganisms with the strongest possibility to form biofilms were part of the *Pseudomonas*, *Acinetobacter*, and *Stenotrophomonas* families.

***Significance:*** Detection and characterization of biofilms, in the concerned food companies, gave useful insights in the potential to cause food spoilage and foodborne infections; and offered a basis for the development of more efficient cleaning and disinfection procedures.

## T5-04 The Importance of Strain Selection to the Conduct of Challenge Testing and Assessment of Food Spoilage

Francesca Valerio[1], Anne-Gabrielle Mathot[2], Marie-Laure Divanac'h[3], Emeline Cozien[3], Noémie Desriac[2], Nadine Henaff[3], Véronique Huchet[3] and **Florence Postollec**[3], [1]Institute of Sciences of Food Production, National Research Council, Bari, Italy, [2]LUBEM UBO university - UMT14.01SPORE RISK, Quimper, France, [3]ADRIA Food Technology Institute - UMT14.01 SPORE RISK, Quimper, France

***Introduction:*** To comply with EC regulation, it is the responsibility of the food business operator to control microbial hazards in foods under foreseeable conditions of production, storage, purchase, and use. For that matter, predictive mathematical models and challenge testing are recognized approaches used to validate control measures within the HACCP system, as well as to assess microbiological food safety and quality.

***Purpose:*** This work aimed at the industrial application and transfer from mathematical models validated for pathogens to *Bacillus* spp., in order to assess potential rope spoilage in bread.

***Methods:*** Growth cardinal values are microbiologically relevant parameters. Even though time consuming, the determination of such values enable the determination of growth/no growth boundaries for given food product formulation and storage conditions (pH, water activity (aw), temperature). Based on known cardinal values of *Bacillus* spp. strains, challenge testing was performed with artificial spore inoculation into bread dough. After baking, the enumeration of *Bacillus* spp. from bread in storage (30°C) was performed. Growth kinetics were fitted to mathematical models to further enable *in silico* simulations during bread shelf life for storage scenarios mimicking Mediterranean temperature.

***Results:*** Growth/no growth boundaries clearly showed variable growth abilities in bread for the different *Bacillus* strains selected. A challenge test was performed with the strain isolated from wheat grain that showed wider growth abilities regarding low aw. *In silico* simulations were performed to quantify *Bacillus* spp. populations during shelf life and to determine the probability of overpassing a given five log/g contamination. Growth simulations underline that rope spoilage showed rapid evolution and, mainly, occurs in the summer season in Mediterranean countries.

***Significance:*** To facilitate the practical use of generic and recognized mathematical models, several user-friendly tools exist for growth prediction. Besides the importance of using real-life strains, this study further underlined the importance of characterized collections for the selection of the bacterial strain(s) to be used in the challenge test to ensure food quality and safety during shelf life.

## T5-05 Early Detection of *Campylobacter* Using Air Sampling and VOC Analysers

**Tim Gibson**[1], Stan Curtis[2], Ben Curtis[2], Lynn McIntyre[3], Frank Vriesekoop[3], Sarah Hardy[4], Simon Lock-Pender[4] and Andrew Stacey[5], [1]RoboScientific Ltd, Leeds, United Kingdom, [2]RoboScientific Ltd, Littleport, United Kingdom, [3]Harper Adams University, Newport, United Kingdom, [4]Banham Group Ltd, Attleborough, United Kingdom, [5]Cellular Systems Ltd, Grantham, United Kingdom

***Introduction:*** *Campylobacter* is a gram negative, micro-aerophilic bacterium, present in the gut and faeces of chickens; yet, it does not cause disease in the chickens. It causes food poisoning in humans; and in the UK, up to 280,000 cases per year, leading to 100 deaths, have been reported. It is, also, the most frequently reported foodborne illness in the EU; costing around 2.4 billion euros per year due to illness. Early detection to help reduce food contamination would be very useful.

***Purpose:*** The aim of this work was to develop and commercialise a simple field detection system that will enable the onset of *Campylobacter* in chicken farms to be detected using the volatile chemical profile produced.

***Methods:*** Air samples from above four chicken flocks were taken, daily, using a µCoriolis air sampler (Bertin Instruments). A volume of 3000 Litres of air was concentrated into 7 ml of distilled water and the resulting aqueous sample was sniffed 4 times using a Bloodhound® volatile analyser (RoboScientific Ltd), containing an array of chemical sensors tuned to the VOCs, associated with presence of *Campylobacter*. The data obtained was correlated with *Campylobacter* detection by molecular analysis, using a DNA amplification system.

***Results:*** *Campylobacter* detection using the Bloodhound® analyser correlated well with the DNA results and, in each flock, indicated the appearance of active *Campylobacter* infections in the chickens. Each flock sampled over a 33 to 38 day period showed a significant change in the VOCs detected when *Campylobacter* was present, indicated by the appearance of different sensor outputs; and, also, after data processing using discriminant analysis, by the clustering of data points remote to the *Campylobacter* negative samples. Mahalanobis distances jumped from 472 for negative samples to 15,777 for *Campylobacter* positive samples.

***Significance:*** Early field detection of *Campylobacter* will provide knowledge to reduce chicken meat contamination by enabling slaughter and processing of negative flocks before positive flocks.

## T5-06* Attribution of *Listeria monocytogenes* Human Cases to Food and Animal Sources in Northern Italy

**Virginia Filipello**[1], Lapo Mughini-Gras[2], Silvia Gallina[3], Ettore Amato[4], Mirella Pontello[4], Lucia Decastelli[3], Marc Allard[5], Eric Brown[5] and Sara Lomonaco[1], [1]University of Turin, Grugliasco, Italy, [2]Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [3]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy, [4]University of Milan, Milan, Italy, [5]U.S. Food and Drug Administration, College Park, MD

***Introduction:*** Human listeriosis is a rare, but serious foodborne disease, with high hospitalization and fatality rates in at-risk population (i.e., the immunocompromised, elderly, newborns, and pregnant women). Source attribution of foodborne diseases, based on microbial subtyping, is widely used to ascertain the main sources of infection by quantifying the relative contributions of different foods to human disease.

***Purpose:*** This study aimed to assess the contribution of different animal and food sources of human listeriosis in the Piedmont and Lombardy regions (Northern Italy) from 2005 to 2016.

***Methods:*** A representative collection of human (n=230) and veterinary/not-human (n=440) *Listeria monocytogenes* isolates was selected and typed with multi-locus sequence typing (MLST) and multi-virulence locus sequence typing (MVLST). Using both MLST and MVLST data, four different source attribution modelling approaches (Asymmetric Island, STRUCTURE, Hald and Dutch models) were applied in a comparative fashion.

***Results:*** In all models, the primary source of listeriosis cases was estimated to be dairy products (either from cattle or mixed species), accounting for up to 73% of human cases; followed by poultry (3-18%) and game meat (3-16%). Sources, like pork (2-3%) and beef (2-4%), seemed to play a minor role. Differences in attributions were observed depending on the modelling approach and typing method. Combining MLST and MVLST data did not, significantly, influence the results of attribution modeling.

***Significance:*** Source attribution, based on microbial subtyping, is a valuable tool for quantifying the contribution of different food-animal sources of human listeriosis and to guide public health interventions. Our results provided strong evidence for dairy products as the most important source of human listeriosis in these regions and underline the lasting need for control measures aimed at reducing *L. monocytogenes* contamination in these products.

# T6 Technical Session 6 – Modeling and Risk Assessment 1

**Thursday, 30 March – 13.30 – 15.00**

## T6-01* Is It Safe to Use Tap Water to Prepare Infant Formula in France?

**Géraldine Boué**[1], Luiza Wasiewska[1], Enda Cummins[2], Sandrine Guillou[1], Jean-Philippe Antignac[3] and Jeanne-Marie Membré[1], [1]UMR1014 SECALIM, INRA, Oniris, Nantes, France, [2]UCD School of Biosystems and Food Engineering, Dublin, Ireland, [3]Laberca USC1329, INRA, Oniris, Nantes, France

***Introduction:*** Powder infant formula, the most consumed food by infants in France, needs to be reconstituted with water before consumption. The use of tap water is permissible (according to the French food safety agency), with the caveat that it is not sterile and may contain chemical and microbiological contaminants.

***Purpose:*** The aim of the study was to develop a microbiological-chemical risk assessment model to quantify the risk associated with the use of tap water in France for preparation of infant formula (during the first six months of life).

***Methods:*** *Cryptosporidium* spp. and arsenic were selected as the hazards of greatest concern in microbiology and chemistry, respectively. The risk assessment model, with uncertainty and variability separated, was built using French data (or European data, alternatively). Outputs were expressed, firstly, at the individual level, as probability of illness; and then, at the population level, by using the DALY (Disability Adjusted Life Year) indicator. Two scenarios of milk preparation were considered: the use of boiled and unboiled tap water.

***Results:*** Consuming infant formula rehydrated with unboiled tap water during the first six months of life led to 6,000 DALY per 100,000 infants (90% uncertainty interval [1500; 12000]) for *Cryptosporidium* spp. causing diarrhea and 2 DALY [1.6; 2.3] for arsenic causing lung and bladder cancers. For the whole infant population, boiling water would suppress the risk from *Cryptosporidium* spp. In contrast, the cancer risk, although low at the population level, was rather high for infants having a high level of exposure to arsenic. For those, exposure would be decreased by changing the tap water supply point.

***Significance:*** This model can help authorities quantify the risk associated with tap water used for preparation of infant formula. More generally, it is an example of a microbiological-chemical risk assessment and, thus, falls into the emerging risk-benefit assessment area.

## T6-02 Risk Factors Selection, Criteria Assessment, and Final Weighting for the Canadian Food Inspection Agency's Establishment-based Risk Assessment Model

Manon Racicot[1], Romina Zanabria[2], Mathieu Cormier[3], Julie Arsenault[4], Cecile Ferrouillet[4], Marie-Lou Gaucher[4], Ann Letellier[4], Anna Mackay[5], Ashwani Tiwari[5], Solomon Aklilu[5], Ryan Currie[5], Mansel Griffiths[6], Richard Holley[7], Tom Gill[8], Sylvain Charlebois[8] and **Sylvain Quessy**[9], [1]Canadian Food Inspection Agency, St-Hyacinthe, QC, Canada, [2]Canadian Food Inspection Agency, Guelph, ON, Canada, [3]Canadian Food Inspection Agency, Montreal, QC, Canada, [4]University of Montreal, St-Hyacinthe, QC, Canada, [5]Canadian Food Inspection Agency, Ottawa, ON, Canada, [6]University of Guelph, Guelph, ON, Canada, [7]University of Manitoba, Winnipeg, MB, Canada, [8]Dalhousie University, Halifax, NS, Canada, [9]University of Montreal, Saint-Hyacinthe, QC, Canada

***Introduction:*** The Canadian Food Inspection Agency (CFIA) is developing a risk-based assessment model to quantify the risk associated with food

establishments. As part of its development, 155 risk factors were initially identified and their significance assessed through an expert elicitation in 2013.

***Purpose:*** To further discriminate risk factors to be included in the model, a refinement process was performed. Also, a second expert elicitation was held in 2014 to assign a weight, based on their relative risk to human health, to estimate the risk associated with specific clusters, and to validate the final list of factors.

***Methods:*** For the refinement process, the availability of data sources and the clarity and measurability of the selected factors were considered. Those that were lower-rated during the expert elicitation were eliminated; the remaining were grouped, based on their focus of attention (clusters). Assessment criteria were, then, defined for each risk factor to allow their individual quantification within the model, and, collectively, presented to experts during a two-round Delphi exercise for their assessment.

***Results:*** Twenty-nine Canadian experts participated in the elicitation, and a very good consensus on the weighting was obtained for most risk factors and clusters. All experts scored the risk factors as significantly affecting the risk related to a food establishment, and none of them expressed formal opposition to their inclusion or to the way they were clustered in the model.

***Significance:*** As a result of this work, the median values assigned to each criterion used to assess the risk factors and clusters will be included in the new CFIA risk assessment model, which will be further implemented as part of the regulatory oversight activities of the Agency.

## T6-03 Insects Fed with Former Foodstuffs for Feed Production: What are the Risks to Public and Animal Health?

**Linda Kox**, Netherlands Food and Consumer Product Safety Authority, Utrecht, Netherlands

***Introduction:*** The need for proteins for human food and animal feed has increased over the last decades and will further, greatly increase in the future; that is why there is a growing focus on less traditional sources of protein, such as cultured insects. Insects are a sustainable protein source, in particular, when using organic residues and waste streams to feed them.

***Purpose:*** The purpose of the study was 1) to assess the risks of the use of insects that were reared on a substrate composed of former foodstuffs as raw material for feed for food-producing animals to animal and public health and 2) to indicate measures to control these risks.

***Methods:*** A literature review aimed at insect species that are suitable for large-scale production, on substrates derived from organic residues and waste streams, was performed. The production of the insects as feed was approached as a chain, where the chemical and microbiological risks were assessed for each step of the production process.

***Results:*** The health risks that are associated with the use of animal feed derived from insects that are reared on former foodstuffs are determined, almost exclusively, by pathogenic microorganisms. The most important critical control point in the production process is the last step, the processing of the insects to the final product.

***Significance:*** When appropriate measures to prevent microbiological contamination of insects are taken, the feeding of food-producing animals with feed derived from insects that were reared on a substrate composed of former foodstuffs posed no health risks for these animals or for humans consuming products from these animals.

## T6-04 Evaluation of the Microbial Risk of Storage at Ambient Temperature of Artisanal Rice Pie

**Els Van Coillie**[1], Koen De Reu[1], Geert Van Royen[1], Claire Verraes[2], Marc Heyndrickx[1] and Lieve Herman[1], [1]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium, [2]Federal Agency for the Safety of the Food Chain (FASFC), Brussels, Belgium

***Introduction:*** Rice pie must currently be stored in the shop at ≤ 7°C. Because of the negative impact of refrigeration on the taste, a study was undertaken to evaluate the possibility of storing rice pie at ambient temperature for 12 h.

***Purpose:*** This study was conducted as a risk evaluation performed on storage of artisanal rice pie at different temperatures.

***Methods:*** A survey was undertaken in 15 Belgian artisanal bakeries to evaluate variations in the production process. Internal microbiological contamination (total aerobic and total aerobic spore count), pH, and water activity ($a_w$) were determined in 31 samples. Pilot experiments were undertaken to determine the internal pie temperature during the baking process. Simulations of *Bacillus cereus* growth were performed using Combase at pH and $a_w$ combinations corresponding to those observed. Results were validated by challenge tests.

***Results:*** A large variation was found in receipt and baking conditions. Bacteria were counted in 5/31 samples (10 to 110 CFU/g), but spores were not found in any of the samples. The pH and $a_w$ of the rice pies varied between 6.3 and 7.03 and between 0.957 and 0995, respectively. During the baking process, it took at least 30 minutes to reach an internal temperature of 90°C. The baking time at >90°C was 0-40 minutes. Simulations using worst case combinations of pH and $a_w$ (pH 6.8 and $a_w$ 0.995) indicated a level of *B. cereus* of < 4 log CFU/ml (considered the limit for food safety) after storage at 20°C for 12h, when starting from 1 CFU/g. Challenge tests resulted in growth similar to that predicted by modelling.

***Significance:*** The data suggested that a maximum level of *B. cereus* (4 log CFU/g) can be guaranteed in rice pie, if stored for 12h at ≤ 20°C.

## T6-05* Burden of Disease of Barbecued Meat: Who is at Risk?

**Lea Sletting Jakobsen**[1], Stylianos Georgiadis[2], Bo Friis Nielsen[2], Anders Stockmarr[2], Elena Boriani[1], Lene Duedahl-Olesen[1], Tine Hald[1] and Sara Pires[1], [1]National Food Institute, Technical University of Denmark, Lyngby, Denmark, [2]Technical University of Denmark, Lyngby, Denmark

***Introduction:*** Consumption of meat, prepared by barbecuing, is associated with risk of colorectal cancer, due to formation of polycyclic aromatic hydrocarbons (PAH). In Denmark, the population is advised to limit consumption of barbecued meat; and, when barbecuing, to avoid charred meat. This advice is based on conservative, semi-quantitative estimates that do not take the variability of consumption patterns into account.

***Purpose:*** We aimed to estimate the disease burden due to barbecuing, in the Danish population, using disability adjusted life years (DALY), as well as the annual number of barbecued meals needed to reach an exposure that is considered a health concern.

***Methods:*** We applied a probabilistic risk assessment model, taking into account the variability of exposure patterns and of human sensitivity to the hazard, as well as the uncertainty in the exposure and dose-response data.

***Results:*** Preliminary results suggested that PAH exposure through barbecuing caused 2.23 x $10^{-7}$ cases of colorectal cancer per 100,000 inhabitants and a disease burden of 0.0004 DALY per 100,000 inhabitants per year in Denmark. Our results also showed that only extreme cases of consumption and contamination resulted in a health risk. These results highlighted the importance of deriving estimates, at the individual level, to be able to deliver advice on the health effects associated with different frequencies of barbecuing.

***Significance:*** We proposed a probabilistic method to quantitatively estimate the disease burden and individual risk of barbecuing, taking into account the variation in the population. This approach is useful to directly advising individuals to adjust behavior to optimize health and, also, with regard to risk factors other than barbecuing.

### T6-06 Conceptual Framework for a Cumulative Risk Assessment of Biogenic Amines in Foods

D. Sylvain Dabadé[1], **Liesbeth Jacxsens**[2] and Bruno De Meulenaer[3], [1]Faculty of Agricultural Sciences, University of Abomey-Calavi, Abomey-Calavi, Benin, [2]Ghent University, Ghent, Belgium, [3]Professor, Ghent, Belgium

***Introduction:*** Excessive exposure and/or inadequate detoxification capacity of biogenic amines (BA) can lead to a toxic effect in the human body. A well known example is scombrotoxin fish poisoning caused by histamines present in certain species of marine fish. However, knowledge on concentration and health risk of this BA, and other BA in other food products, is limited.

***Purpose:*** The purpose of this study was to assess the health risks associated with exposure to BA through the consumption of foods, using a cumulative risk assessment approach.

***Methods:*** Concentrations of six BA (histamine, cadaverine, putrescine, tyramine, β-phenylethylamine, tryptamine) were determined using UHPLC in six different food groups, namely meat and meat products, (processed) fruits and vegetables, chocolate, beer, diary, and non-scombroid fish (NSF) from the Belgian market (461 samples). Food consumption data were obtained from a Belgian national food consumption survey. BA were grouped, based on their main detoxification enzymes: monoamine oxidase (MOA) and diamine oxidase (DOA). A probabilistic exposure assessment was performed for each BA, for all MOA BA, and all DAO BA (dose additive) using the software @Risk version 7.0.

***Results:*** Each food group demonstrated a specific pattern of BA; not only histamine was detected. NSF, cheese, and sauerkraut contributed, mostly, to the exposure to BA. For example, 43% of NSF consumers were exposed to a dose higher than the established NOAEL for histamine. Although the exposure to a single BA in other foods was low, consumers might still be at risk, due to the presence of other BAs using the same detoxification enzyme or the combination of foods in a diet.

***Significance:*** This study provided a basis for the development of cumulative risk assessment of BA in foods and pinpointed the importance of BA, other than histamine, and the need for further toxicological data collection on BA.

## T7 Technical Session 7 – Food Processing Technologies

**Thursday, 30 March – 15.30 – 17.00**

### T7-01 *Listeria monocytogenes* Control Strategies Applied to Fresh and Cold-smoked Salmon

**Even Heir**, Kristian H. Liland and Askild L. Holck, Nofima AS, Ås, Norway

***Introduction:*** Salmon products with high levels of *Listeria monocytogenes* represent a potential health threat for consumers and is a serious microbial challenge for the salmon industry. Hygienic processing is essential for *Listeria* control, but cannot ensure absence in salmon and salmon products. Therefore, the salmon industry has shown an increasing interest in methods with documented effects for elimination or reduction of *Listeria,* when applied directly on salmon and salmon products.

***Purpose:*** This investigation was undertaken to evaluate selected methods/technologies that are used in the industry, or are regarded to have potential for industrial use, for their effect and limitations on *Listeria* elimination or reduction on fresh and cold-smoked salmon.

***Methods:*** Salmon was contaminated with a 10-strain mixture of *L. monocytogenes*. Control strategies providing both *Listeria* kill and growth inhibiting effects were tested on fresh, raw salmon and cold-smoked salmon. Tested methods included UVC and pulsed UV light, $H_2O_2$-based desliming, and treatments using organic acid salts/fermentates and acidified sodium chlorite. Analyses determined *Listeria* reductions, growth inhibition, and investigated parameters affecting robust *Listeria* reductions under industry-relevant conditions.

***Results:*** Reductions of *L. monocytogenes* on salmon treated with UVC and pulsed UV light were similar (0.5-1.5 log), with higher effects on the skin of raw salmon and on fillets of smoked salmon compared to fresh salmon fillets. Organic acid salts and fermentates provided concentration and temperature dependent growth inhibition on cold-smoked salmon during storage. Growth inhibition, without killing *Listeria*, was also obtained on fresh salmon rinsed in fermentate solution. Limited effects were obtained using desliming and rinsing of fresh salmon in acidified sodium chlorite.

***Significance:*** The results provide the industry with knowledge-based information for selection of cost-effective, *Listeria* control strategies for raw and processed salmon.

## T7-02* Resistance of *Bacillus subtilis* Endospores to Cold Plasma

**Christian Hertwig**, Kai Reineke and Oliver Schlüter, Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany

***Introduction:*** Bacterial spores are extremely resistant towards multiple environmental stress conditions. The involved resistance factors include the outer layers of the spores, DNA saturation with small acid soluble proteins (SASP), DNA repair systems, and high levels of dipicolinic acid (DPA) in the cores.

***Purpose:*** In this study, factors involved in spore resistance to cold plasma were investigated.

***Methods:*** *Bacillus subtilis* spores and isogenic mutant strains were treated using a dielectric barrier discharge plasma system. The strains PS578 ($\alpha^- \beta^-$), which lacks the genes encoding the two major α/β-type small acid soluble proteins (SASP); FB122 (*sleB spo VF*), which lacks dipicolinic acid (DPA); PS3328 (*cotE*), which lacks the outer coat, and the wild type PS832 were treated up to five minutes in a static atmosphere using different process gases (air, $N_2$, $O_2$). Plasma was characterized using optical emission spectroscopy and by the quantification of ozone.

***Results:*** Air-plasma showed high emission intensities in the UV-A and UV-B range. $N_2$-plasma emitted, mainly, UV-C photons. $O_2$-plasma generated a high amount of reactive oxygen species (ROS) and up to 22,000 ppm ozone. The strains PS3328 and FB122 were sensitive to the $O_2$-plasma, with an inactivation of 4.1 and 3.8 $\log_{10}$ after 5 min; the other strains showed a reduction of 2.7 $\log_{10}$. Strain PS578 was sensitive to the $N_2$-plasma treatment, with a reduction of 4.8 $\log_{10}$ after 0.5 min. The other strains were inactivated by 3.1 (PS832), 2.8 (PS3328) and 1.7 (FB122) $\log_{10}$. When air was the process gas, strain PS578 showed the highest inactivation with 4.8 $\log_{10}$ after 5 min; PS832 and PS3328 were inactivated by 3.1 and FB122 by 2.1 $\log_{10}$, respectively.

***Significance:*** The results indicated the different factors involved of *B. subtilis* spore resistance to cold plasma. The α/β-type SASP play a significant role in spore resistance to the emitted UV-C photons and, furthermore, protect the outer coat and the spore DPA against generated ROS.

## T7-03* Inactivation of MS2 Bacteriophage, Murine Norovirus-1, *Salmonella* spp., and *Enterococcus faecium* on Strawberries by Using Gaseous Ozone

**Zijin Zhou**[1], Frédérique Cantergiani[2], Frank Devlieghere[1], Sophie Zuber[2] and Mieke Uyttendaele[1], [1]Ghent University, Ghent, Belgium, [2]Nestlé Research Center, Lausanne, Switzerland

***Introduction:*** Sufficiently mild processing technologies need to be investigated to improve the safety of berry products. Ozone technology has been used as a sanitation tool in the food industry; however, limited publications are available on the inactivation of foodborne bacteria and viruses on fresh produce by using gaseous ozone.

***Purpose:*** This study was an evaluation of the effect of gaseous ozone on foodborne pathogens and their surrogates on strawberries.

***Methods:*** Fresh strawberries (25 g) were inoculated with MS2, MNV-1, *Salmonella* spp. and *Entercoccus faecium,* separately, and treated with gaseous ozone at concentrations of 1% (ca. 15 g/m$^3$) and 6% (ca. 80 g/m$^3$) for 5 and 30 min. Oxygen was used to flush away residual ozone. Ozone (6%) for 5 min and nitrogen flushing were applied to frozen samples. After treatment, samples were transferred into a filter-stomach bag containing either 50 ml virus elution buffer or 225 ml buffered peptone water to recover and enumerate viruses and bacteria, respectively. All experiments were conducted in triplicates.

***Results:*** On fresh strawberries, 1%/5 min, 1%/30 min and 6%/5 min ozone treatments resulted in MS2 reductions of 1.78, 2.10 and 2.23 $\log_{10}$ units, respectively. MNV-1 showed reductions of 1.12, 1.58 and 1.14 $\log_{10}$ units, respectively. The highest reductions were achieved using 6%/30 min (3.30 $\log_{10}$ for MS2 and 1.76 $\log_{10}$ for MNV-1). Reductions of *Salmonella* spp. ranged from 1.00 to 2.06 $\log_{10}$ units. *Enterococcus faecium* appeared to be more resistant ($P < 0.05$), producing reductions ranging from 0.45 to 1.52 $\log_{10}$ units. For frozen strawberries, treatment with ozone resulted in $\log_{10}$ reductions of 1.60, 0.72, 0.67 and 1.77 of *Salmonella* spp., *E. faecium*, MNV-1, and MS2, respectively.

***Significance:*** This is the first study showing the inactivation effect of gaseous ozone against both viral and bacterial strains on frozen strawberries.

## T7-04* The Synergistic Effect of High Pressure and Nisin on the Inactivation of Heat-resistant and Pathogenic Spores in Food Matrices

**Chloe Modugno**[1], Souhir Kmiha[2], Hélène Simonin[1], Stéphane AndrÉ[3], Chedia Aouadhi[2], Slah Mejri[4], Abderrazak Maaroufi[2] and Jean-Marie Perrier-Cornet[5], [1]Unité Mixte de Recherche - Procédés Alimentaires et Microbiologiques (UMR PAM), Dijon, France, [2]Laboratory of Epidemiology and Veterinary Microbiology, Group of Bacteriology and Biotechnology, Pasteur Institute of Tunisia (IPT), Tunis, Tunisia, [3]CTCPA, Avignon, France, [4]Laboratory of Animal Resources and Food, National Institute of Agronomy, Tunis (INAT), Tunis, Tunisia, [5]Unité Mixte de Recherche – Procédés Alimentaires et Microbiologiques (UMR PAM), France, Dijon, France

***Introduction:*** The high demand for minimally processed food makes high pressure processing (HPP) one of the most promising non-thermal technologies for food application. While HPP efficiently inactivates vegetative bacteria, bacterial spores show strong resistance, especially at mild temperatures. To ensure spore inactivation in food matrices, the addition of other hurdle(s), such as mild heat or antimicrobial agents, is necessary.

***Purpose:*** HPP in combination with nisin was investigated as a non-thermal method for the inactivation of pathogenic and thermoresistant bacterial spores in food matrices.

***Methods:*** Six bacterial strains were studied with regard to the diversity of their origins and properties: *Bacillus pumilus*, *Bacillus sporothermodurans*, *Bacillus licheniformis*, *Bacillus weihenstephanensis, Bacillus subtilis* and *Clostridium* sp. (*botulinum* type E-like). Spores were treated in a buffer, skim milk or a liquid

medium simulating cooked ham. Pressure levels ranging from 200 MPa to 600 MPa were applied for 10 min at 20°C or 50°C. Nisin was added during and/or after HPP to a final concentrations of 50 or 20 UI/mL.

***Results:*** While no significant reduction of spore cultivability was observed at any pressure at 20°C, the addition of nisin at low concentration (ten times lower than the legal concentration) during and after HPP treatment induced a highly synergistic effect on *Bacillus* spp. inactivation, with spore count below the detection limit (inactivation > 6 log/mL). Moreover, spores remained sensitive to nisin up to 6h after HPP.

***Significance:*** The food industry usually pressurizes foods at room temperature, resulting in, only, inactivation of vegetative cells. The present work shows that combining HPP with nisin can lead to a synergistic *Bacillus* spp. spore inactivation, even after treatments at 20°C. The addition of nisin in foods before their pressurization can, therefore, be an efficient way to ensure the inactivation of bacterial spores.

## T7-05 Application of UV-C Light Processing on Fresh and Frozen Strawberries, Raspberries, and Blueberries to Compare the Inactivation of Viral and Bacterial Pathogens and Their Surrogates

**Frederique Cantergiani**, Sophie Butot, Thierry Putallaz, Lise Michot, Mireille Moser and Sophie Zuber, Nestlé Research Center, Lausanne, Switzerland

***Introduction:*** Several foodborne outbreaks associated with strawberries have raised safety concerns about various fresh and frozen berry products, in recent years. UV-C is considered a promising technology for a wide range of beverages and food products. The berry industry needs novel approaches to address the current microbiological issues, especially viruses.

***Purpose:*** The objective of this study was to evaluate the sensory effects and to compare the inactivation of viral and bacterial pathogens and their surrogates on fresh and frozen berries using UV-C light and to critically assess the potential of this technology for the berry supply chain.

***Methods:*** Fresh and frozen strawberries, raspberries, and blueberries were spot-inoculated with Hepatitis A virus (HAV), Murine Norovirus (MNV), *Listeria* spp., *Salmonella* spp., STEC, and their surrogates (MS2 bacteriophage, *Listeria innocua*, *Enterococcus faecium*, and *Escherichia coli*) and treated with UV-C using a 95 W high output UV-C emitter. Samples were exposed to UV-C for up to two minutes. After treatments, viruses and bacteria were recovered and quantified using infectivity assays and selective media, respectively.

***Results:*** A sensory evaluation demonstrated the same sensory properties in treated versus non UV-C treated berries. Results were analysed by determination of tolerance intervals, instead of standard deviation. One log reduction of HAV and MS2 was ensured in 95% of cases, for fresh and frozen strawberries, raspberries, and blueberries with a treatment of 120 sec. Against MNV, the exposure of fresh blueberries for 120 sec was the only UV-C treatment that ensured a reduction of more than one log. For all three berry types, inoculated with the three bacterial cocktails, a one log reduction could not be ensured in 95% of cases.

***Significance:*** The present study shows the opportunities and weaknesses of the UV-C technology when applied to complex food matrices, such as berries.

## T7-06 Effect of a Novel Supercritical Carbon Dioxide ($CO_2$) Drying Process on Foodborne Pathogens Inoculated onto Coriander and Strawberries

**Siméon Bourdoux**[1], Stijn De Sutter[1], Sara Spilimbergo[2], Alessandro Zambon[2], Filippo Michelino[2], Mieke Uyttendaele[1], Frank Devlieghere[1] and Andreja Rajkovic[1], [1]Ghent University, Ghent, Belgium, [2]University of Padova, Padova, Italy

***Introduction:*** Dried foods are considered microbiologically stable foods, having a long shelf life at ambient temperature. However, even though dried foods show adverse conditions toward microbial growth, they may still host pathogenic microorganisms, which may proliferate upon rehydration. Although it is often assumed that drying processes have a lethal effect on vegetative organisms, little is known about bacterial pathogens survivability throughout drying processes.

***Purpose:*** In this study, we investigated the inactivation of three pathogens inoculated onto coriander and strawberries, after drying with supercritical $CO_2$.

***Methods:*** The fresh products, inoculated with three strainsof *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Salmonella* Thompson or *Listeria monocytogenes*, were treated with pure, supercritical $CO_2$ in the following conditions: (1) pressurization to 80 bar at 35°C, immediately followed by depressurization; (2) pressurization to 100 bar at 40°C, immediately followed by depressurization; and (3) pressurization to 80 bar at 35°C, followed by depressurization after 150 min. The depressurization rate was kept at 5 bar/min. Enumeration of *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* was performed by standard plate count.

***Results:*** After pressurization/depressurization, coriander lost less than 10% of its initial weight, whereas, after 150 min at 80 bar/35°C, the average mass loss was 88.7%. Independent of the type of treatments, four to six log inactivation of *E. coli* O157:H7 and *Salmonella* spp. strains were noted for coriander. *Listeria monocytogenes* strains were found to be more resistant, showing a four log reduction. On strawberry, *E. coli* O157:H7 and *Salmonella* spp. strains were only reduced by two to three log units, whereas in this case, *L. monocytogenes* strains were more susceptible, and again a four log reduction was noted.

***Significance:*** This study indicates that supercritical $CO_2$ can be used for drying, while offering a good inactivation of bacterial pathogens. Moreover, microorganisms resistance to the process were strongly influenced by the food matrix.

## T8 Technical Session 8 – Modeling and Risk Assessment 2

**Friday, 31 March – 8.30 – 10.00**

### T8-01 Applicability of Culture Medium-based Predictive Models to Food Scenarios Using *Bacillus cereus* as a Model Organism

**Nathália B. Silva**[1], Bruno A. M. Carciofi[1], Gláucia M. F. Aragão[1], Jozsef Baranyi[2] and Mariem Ellouze[3], [1]UFSC - Universidade Federal de Santa Catarina, Florianópolis, Brazil, [2]Imperial College, London, United Kingdom, [3]Nestlé, Lausanne, Switzerland

***Introduction*:** One of the issues that hinders practical applications of predictive microbiology models is the question of extent to which culture medium-based models can be used for real food systems. One possibility is to make use of the so-called bias factor, by which culture medium-based predictions on the microbial growth rate can be adjusted to a specific food scenario. The basis of this method is the assumption that this factor can be considered constant, in the region of interest of the studied environmental variables.

***Purpose*:** We investigated the validity of the above assumption in a sytematic way, using *Bacillus cereus* as a model organism.

***Methods*:** Three strains of *Bacillus cereus* were grown in RIF (Reconstituted Infant Formulae) and the maximum specific growth rates were estimated using viable count measurements at temperatures ranging from 12 to 25°C. Both the (i) square-root and the (ii) natural logarithm transformations, as link functions, were applied to the rates, in order to model the effect of temperature on them by (i) Ratkowsky-type square root and (ii) quadratic polynomial models, respectively. The results were compared with each other and with predictions from ComBase Predictor, using an appropriate bias factor.

***Results*:** The bias factor was fairly constant, 1.3 in our case, providing a convenient simplification that can be used, with confidence, between 12 and 25˚C, as a generic rule for the three strains. We also showed that the answer to the question, which link function should be preferred, depends on various other considerations; notably, how generic the model should be in terms of applicability to more strains and bigger range of environmental factors.

***Significance*:** These findings strengthen confidence in using culture medium-based predictive models for food scenarios, adjusted by constant (but food-dependent) bias factors, which can bring significant saving to the food industry.

### T8-02* Modelling the Effect of Different Storage Temperatures on the Growth and Toxin Production of *Staphylococcus aureus* in Milk

**Varalakshmi Sudagar**, Liesbeth Jacxsens and Mieke Uyttendaele, Ghent University, Ghent, Belgium

***Introduction:*** In India, 80% of the milk is produced by the rural producer and handled by an unorganized sector. The remaining 20% is handled by an organized sector. The maintenance of the cold chain is a difficult task; as most of the milk chilling centers are nonfunctional or underutilized. This leads to exposure of consumers to pathogenic bacteria and their toxins.

***Purpose:*** The purpose of this study was to assess the growth of *Staphylococcus aureus* and its toxin production using a Quantitative Microbial Risk Assessment (QMRA) model under different storage temperatures.

***Methods:*** A QMRA model was constructed using the published literature data, data for India and consumption from NDDB, India using two scenarios viz., high temperature and low temperature storage scenarios, and by using three modules to assess the exposure of *S. aureus* and SEA toxin in the population (India) that consumes the milk contaminated with *S. aureus*.

***Results:*** The mean dose exposure to toxins was calculated as 159.12 ng/serving. It was also found that in 95% of the cases, the probability of risk of illneess to at least one recipient was equal to or less than 0.00004. In 99% of the cases, the probability of illness due to the consumption of *S. aureus* contaminated milk is 0.00008.

***Significance:*** The results predicted that *S. aureus* levels could surpass the $10^5$ CFU/ml level of concern, at the 70th percentile of servings; and therefore, may represent a potential consumer risk to the SEA enterotoxin.

### T8-03 Quantification of the Survival of Pathogenic *Escherichia coli* during Meat Preparation

**Lucas Wijnands**[1], Ellen Delfgou-van Asch[1], Angelina Kuijpers[1], Jurgen Chardon[1], Annemarie Pielaat[2] and Eric Evers[1], [1]cZ&O/RIVM, Bilthoven, Netherlands, [2]Unilever R&D, Vlaardingen, Netherlands

***Introduction:*** Meat is, based on epidemiological data, considered an important transmission route for pathogenic bacteria. Although inactivation through heating has been a subject of investigation, with respect to evaluation of possible health risks, quantification of inactivation is often lacking.

***Purpose:*** The goal of this project was to quantify survival of pathogenic bacteria during meat preparation, for risk assessment calculations, with a special interest on the side of the steak. The methods for preparation of the steaks were those most commonly recommended for the various types of readiness.

***Methods:*** Steaks were contaminated with *Escherichia coli* O111 (cured, i.e., lacking the genes for shiga toxins) and fried in butter according to a standardized protocol. Frying times were 2 minutes per side (rare), 4 minutes per side (medium) and 6.5 per side minutes (done). After frying, steaks were left for three minutes and, subsequently, the number of surviving bacteria was determined by culture method. During frying, the temperature at the top and side of the steak, was determined with an Infrared Thermal Imager. Appropriate controls were part of the investigations.

***Results:*** Increase in frying time led to a fast decrease in survival of *E. coli*. Decrease in survival on top/bottom of the steaks was two to three $\log_{10}$ units higher than on the side of the steaks. Overall, a decrease in numbers of *E. coli* ranged from two to three $\log_{10}$ units. Two samples from the 2 minutes per side frying time and two samples from the 6.5 frying time per side samples showed seven to eight $\log_{10}$ unit reductions.

***Significance:*** The temperature profile, in combination with the inactivation data, allowed for the calculation of D/z-values. This is important for risk assessment calculations, for any meat type and preparation method.

## T8-04 A Simple Concept Allowing the Prediction of Microbial Inactivation under Non-isothermal Process, Taking into Account Non-log-linear Inactivation Kinetics

**Noémie Desriac**[1], Mikael Vergos[2], Ivan Leguerinel[3], Leguerinel[3], Veronique Huchet[4], Louis Coroller[5] and Olivier Couvert[2], [1]LUBEM UBO University – UMT14.01SPORE RISK, Quimper, France, [2]LUBEM – University of Brest- UMT 14.01 SPORERISK, Quimper, France,[3] Universite De Brest, Quimper, France,[4] ADRIA Development–UMT 14.01 SPORERISK, Quimper, France, [5]University of Brest- UMT 14.01 SPORE RISK, Brest, France

***Introduction:*** During pasteurization and sterilization, microorganisms are exposed to non-isothermal process, as a consequence of the heating penetration. Nevertheless, nowadays, microorganism heat parameters are obtained under isothermal conditions; and furthermore, the prediction becomes more complicated, when the inactivation kinetics are non-log-linear. Thus, the investigation of thermal models that may accurately predict heat inactivation for non-isothermal heat treatments is a topic of interest for food industry.

***Purpose:*** In this study, we investigated the performance of two published models (Valdramidis et al., 2011 and Peleg et al., 2000) and one new one.

***Methods:*** Briefly, the proposed model converts the non-isothermal profile into an isothermal profile for a given temperature and could be combined with both a linear or Weibull primary model for further prediction. To compare the different models, heat resistance parameters of *Bacillus pumilus* E71 were estimated from 11 non-log-linear inactivation kinetics (68°C-101°C), obtained under isothermal conditions. Thereafter, inactivation kinetics were acquired under seven temperature profiles and performances of the simulation were assessed statistically (RMSE, $A_f$ and $B_f$).

***Results:*** Calculated RMSE varied from 0.14 to 2.25 for all predictions and for six temperature profiles. The new model combined with a Weibull model gave the lowest forecasting error. According to the bias factor, Peleg model 'fail-safe' for six temperature profiles, Valdramidis 'fail-dangerous' for all, and the proposed model over and under predicted three and four times, respectively. The accuracy factors closest to one, obtained for the new model, were mixed; indicating that the average estimate was more accurate for this model. All these results underlined that the proposed model was more robust than the others for prediction of heat inactivation under non-isothermal treatment.

***Significance:*** In conclusion, the present study provides a new model, which could be used to predict the microbial heat inactivation under non-isothermal process and, thus, can lead to effective management systems for the optimization of the pasteurization or sterilization steps.

## T8-05 Accurate Quantification of *Campylobacter* Contamination on Chicken Carcasses Including Variability and Uncertainty

Benjamin Duqué, Samuel Daviaud, Sandrine Guillou, **Nabila Haddad** and Jeanne-Marie Membré, UMR1014 SECALIM, INRA, Oniris, Nantes, France

***Introduction:*** *Campylobacter* is a foodborne pathogen, highly prevalent in poultry, and the primary cause of enteritis in humans. In this context, a project between academics and industrials was set up to evaluate, accurately, the level of contamination at different steps of process in different slaughterhouses.

***Purpose:*** The purpose of this study was to evaluate, accurately, the contamination level of *Campylobacter* spp. on chicken carcasses.

***Methods:*** From a large dataset with censored data (concentration less than 10 CFU/g), several factors were considered; including, the month of sampling, the age of chickens (<50 days *vs* >50 days), the farming method (standard *vs* quality label), and the sampling area (neck *vs* leg). First, data were fitted by different distributions considering uncertainty and variability, separately. Then, the effect of factors was assessed taking the uncertainty into account. All analyses were performed in R.

***Results:*** Among factors studied, only those associated with the sampling period and area were shown to significantly affect the contamination level of *Campylobacter* spp. Thereby, two distributions of contamination levels were obtained per season (cold *vs* warm) and per sampling area. During the warm season, the mean contamination level was 2.6 (2.4; 2.8) log CFU/g and 1.8 (1.5; 2.0) log CFU/g for neck and leg, respectively. In contrast, during the cold season, the contamination level was 1.0 (0.6; 1.3) log CFU/g and 0.6 (0.3; 0.9) log CFU/g for neck and leg, respectively. Uncertainty was small (ca. 0.5 log) in comparison to variability (3 log or more), showing an accurate quantification of contamination, even with censored data.

***Significance:*** An accurate quantification of contamination level could enable industrials to better adapt their processing and hygiene practices. These results will help in refining exposure assessment models.

## T8-06 An Integrated Approach to Process Qualification

**Bertrand Colson**, QuoData GmbH, Dresden, Germany

***Introduction:*** In order to ascertain that processed food conforms to safety regulations, producers are required to conduct process qualification trials, in which a battery of parallel data are collected. If, for each data series, a separate analysis is conducted, it is not unlikely that some series will not comply with the criteria. Since the statistical tests are conducted in parallel, however, there is no way of determining whether the non-conformities are purely random or manifestations of an out-of-control process. The question, thus arises, whether a more efficient process qualification can be achieved, if the data analysis is carried out simultaneously on the basis of all available data series.

***Purpose:*** Accordingly, an "integrated approach" to process qualification was implemented in the statistical analysis of a process qualification trial. The aim was to examine whether the integrated approach affords deeper insights into the causes of observed non-homogeneities.

***Methods:*** Data were collected for four different parameters, across settings corresponding to three different production factors; three different time points; and three different sampling locations. An appropriate variance components model was applied to the data in order to obtain estimates of the influence the different settings had on the homogeneity.

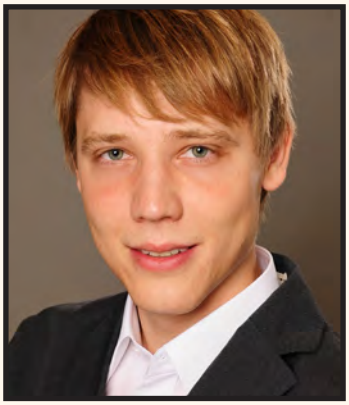
***Results:*** For some parameters, the approach allowed the identification of a fundamental variability in the analytical component of total variability. Since this source of error does not concern the process, it was deemed legitimate to subtract the corresponding heterogeneity in the assessment of the quality of the process per se. Moreover, the variability of one of the production factors was found to be significantly higher than the others; thus, providing crucial information in terms of improving process homogeneity.

***Significance:*** It was concluded that the implementation of effective statistical approaches in process qualification can play an important role in enhancing the reliability of process qualification; thus, ensuring that food safety criteria are satisfied.

# EUROPE STUDENT TRAVEL SCHOLARSHIP


## Christian Hertwig

**Technical University of Berlin**
**Berlin, Germany**

Christian Hertwig is a Ph.D. student at the Technical University of Berlin in the Department of Food Biotechnology and Food Process Engineering, located in Berlin, Germany. Since 2013, he has worked as a research associate at the Leibniz Institute for Agricultural Engineering in the Department of Horticultural Engineering and Bioeconomy in Potsdam, Germany, and in the Quality and Safety of Food and Feed Research Program.

Mr. Hertwig's research is focused on novel non-thermal preservation technologies, with an emphasis on the inactivation of bacterial spores using cold atmospheric pressure plasma. His research was awarded with the "Best Poster Presentation Award" at the 12th International Congress on Engineering and Food (ICEF12) in Quebec, Canada in 2015. He was also awarded third place of the "Bühler Food Engineering Award" at the 10th European Workshop on Food Engineering and Technology in Uzwil, Switzerland in 2016, and won the "Non-thermal Processing Division Graduate Paper Competition" at the Institute of Food Technologists' (IFT) 2016 Annual Meeting in Chicago, Illinois. In addition, he is a Student Representative in Europe for IFT's Non-thermal Processing Division.

Mr. Hertwig is grateful to receive IAFP's European Student Travel Scholarship, which provides an excellent opportunity to attend the European Symposium on Food Safety in Brussels, Belgium. He looks forward to meeting and networking with professionals from academia, government and industry in the field of food safety to exchange knowledge and establish new collaborations.

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# POSTER ABSTRACTS

29–31 March 2017 – Brussels, Belgium

# POSTER ABSTRACTS

## Wednesday, 29 March – 10.30 – 16.00

### P1 Poster Session 1 – Antimicrobials; Beverages and Acid/Acidified Foods; Communication Outreach and Education; Dairy; Epidemiology; Food Chemical Hazards and Food Allergens; Food Defense; Food Law and Regulation; Food Processing Technologies; Food Safety Systems; Food Toxicology'

### P1-01* Antibacterial and Resistance-modifying Activities of Carvacrol and p-Cymene against *Listeria monocytogenes*

**Ahmad Mouwakeh**[1], Sonja Smole Možina[2], Anja Klančnik[2], Katarina Šimunović[2], Gabriella Kisko[1] and Csilla Mohacsi-Farkas[1], [1]Szent Istvan University, Budapest, Hungary, [2]University of Ljubljana, Ljubljana, Slovenia, [3]

*Introduction: Listeria monocytogenes*, a Gram-positive, facultative intracellular bacterium, is capable of causing serious human infections and is, generally, susceptible to a wide range of antibiotics. However, during the last few years, increasing numbers of strains have been reported as resistant to one or more antibiotics. Carvacrol and p-cymene are two monoterpenes that are constituents of many essential oils, including oregano and thyme. They have been proven as efficient antimicrobials.

*Purpose:* The aim of this study was to investigate the effect of carvacrol and p-cymene on *L. monocytogenes* by studying their antimicrobial and resistance-modifying activity.

*Methods:* In this study the MICs of carvacrol, p-cymene and ampicillin (Amp) were determined in the absence and presence of sub-inhibitory concentration of carvacrol and p-cymene (1/2 MIC) on 10 *L. monocytogenes* strains. In addition, ethidium bromide accumulation assays were carried out to determine the influence of carvacrol and p-cymene (1/2 and 1/4 MICs) on the cell efflux activity, where reserpine (chemical efflux pump inhibitor) was used as control. The LIVE/DEAD *Bac*Light cell viability assay was carried out for determining cells membrane integrity in the absence and presence of carvacrol and p-cymene (1/2 and 1/4 MICs).

*Results:* Carvacrol and p-cymene inhibited the growth of all *L. monocytogenes* strains. Carvacrol, in combination with Amp, increased the susceptibility of *L. monocytogenes*; a reduction in the MIC was observed (up to 16-fold). No effect was observed with p-cymene in combination with Amp. The ethidium bromide accumulation increased in the presence of carvacrol or p-cymene and was comparable to reserpine. Membrane integrity disintegrated.

*Significance:* These data warrant further studies on the use of carvacrol and p-cymene in the control of antibiotic resistance in *L. monocytogenes*.

### P1-02 Effect of Hurdle Technology on Caviar Microbial Flora

**Maryam Khakbaz Heshmati**[1], Mohammad Amin Hejazi[2], Hajie Lotfi[3], Nasser Hamdami[4] and Somayeh Zolfaghari[4], [1]University of Tabriz, Tabriz, Iran, [2]Agricultural Biotechnology Research Institute of Iran (ABRII), Tabriz, Iran, [3]Tabriz University of Medical Sciences, Rabriz, Iran, [4]Isfahan University of Technology, Isfahan, Iran

*Introduction:* Besides the traditional processing method, there are various methods to extend the shelf life of caviar.

*Purpose:* The main objective of this study was to evaluate the capabilities of *Zataria multiflora* essential oil (0-0.06%(W/W)), nisin (0-18mg/kg), and potassium sorbate (0-1000mg/kg) combinations on caviar processing.

*Methods:* Efficacy of the above hurdles was assessed using response surface methodology (RSM) and identification of caviar microbial flora.

*Results:* Results revealed that potassium sorbate, *Zataria multiflora* essential oil, and nisin were effective treatments for inhibiting the total count of caviar samples; although, the nisin was not as effective as potassium sorbate and *Zataria multiflora* essential oil. The RSM study indicated that the quadratic model for total count was sufficiently accurate to predict the corresponding response as a function of variable concentrations. Optimum concentration for caviar processing, with the least total count, was determined to be 1000 mg/kg potassium sorbate, 0.06% *Zataria multiflora* essential oil, and 6 mg/kg nisin. For identification of caviar microbial flora, one of the caviar colonies was isolated and a total of 1356 nucleotides from the 16S rDNA gene was sequenced.

*Significance:* Through performing BLAST, the sequence showed significant similarity (≥99%) to the 16S rDNA of known *Rahnella aquatilis* strains. The 16S rDNA gene sequence from the isolate was submitted to NCBI (accession number JQ968608).

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## P1-03 Analysis of the Activity of the Essential Oil of Oregano against Planktonic and Biofilm Forms of *Listeria monocytogenes* and *Staphylococcus aureus* Strains

Maria Grazia Cusimano[1], Domenico Schillaci[1], Sergio Migliore[2], Piera Nocera[2], Benedetta Amato[3], Vincenzo Di Marco Lo Presti[3], Vincenzo Arizza[1] and **Maria Vitale**[2], [1]University of Palermo Dept. of Biological, Chemical and Pharmaceutical Science and Technology (STEBICEF), Palermo, Italy, [2]Istituto Zooprofilattico Sperimentale of Sicily, Palermo, Italy, [3]Istituto Zooprofilattico Sperimentale of Sicily, Barcellona P.G., Italy

*Introduction:* *Listeria monocytogenes* is widely spread in the environment and its pathogenic strains are dangerous contaminants in the food industry. In this community, bacteria can become intrinsically resistant to antimicrobial drugs and/or sanitation procedures, causing persistent infection and/or environmental contamination.

*Purpose:* The aim of research was to test essential oil from oregano, as a new antimicrobial agent, against pathogenic bacteria using strong biofilm-producing strains of *Listeria monocytogenes* and *Staphylococcus aureus.*

*Methods:* Biofilm assays were performed, using 20 *L. monocytogenes* isolates plus the reference strain *Listeria* ATCC 7644 with crystal violet stain and 20 isolates of S. *aureus* plus the reference strains *Staphylococcus epidermidis* RP62A and *S. aureus* ATCC 25923 with safranin stain, to test biofilm production capabilities. The essential oil from *Origanum vulgare* subsp. *hirtum* was tested against the stronger biofilm-producing strains of both pathogens, in planktonic and sessile forms.

*Results:* MIC values of 800µg/ml were obtained in planktonic forms of both pathogens, but some strains of *L. monocytogenes* showed a MIC of 400µg/ml. Sub-MIC levels were used to analyze inhibition of biofilms. An average of 25% inhibition was obtained for *L. monocytogenes* in contrast to 68% in *S. aureus*. Each assay was performed in triplicate and repeated at least twice.

*Significance:* A slightly better activity of essential oil from oregano was observed against planktonic forms of *L. monocytogenes* compared to *S. aureus;* however, biofilm inhibition activity was more effective in *S. aureus* than in *L. monocytogenes* biofilms. New antimicrobial strategies, addressing both planktonic and sessile forms of food transmitted pathogenic bacteria, are important to counteracting the overgrowing diffusion of multidrug resistance.

## P1-04 The Inhibitory Effect of a Sodium-free Powder Preservative on the Growth of *Listeria monocytogenes* and Lactic Acid Bacteria in Turkey Ham Applications

**Eelco Heintz**, Niacet, Tiel, Netherlands

*Introduction:* Organic acids are effective antimicrobials against *Listeria monocytogenes* and Lactic Acid Bacteria (LAB). Current organic acid based preservatives are, mostly, liquids; powdered forms contain sodium. This document compares the antimicrobial efficacy of an organic acid-based, sodium-free powder preservative (Provian®K) to the commonly used liquid preservatives.

*Purpose:* This study aimed to evaluate the antimicrobial efficacy of a sodium-free preservative powder in cured and uncured turkey ham applications.

*Methods:* Deli-style turkey formulations were prepared (75% moisture, 1.9% salt, 6.2-6.4 pH). Analysed treatments: 1.) Control (no antimicrobials); 2.) 2.5% potassium lactate –sodium diacetate 60% solution (PL-SD); and 3.) sodium-free acetate/diacetate powder (Provian®K): 0.25%, 0.5%, and 0.75%. Surfaces were inoculated with approximately 3 log CFU/g using a cocktail of various serotypes of *L. monocytogenes* or different species of LAB isolated from meat products. Packages were stored at 4°C and 7°C.

Inoculated samples from each treatment (n = 3) were assayed by rinsing and massaging the meat in Butterfield's phosphate buffer for three minutes. Rinsates were serially diluted and enumerated on appropriate media. *Listeria* populations were determined by surface plating onto MOX agar (35°C, 48h); LAB plate counts by plating onto APT agar with bromcresol purple (25°C, 48h).

*Results:* All treatments showed clear growth inhibition of *Listeria* and LAB. ANOVA with n = 3 was used to determine statisitcal significance. At 4°C all treatments show complete inhibition of *Listeria* growth for 12 weeks. At 7°C, 0.5% Provian®K samples showed comparable inhibition to formulations with 2.5% PL/SD ($P > 0.05$). In uncured meat, 0.25% Provian®K and 2.5% PL/SD performed comparably ($P > 0.05$). Shelf-life extension (LAB inhibition) was most effective in when formulations included Provian®K ($P > 0.05$).

*Significance:* This research shows excellent antimicrobial efficacy of a sodium-free powder antimicrobial in improving the safety and shelf life of processed meats. Besides improvements in handling, shipping, and storage, compared to liquid forms, this powder is sodium-free, making it suitable for sodium reduction programs.

## P1-05 Fungal Strains and the Development of Tolerance against Natamycin

**Jan Dijksterhuis**[1], Alex E.E. Verkennis[1], Robbert Jacobs[1], Angelina Dekker[2], Jacques Stark[3] and Hugo Streekstra[2], [1]CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan, Utrecht, Netherlands, [2]DSM Food Specialties, Delft, Netherlands, [3]NZO, Den Haag, Netherlands

*Introduction:* Antimicrobial resistance is a relevant theme with respect to both antibacterial and antifungal compounds.

*Purpose:* In this study, we address the possible development of tolerance against, the antifungal food preservative, natamycin.

*Methods:* A selection of 20 fungal species, originating from a medical, as well as a food product context, was subjected to increasing concentrations of natamycin for prolonged time, a procedure designated as "training".

*Results:* The range of Minimum Inhibitory Concentrations (M.I.C.) before (1.8-19.2 μM) and after (1.8-19.8 μM) training did not change significantly; but, natamycin exposure caused an increase of M.I.C. in 13 out of 20 tested strains. The average M.I.C. increased from 6.1 to 8.6 μM and four strains showed a two-fold increase of tolerance after training. One strain of *Aspergillus ochraceus*, also, showed increased tolerance to amphotericin B and nystatin. However, two *Fusarium* strains showed similar or decreased tolerance for these other polyene antifungals.

*Significance:* The work reported here shows that a continuous and prolonged increasing selection pressure induced natamycin tolerance in individual strains. This implies that such a selection pressure should be avoided in the technical application of natamycin to continue to ensure its safe use as a food preservative.

## P1-06 Characterisation of the Antimicrobial Effects of Liquorice Extract: Selective Inhibition of a Broad Range of Gram-positive Bacteria

**Madiha EL Awamie** and Cath Rees, The University of Nottingham, Nottingham, United Kingdom

*Introduction:* Liquorice extract is commonly used as a food flavouring and we have, previously, shown that a waste material from the production of liquorice extract has antimicrobial activity against the Gram-positive *Listeria* spp. Reduction in the bioluminescence levels of strains carrying the bacterial *luxABCDE* genes indicated that growth inhibition was due to a reduction in levels of metabolism. *Bacillus* spp., *Brochthrix* spp., and Lactic Acid Bacteria (all Gram-positive) commonly contribute to the spoilage of cooked meats and *Listeria monocytogenes* contamination is a particular problem associated with sliced deli meats.

*Purpose:* The purpose of this study was to further investigate the mechanism of action of the liquorice extract and towards its application as a food preservative for sliced meats.

*Methods:* Bacteria were treated with an inhibitory (50 μg $ml^{-1}$) or sub-inhibitory (12.5 μg $ml^{-1}$) concentration of the extract. Growth was assessed by viable count or optical density. Metabolic levels were monitored by measuring bioluminescence levels of strains carrying the bacterial *luxABCDE* genes. Effects on membrane integrity were monitored using a LIVE/DEAD®viability stain and fluorescence microscopy.

*Results:* LIVE/DEAD® staining of *L. monocytogenes,* exposed to 50 μg $ml^{-1}$, resulted in an increasing proportion of red-stained (dead) cells over time. Challenge tests indicated that 50 μg $ml^{-1}$ of extract, also, inhibited the growth of a range of Gram-positive bacteria including *Bacillus subtilis, Staphylococcus aureus* and *Enterococcus faecalis*. LIVE/DEAD® staining of *B. subtilis* confirmed that this was, also, due to damage to the membrane integrity. No effect was seen on the growth of the Gram-negative bacteria *Escherichia coli*, *Salmonella* Typhimurium or *Pseudomonas fluorescens*.

*Significance:* This novel antimicrobial extract could be of value to inhibiting the growth of *L. monocytogenes* and other Gram-positive spoilage organisms, when applied to the surface of sliced deli meats.

## P1-07 Elucidating the Probiotic and Technological Potential of Yeasts Isolated from Fermented Table Olives

Stamatoula Bonatsou, Marina Karamouza, George-John Nychas and **Efstathios Panagou**, Agricultural University of Athens, Athens, Greece

*Introduction:* Many yeast species have been reported to exhibit important probiotic characteristics, which may exert beneficial effects on the host. More specifically, resistance during the passage through the gastrointestinal tract, adhesion to intestinal cells, inhibition of pathogens, degradation of non-assimilated compounds, and reduction of cholesterol levels are some of the most important features.

*Purpose:* The purpose of this study was to assess, on the laboratory scale, the probiotic potential and technological characteristics of 50 yeast strains, previously isolated from spontaneously fermented Greek natural black table olives.

*Methods:* All yeast strains were studied for their survival in conditions simulating the passage through the human gastrointestingal tract. The estimation of the survival was performed by enumeration of viable colonies on Yeast – Mold agar, before and after incubation in synthetic gastric and pancreatic juice. Furthermore, enzymatic activities were determined using the API-ZYM test (bio-Mérieux) according to the instructions of the manufacturer. Finally, the resistance to different salt concentrations under diverse pH values was assessed by estimating the non-inhibitory and minimum inhibitory concentration values (NIC, MIC) by spectrophotometry.

*Results:* From the total number of the studied yeast isolates, 23 strains (> 70%) showed a high rate of survival under the simulation of gastric and pancreatic digestion. Moreover, a strain belonging to *Saccharomyces cerevisiae* showed a survival rate up to 90% in the overall digestion. All strains showed lipolytic and proteolytic properties; whereas, 27 strains exhibited β-glucosidase activity and 14 strains exhibited α-glycosidase activity. For the three estimated pH values (3.5, 5.0 and 6.5), NIC median values were 4.8, 6.1 and 7.7, respectively, and MIC values were 10.9, 13.3, and 11.6, respectively.

*Significance:* These data suggest the potential use of yeasts as probiotics to enhance the potential of fermented table olives as functional food.

## P1-08 The Effect of *Lactobacillus plantarum* on the Ochratoxigenic Potential of *Aspergillus carbonarius* at the Gene Expression Level

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*Introduction:* From a food safety perspective, Ochratoxin A (OTA) remains a challenge in the continuous effort to eliminate toxins from the food chain. *Aspergillus carbonarius* stands among the dominant toxigenic producers in a variety of foodstuffs, including table grape berries.

*Purpose:* In this work, the inhibitory effect of *Lactobacillus plantarum* against *Aspergillus carbonarius* was investigated in terms of anti-ochratoxigenic activity, along with of OTA-related gene expression.

*Methods:* One wild fungal isolate and a reference strain of *Aspergillus carbonarius* were co-cultured with four bacterial strains of *Lactobacillus plantarum* on MRS agar plates, incubated at 30°C. Mycelia were collected for toxin quantification after 3 days growth. Gene expression of OTA biosynthetic key genes *nrps* and *pks* was monitored at the same time using Real-Time PCR.

*Results:* HPLC analysis of OTA presentedown-regulationd inter-strain differences among bacterial efficacy for toxin reduction. OTA production was decreased by 51-100%. Gene expression analysis showed differences between the fungal strains. In *pks* gene, up to three-fold down-regulation was observed in almost all bacterial treatments. In contrast, the *nrps* transcripts showed up to a three-fold down-regulation in the wild strain and up to a nine-fold up-regulation in the reference strain.

*Significance:* According to the above findings, the specific microbial strains have been proven to successfully control mycotoxin and, also, reveals a possible mode of action at the molecular level.

## P1-09 Educational Instruments for Food Safety and Nutrition

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*Introduction:* One strategy to the reduce foodborne diseases (FD) burden is prevention and health promotion; increasing the student population's health literacy.

*Purpose:* To empower students to develop healthier life styles, we developed and implemented school educational materials on food safety and nutrition. The curricula was adapted at different school levels, in collaboration with the respective teachers.

*Methods:* Development and implementation of school educational materials: 1) Foodborne outbreaks investigation data from 2009 to 2013, obtained at National Institute of Health (INSA), were compiled and analysed. This led to the identification of their risk occurrence and contributing factors. Good practices that elaborated the educational materials were scientifically evidenced. 2) The Portuguese Food Composition Table (FCT) edited and disclosed by INSA was used in order to learn how to assess nutritional intake and how to gain understanding of the consequences of food choices. 3) The developed materials aimed to highlight the importance of understanding the information contained in the labelling of foodstuffs, in order to make informed choices. Educational materials on food safety, nutrition, and labelling, adjusted for different educational stages, were prepared and disseminated on the INSA website and in schools near the selected teachers.

*Results:* Currently, FD prevention materials (a Guide for Consumer Good Practices, two slide presentations, one flyer and one questionnaire), are available on the INSA website and were presented to several teachers in the Lisbon area, to inform them about the importance of sharing this knowledge in their classes.

*Significance:* Education in healthier and safer food practices is crucial for the prevention of FD and to help students make better and more informed food choices. The understanding of the concepts and importance of all aspects of food safety and nutrition, from early stages and along all levels of undergraduate school, is the key to a healthier next generation of adults.

## P1-10 FSMA Rules: For the Right Approach, Small and Medium-sized Enterprises (SME) Need the Food Safety Culture Method

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*Introduction:* Within the seven iimplementing regulations of the current Food Safety Modernization Act, we, repeatedly, read the phrase: "Individuals must be qualified by education, training, or experience to manufacture, process, pack or hold vertical food." This highlights an important concept that is still little known in Europe: the food safety culture (FSC).

*Purpose:* The heart of the new US regulations is strongly related to this new concept of FSC. FSC involves the whole company, from top management to the workers, in order to produce the range of preventive procedures necessary to give evidence of their behavior and to trace their responsibility. In Europe, this approach has been recently introduced by a voluntary standard, the BRC V. 7. Clarification of this method to European small and medium-sized enterprises (SME) will be treated in this work.

*Methods:* The level of FSC in 60 European manufacturing companies was measured according to the matrix of the FSC proposed by Lone Jespersen (Food Safety Culture: Measure What You Treasure, 2015). Between June to December, 2016, these companies were active in training, qualification of their PCQI for PCHF, and drafting their own Food Safety Plan.

*Results:* About 85% of companies did not understand the concept of "Education" related to FSC; 13.5% of companies demonstrated a low level of FSC, and only 1.5% showed a good level of involvement throughout the entire company in demonstrating behaviors and responsibilities in line with the FSC. Procedures, documentation, and reviews have been poorly implemented.

*Significance:* The large volume of documents requested by the FSMA can be supported, only, with the understanding of the importance of implementation of an appropriate/right FSC method. Without that, domestic and foreign companies will find themselves in trouble.

## P1-11 Probiotic Substances: Comparison between Yak Milk and Camel Milk

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*Introduction:* In recent years, the concept of providing functional foods, including beneficial probiotic components, has been gaining attention. Fermented camel milk, named shubat, and fermented yak milk, are known for their medicinal and dietary properties.

*Purpose:* The aim of this work was to identify the microflora of fermented milks that have a role in the aroma, texture, and acidity. The therapeutic role can be explained by improvement of digestive properties and the biological activity linked to the antimicrobials properties.

*Methods:* This analysis was the first to produce proteomic data for milks from the above-mentioned animal species. The results of principal component analysis showed significant differences in proteomic patterns among camel, cow, and yak milk.

*Results:* Microflora of traditional fermented milk can be applied for organizing industrial production of traditional fermented products. Probiotic microflora was able to inhibit the growth of the Gram negative, tested bacteria, as shown by production of an inhibition zone.

*Significance:* This study highlighted the biodiversity of microflora available in fermented milk and can help us establish a starter kit with probiotic Lactic Acid Bacteria for activation of the fermentation process. Probiotic activities are, interestingly, able to prevent some bacterial adhesion and inhibit bacterial growth in the mouth (oral health prevention) and in the gastrointestinal tract (prevention of inflammation). Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may, ultimately, influence health.

## P1-12 Investigation of the Effect of Initial Contamination Level of *Salmonella* on the Behaviour of Yoghurt Starter Cultures during Milk Fermentation

**Derya Savran** and A. Kadir Halkman, Ankara University, Ankara, Turkey

*Introduction:* The behaviour of yoghurt starter cultures influences the microbial and physicochemical properties of the product. If hygiene practices are not adequate, contamination by foodborne pathogens can occur during the production of fermented milk products.

*Purpose:* This work was conducted to investigate the effect of initial contamination level of *Salmonella* Enteritidis on the behaviour of yoghurt starter cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) during milk fermentation.

*Methods:* The counts of yoghurt cultures were determined every 30 min by plate count method. To find the significance among the initial contamination levels of *Salmonella* Enteritidis on the behaviour of yoghurt starter cultures, the one-way ANOVA was utilized using the SPSS software (version 11.5). The experiments were repeated three times.

*Results:* The counts of yoghurt cultures contaminated by *Salmonella* (approximate level of 3, 5, 7 log CFU/mL) were found to be 7.07 ± 0.07, 7.17 ± 0.15 and 7.00 ± 0.12 log CFU/g, respectively, for *Streptococcus thermophilus* and 6.74 ± 0.31, 6.88 ± 0.22 and 6.58 ± 0.05 log CFU/g, respectively, for *Lactobacillus bulgaricus* at the beginning of the fermentation. For the same initial *Salmonella* contamination levels, the counts were found to be 8.25 ± 0.06, 8.30 ± 0.14 and 8.18 ± 0.20 log CFU/g, respectively, for *Streptococcus thermophilus* and 8.81 ± 0.11, 8.86 ± 0.06 and 8.89 ± 0.14, respectively, for *Lactobacillus bulgaricus* at the end of the fermentation. On one hand, the initial contamination level of *Salmonella* did not significantly influence the behaviour of yoghurt starter cultures during fermentation ($P > 0.05$). On the other hand, *Salmonella* survived in milk throughout the fermentation process for all contamination levels.

*Significance:* Even though the behaviour of yoghurt starter cultures are not affected by high contamination level of *Salmonella,* the pathogen may still survive during fermentation, even at a low contamination levels, leading to significant risk to consumers.

## P1-13 Does *Campylobacter* in Raw Milk Pose a Threat to Human Health?

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*Introduction:* Besides poultry meat, raw milk is a potential source of human *Campylobacter* infections. Nowadays, raw milk consumption is increasing, as some people claim that raw milk is more nutritious than heated milk. However, raw milk may contain pathogens.

*Purpose:* This study determined the prevalence of *Campylobacter* in raw cow milk in Flanders, both by conventional culturing and molecular techniques to include detection of nonculturable strains/species.

*Methods:* Two hundred raw milk tank samples were collected in Flanders. Bacteriological analysis was performed according to ISO10272-1, but with Bolton Broth and Preston Broth, in parallel. To detect nonculturable campylobacters, DNA was extracted from the raw milk samples. Beforehand, two commercial kits (PowerFood, Mobio Laboratories and AdiaPure, Biomérieux) were evaluated to determine their detection limit for *Campylobacter* DNA extracted from inoculated milk. The kit that performed best was used to extract DNA from the 200 milk samples. This DNA was used for *Campylobacter* genus detection.

*Results:* By conventional culturing, a prevalence of 0.5% was noted with Bolton Broth and 1% with Preston Broth. The isolates were identified as *Campylobacter jejuni*. The AdiaPure kit, with a detection limit of 1,000 CFU *Campylobacter*/ml raw milk, performed best. But, none of the 200 samples were positive after DNA extraction and PCR; meaning that the number of *Campylobacter* in the milk samples was below the detection limit of the kit and PCR.

*Significance:* By conventional culture, a prevalence of 1% was noted, which is quite low compared to the prevalences reported in other countries. Further, this study showed that molecular methods were not able to detect *Campylobacter*, culturable or nonculturable, in raw milk. So, the true prevalence may be higher,

although the number of *Campylobacter* may be low. Considering the low infection dose of *Campylobacter*, consuming raw milk is not recommended.

## P1-14 Reduction of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in Colostrum: Positive Effects for the Public Health?

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*Introduction: Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes a chronic enteritis in cows that reduces milk yields and is often lethal. MAP is, possibly, associated with Crohn's disease in humans. To reduce MAP intake of consumers, the MAP presence on a farm should be reduced. A contamination route for calves is ingestion of MAP contaminated colostrum, the first milk after parturition, which is rich with viital immunoglobulins.

*Purpose:* This study aimed to reduce MAP in colostrum using curdling (for on-farm treatment) and centrifugation (for off-farm treatment at a central location).

*Methods:* Different conditions for curdling and centrifugation were evaluated, such as rennet type and concentration; centrifugation speed and time; and diluting colostrum and skimming before treatment. The total protein and immunoglobulin concentration were determined by the Coomassie Bradford assay and gel filtration, respectively. An animal experiment was conducted (24 calves; 4 groups; health parameters, IgG blood value, and consumability).

*Results:* The on-farm protocol included diluting the colostrum with water (2/1), curdling with 2% calf rennet, separation of whey and curdle, and adding milk powder to the whey before administration to the calf. With this protocol, one log MAP reduction/ml colostrum was observed. The off-farm protocol included diluting the colostrum in a two to one ratio with skimmed colostrum and subsequent skimming and clarifying. On average more than 1.5 log reduction in MAP presence and a limited reduction in proteins and IgGs was seen. We found no significant differences between test and control groups, during the animal experiment.

*Significance:* We developed two protocols for MAP reduction in colostrum, which could lead to a reduction of MAP presence in cows, and could be, successfully, used in the Belgian MAP reduction program on dairy farms. This program is an important measure for reducing the risk of MAP intake by consumers.

## P1-15 Thermal Death Kinetics of *Bacillus sporothermodurans* Spores Isolated from UHT Milk

Rodney Owuso-Darko and **Elna Buys**, University of Pretoria, Pretoria, South Africa

*Introduction: Bacillus sporothermodurans* is a rod shaped mesophilic bacteria producing highly heat resistant spores (HRS), which can survive ultra-high temperature (UHT) milk processing. The presence of these spores may have implications on the quality and safety of UHT milk and other thermally processed food products. Under UHT conditions, *B. sporothermodurans* has been found to be more resistant than other heat resistant spores, with $D_{140}$ranging from 3.4 s – 7.9 s.

Purpose: The purpose of this study was to establish the heat inactivation kinetics of selected *B. sporothermodurans* strains with the aim of improving thermal validation during UHT food processing. And consequently, evaluate the performance of the linear, Weibull, and biphasic models of inactivation with the aim of establishing the best fit for *B. sporothermodurans*in UHT milk processing.

*Methods: Bacillus sporothermodurans* spores were prepared by dispensing 1 mL of culture onto the sporulation medium (nutrient broth (25 g/L), bacteriological agar (15 g/L), vitamin $B_{12}$ (1 mg/L), $MnSO_4.H_2O$ (8.4 mg/L) and $CaCl_2.2H_2O$ (1 g/L), pH 6.8)), and harvested using physiological saline (8.5 g/L). Sterile milk was inoculated with the bacterial spores to a concentration of approximately $2x10^7$spores/mL. Thermal inactivation analysis was undertaken and subsequent modelling of the data using the linear, Weibull and biphasic models.

*Results:* The survival curves indicated a good fit for the nonlinear models for the selected strains. At 130°C tailing of curves started after approximately 30 s and 35 s treatment time, corresponding to a two-$log_{10}$ reduction. The Weibull model consistently proved a better fit than the biphasic and linear models after computation of the mean square error (0.10, 0.14, and 0.77, respectively) and correlation coefficient (0.99, 0.98, and 0.86, respectively).

*Significance:* The Weibull model should provide the best model for use in thermal inactivation of *B. sporothermodurans* in UHT milk processing in the food industry.

## P1-16 Modelling the Effect of Acid and Salt Stress on the Survival and Diversity of *Listeria monocytogenes* in a Lactic Soft Cheese

Thulani Sibanda and **Elna Buys**, University of Pretoria, Pretoria, South Africa

*Introduction: Listeria monocytogenes* is widely present in many food processing plants where it causes product contamination. Continued exposure to stress results in the selection of stress resistant variants with enhanced survival. Among dairy products, soft cheeses are the leading cause of listeriosis outbreaks.

*Purpose:* The aims of this work were: 1) to model the survival response of *L. monocytogenes* strains in a lactic soft cheese stored at 4°C, after exposure to acid and salt stress; and 2) to evaluate the effect of lactate and diacetate salts on the inactivation kinetics.

*Methods: Listeria monocytogenes* strains T69, 159/10, 243 and ATCC19115 were subjected to acid and salt (NaCl) stress; then inoculated into soft cheese, which was stored at 4°C for 15 days. Survival data was fitted into four primary inactivation models. A secondary, second order polynomial

function was used to model the effect of sodium lactate and diacetate addition on the rate parameter of the Weibull model. Suvivor diversity was assessed by (GTG)5-rep-PCR fingerprinting.

*Results:* Inactivation of stress treated cells was described by nonlinear models ($R^2 > 0.90$). When unstressed, inactivation was best described by a convex double Weibull model ($R^2 = 0.86$). Stressed cells significantly reduced ($P < 0.05$) inactivation rates. Addition of sodium lactate and diacetate (0.5 – 2.5% (m/v)) to the soft cheese resulted in an increase in the rate parameter in the Weibull model described by a second order, polynomial function ($R^2 = 0.84$). Of 40 isolates, strain 159/10 remained the dominant strain representing survivors of acid and salt treated *L. monocytogenes* after 15 days.

*Significance:* Stressed *L. monocytogenes* have enhanced survival in acid soft cheese. Lactate and diacetate salts are not effective in controlling *L. monocytogenes* in lactic soft cheese. In contaminated foods, recent food isolates survived better than laboratory strains and could be more useful in predicting *L. monocytogenes* survival response.

## P1-17 Fast and Reliable Screening and Identification of the Most Relevant Beer Spoilage Bacteria Plus Detection of Spoilage Yeasts in Beer by Real-time PCR

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*Introduction:* In general, beer is a hostile environment for most microorganisms. Only a few kinds of bacteria are able to grow under such conditions and are able to spoil beer. The detection of beer spoilage bacteria by conventional methods is a time consuming and laborious task.

*Purpose:* This study attempts to clarify that real-time PCR provides an easy, fast, and reliable alternative testing solution.

*Methods:* For this study, foodproof® Beer Screening Kit hybridization probes were used.

*Results:* This kit allowed the detection of 31 beer-spoilage bacteria, including identification of 12 species, in one test within 24–48 h. The range of detected organisms includes the most beer-relevant species of the genera *Lactobacillus, Pediococcus, Pectinatus* and *Megasphaera*. The method was adjusted to the routine lab with throughput up to 94 samples per PCR run. The absence or presence of beer spoilers was detected immediately after the PCR run. Subsequent melting curve analysis allowed the identification of the most important spoilage bacteria, such as *Lactobacillus brevis*, *Lactobacillus lindneri*, or *Pediococcus inopinatus* from a positive result without further testing. *Lactobacillus acetotolerans*, another beer spoiling bacteria, was the most recent addition to the list of target organisms.

The new foodproof® Beer Screening LyoKit is, also, available and allows the detection of the above mentioned 31 different beer-spoilage bacteria. In addition, it detects hop tolerance genes in a separate fluorescence channel. *Lactobacillus brevis*, the most important bacterial beer spoiler can be identified. To detect the most important spoilage yeasts, BIOTECON Diagnostics has developed the foodproof Spoilage Yeast Detection 1 and 2 Kits, which detect the most important genera using two tests.

*Significance:* The combination of the foodproof® Beer Screening Kits, liquid and lyophilized, and the foodproof Spoilage Yeast Detection Kits provides the most complex testing solution available to brewers.

## P1-18 Quantification of Yeasts and Molds in Dairy Products By Real-time PCR

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*Introduction:* Since yeasts and molds are ubiquitous in the environment, foods can be easily contaminated through raw material, inadequately sanitized equipment, or simply by airborne contaminants. When conditions for bacterial growth are less favorable, yeasts and molds may become predominant spoilers in dairy products. Conventional methods for the detection of yeasts and molds take up to 14 days. During this period, the product is not available for the market. A rapid test would prolong the distribution time.

*Purpose:* To develop the foodproof® *Yeast & Mold Quantification* LyoKit; a fast and sensitive assay for the detection and quantification of yeasts and molds in dairy products, with a time to result of less than 5 hours.

*Methods:* Food samples were diluted 1:10 in a stomacher bag. After homogenization, 800 µl of sample was used for DNA extraction, which included live/dead differentiation and a mechanical disruption step. Purified DNA was used directly for qPCR. The efficiency of live/dead differentiation, sensitivity, specificity, and robustness were determined. The assay was compared to the ISO-method.

*Results:* Genomic DNA from 15 different species was tested in 11 replicates. Even at the lowest concentrations (0.39 GE/reaction), 100% of all replicates were positive. Ten different samples were spiked with $10^2$ and $10^3$ CFU *Yarrowia lipolytica*/g . Deviation of the threshold cycle was less than one Ct. Samples spiked with 6000, 600, 60, and 6 CFU/g showed positive results. The lower detection limit of < 10 CFU/g was fulfilled. Comparison of quantification between ISO-method and PCR of spiked samples showed good correlations. Specificity (inclusivity and exclusivity) is 100%. Two hundred-sixty (260) species were tested, positively, with the kit. More than 60 bacteria, plants, and mammal cells were tested negative.

*Significance:* The foodproof® *Yeast and Mold Quantification* LyoKit is the first qPCR test on the market, which detects and quantifies yeasts and molds, in a single test, in less than 5 hours.

## P1-19 Novel *Vibrio* Detection Method for Species and Toxigenicity Genes Identification Using Real-time PCR

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*Introduction: Vibrio parahaemolyticus, Vibrio vulnificus,* and *Vibrio cholerae* are known as potential waterborne contaminants of seafood and cause severe health problems, worldwide. Traditional methods for detection are time consuming and error-prone, while real-time PCR can be conducted in < 24 hours with high specificity and sensitivity.

*Purpose:* The target was to design a real-time PCR assay that discriminates between *V. parahaemolyticus*, *V. vulnificus,* and *V. cholerae* and simultaneously detects and individually identifies the pathogenicity factors *ctx*, *tdh*, *trh*1, and *trh*2 by melting curve analysis, in a single reaction using sequence specific 5' nuclease-probes. For convenience, the assay must be lyophilized.

*Methods:* Specificity (inclusivity/exclusivity) was tested with DNA extracts. Sample matrix compatibility, sensitivity, and viability PCR were tested with genomic DNA and spiked samples.

*Results:* With novel targets, false-positive and false-negative results, known from other methods using targets like *tlh* or *hly*A, were avoided. A total of 149 strains (74 *V. parahaemolyticus*, 26 *V. vulnificus,* and 49 *V. cholerae*) were tested for inclusivity. With 100% specificity (inclusivity/exclusivity) for the detection of species and pathogenicity factors, the assay was superior to other *Vibrio* detection methods. There were no false positive results for all 73 tested samples of 54 closely-related species and bacteria from the same habitat. The sensitivity of the foodproof® *Vibrio* Detection LyoKit is one genomic equivalent (GE)/reaction for species detection and 10 – 25 GE/reaction for toxin detection. The assay is compatible with all 21 tested raw and processed seafood matrices, such as whole squid, raw oysters, or smoked salmon. The sample preparation includes a live/dead discrimination by using Reagent D, which efficiently removes DNA of at least $10^3$ CFU/ml dead *Vibrio*.

*Significance:* This assay met the demands of testing laboratories by demonstrating 100% specificity and high sensitivity. As seafood, often, is contaminated with dead cells of *Vibrio* spp., Reagent D treatment prevents false positive results which may be encountered with other PCR methods.

## P1-20 Screening for Genetically Modified Plants and Identification of Non-marker Events in Food and Feed

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*Introduction:* Screening for the transgenic regulatory elements P-35S and T-NOS in food and feed samples has been the standard for testing presence or absence of genetically modified (GM) plants. Several GM plants have been designed that contain neither P-35S nor T-NOS, which outdates this approach. BIOTECON Diagnostics' new GM organism (GMO) screening strategy consists of a sophisticated combination of screening and identification assays for maximum coverage of present GMOs.

*Purpose:* BIOTECON Diagnostics has developed a new, real-time PCR, GMO screening and identification assays, reducing time, effort, and cost of analysis.

*Methods:* The foodproof® GMO Screening 1 and 2 LyoKits target a total of eight different transgenic regulatory elements. Additionally, three, new, multiplex GMO Soya and Maize identification assays, which detect events that are missed, using common regulatory sequences for screening, have been developed. The foodproof® Plant Detection LyoKit can be used to check for integrity of DNA and as a process control. The assays comply with ISO 21569 and the German Food Law § 64 LFGB for the detection of GM DNA sequences. An internal amplification control is included.

*Results:* Specificity (inclusivity/exclusivity) was verified against different modified and non-modified plants. Forty-five different matrices were tested successfully, including vegetable burger, soya products, and fat. A new automated extraction protocol for the KingFisher Flex enables the analysis of matrices with low DNA content, like soya lecithin. The assays were shown to be robust enough for sample volume variation between 20 and 30 µl. The absolute and relative limit of detection were determined.

*Significance:* The flexible GMO LyoKits screening and identification assays offer an easy and cost-effective approach for the analysis of GM foods.

## P1-21* Validation of Predictive Models for the Growth of *Listeria monocytogenes* in Indian Cottage Cheese (Paneer) by Challenge Testing under Homemade Preparation Scenario

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*Introduction:* There is an increasing food safety concern regarding the consumption of fresh cheese due to *Listeria monocytogenes*. Paneer is a cottage cheese, popular among Asian and Middle Eastern countries. *Listeria monocytogenes* is the major cause of foodborne illness associated with consumption of fresh, soft cheese. Several outbreaks and recalls have been reported.

*Purpose:* The goal of this work was to validate predictive growth models for *L. monocytogenes,* based on challenge test data performed in paneer under a homemade preparation scenario.

*Methods:* The paneer cheese was prepared in the laboratory. Three different pathogen contamination scenarios were simulated: i) contamination during the coagulum preparation stage; ii) contamination from the water during immersion of the coagulated milk; and iii) post-processing surface contamination. Each trial was conducted using three batches with two replicates each. The initial concentration of *L. monocytogenes* was ca. 500 CFU/g. Results of challenge testing were compared with Combase and FSSP, predictive modeling software for *L. monocytogenes* growth.

*Results:* The results showed that the models predicted the growth of *L. monocytogenes,* if contamination occurred at the surface or immersion stage. There was an increase of more than two log CFU/g, during 10 days storage at 4°C. The models were unable to

predict the growth, if contamination occurs during coagulum preparation stage. It was found that there was no growth of *L. monocytogenes* and the presence of *L. monocytogenes*/25 g was only detected occasionally.

*Significance:* This work is the first validation of predictive models used to assess the growth of *L. monocytogenes* in the paneer (Indian cottage cheese). Results indicate that the models are valid, only, if *L. monocytogenes* contamination occurs after coagulum preparation.

## P1-22 Epidemiological Study of *Salmonella* spp. Strains Isolated in Lombardia from 2012-2016

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*Introduction:* In cases of foodborne illness, the biggest challenge is to identify the offending food. Symptoms could appear after a few hours, but, sometimes four to five days after ingestion of a contaminated food. As a consequence, it is difficult to have a complete medical history of the potentially responsible foods; and in most cases, they are not available for diagnostic confirmation because they have been completely consumed or thrown away.

*Purpose:* The objects of this study were to analyze the data obtained from molecular characterization of *Salmonella* strains, isolated from food and environmental farm samples, rather than from humans; to evaluate the possible correlations; and to speculate on possible reservoirs for *Salmonella* that can cause human illness.

*Methods:* A total of 1809 *Salmonella* spp. strains were isolated from food (1169), farm (464), and the Lombardia environment (176), along with 148 human strains made available by Hospitals from the same region were serotyped according to ISO 6579-3:2014 and molecular typed by PFGE as per the CDC (Atlanta) Pulsnet System. The data obtained were elaborated and compared using BioNumerics software (Applied Maths).

*Results:* Among human strains monophasic *Salmonella* Typhimurium was the serotype most frequently isolated (42%), followed by *Salmonella* Typhimurium (7.4%), and *Salmonella* Enteritidis (4.7%). Monophasic *Salmonella* Typhimurium was, also, the serotype most isolated from other sources (25.4% of strains considered); *Salmonella* Derby and *Salmonella* Infantis, (16.4% and 11.2%, respectively), were the other prevalent serotypes. Molecular comparison was able to point out some situation of close affinity between strains isolated from human and food.

*Significance:* This work demonstrates a good approach for obtaining a picture of circulating strains of *Salmonella* in Lombardia. The approach could be used to create a system of epidemiological surveillance and to schedule monitoring plans for production chains that represent the greatest potential as reservoirs for human infections.

## P1-23 Factors Associated with Food Safety Behaviors in Cancer Patients Seeking Treatment

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*Introduction:* Over 14 million Americans suffer from cancer and are at a greater risk of foodborne disease due to weakened immune systems. However, food safety practices and factors that may lead to risky food safety behaviors in patients are not well understood.

*Purpose:* The objective of this study was to determine the risk perception and demographic factors associated with food safety behaviors in cancer patients seeking chemotherapy and radiation treatment.

*Methods:* This was a cross-sectional study that recruited participants from two cancer-specific hospitals in Ohio. A 173-item questionnaire assessed the food acquisition and preparation practices and attitudes toward different aspects of food safety in the cancer population. It included sociodemographic factors, food insecurity status, and disease factors. The data were analyzed using SPSS.

*Results:* Participants (n = 120), were mostly breast cancer patients (48.1%), female (84.6%), older than 50 (73.1%), and white non-Hispanic (83.5%). About 38% had a college degree or more and 26.2% had at least one year of college education. A total of 2.9% participants had low food security, while 8.7% were marginally food insecure. Low income was associated with risky food acquisition practices ($r = 0.310$, $P < 0.01$), like eating other people's leftovers (33%), roadkill (3%), or spoiled foods (2%). Low income was associated with unsafe food storage and preparation, such as storing eggs at room temperature ($r = 0.434$, $P < 0.01$) and leaving cooked foods left on the stovetop overnight ($r = 0.380$, $P < 0.01$). Half of the participants were aware that they are at increased risk of con-tracting foodborne diseases due to cancer. Food insecurity in cancer patients was associated with a decreased risk perception ($r = 0.262$, $P < 0.01$).

*Significance:* The findings can be used to develop effective food safety education programs for cancer patients and highlight the need for food safety education for patients during cancer treatment.

## P1-24 Lead, Cadmium, Copper, and Zinc Residual Levels in Seafood on the Hurghada Coast of Egypt

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*Introduction:* Fish is a good source of protein, vitamins, and minerals. Some heavy metals in fish, even in trace amounts or at certain limits, may be toxic and cause serious hazards to human health.

*Purpose:* The aim of this study was to determine heavy metal residues in *Mugil cephalus* and shrimp collected from the markets of Hurghada (city), Egypt.

*Methods:* A total of 50 *Mugil cephalus* and 150 frozen shrimp samples were collected from the Markets of Hurghada. Samples were analyzed by Flame Atomic Absorption Spectrophotometry for determination of lead, copper, zinc, and cadmium.

*Results:* The lead level in *Mugil cephalus* ranged from 0.000 – 0.718 ppm, with a mean value of 0.158 ppm. Lead levels in shrimp ranged from 0.000 – 1.328 ppm, with a mean value of 0.281 ppm. Cadmium levels in fish samples ranged from 0.357 to 1.826 ppm, with a mean value of 1.205 ppm; while Cadmium levels in shrimp ranged from 1.957 to 4.744 ppm, with a mean value of 3.549 ppm. Copper levels in fish ranged from 0.000 – 0.430 ppm, with a mean value of 0.313 ppm. Copper levels in shrimp ranged from 0.00 – 0.747 ppm, with a mean value of 0.382 ppm. Zinc levels in fish samples ranged from 0.268 to 3.412 ppm, with a mean value of 1.731 ppm; while zinc levels in frozen shrimp ranged from 2.746 to 7.060 ppm, with a mean value of 5.065 ppm.

*Significance:* High concentrations of lead and cadmium in fish muscles may have been derived from shipping and containers, which pass through the Red Sea, where a potential source is pollution caused by waste. Butteries and other sources of heavy metal pollutions, such as mining, refining, and industrial oil pollution in the Red Sea area may, also, be playing a role in this pollution.

## P1-25 Withdrawn

## P1-26* Comparative Study of Pyrethroid Residues in Fruit Peels and Flesh Using Solid-liquid Extraction Combined with Magnetic Solid Phase Extraction

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*Introduction:* Many fruits show high amount of nutrients in their peels as compared to flesh. Thus, nutrition specialists recommend consuming the whole fruit rather than the peeled. However, the majority also assumes that peels are more susceptible to pesticide residues, if the fruits are not properly cleaned. To achieve the maximum benefits of fruit consumption, it would be helpful to e able to make comparisons between the pyrethroid levels in peels versus flesh.

*Purpose:* This study aimed to develop a fast and convenient detection method for pyrethroid residues in fruit peels and flesh and to compare the pyrethroid residues in fruit samples.

*Methods:* The application of a laboratory prepared polystyrene magnetic nanoparticles (PSt@MNPs) based magnetic solid phase extraction technique combined with liquid-solid extraction helped clean the sample and preconcentrate the targeted analytes prior to HPLC quantification. Optimization of the parameters affecting extraction efficiency was carried out.

*Results:* Analytical performances were evaluated by carrying out experiments at optimum conditions. Results showed that the LODs and LOQs were below 0.1445 and 0.5116 ng $g^{-1}$, respectively, for the six pyrethroids tested. The recovery rates were within the range of 73.6 – 123.1% with intra-day and inter-day RSD being less than 16.5 and 15.4%, respectively; suggesting satisfactory reproducibility of the proposed method. Real sample analysis was performed using six commonly consumed fruits, obtained from local supermarkets in Singapore; including apples, pears, oranges, peaches, and nectarines. Peels and flesh were tested separately to study the difference of pyrethroid residues in different parts of fruits. Permethrin residue was detected on the grape peel sample. No violation was reported, since the residue amount detected was far below the maximum residue limit set by the government.

*Significance:* The results suggest that the proposed method is a promising, rapid, and convenient way to analyse pyrethroid residues in fruit peels and flesh.

## P1-27 Changes of Histamine Content in Soft and Hard Cheeses during Their Storage at Different Temperatures

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*Introduction*: Biogenic amines, including histamine, belong to compounds that can affect human health. Histamine naturally occurs in many types of cheeses. Cheeses are a favorable environment for the formation of histamine, due to the presence of free amino acids and microorganisms, especially during processing and ripening.

*Purpose*: The aim of this study was to evaluate the effects of temperature and storage time on the formation of histamine, in hard and soft cheeses, during their storage at refrigeration and room temperatures.

*Method*s: Quantification of the histamine was performed by HPLC-DAD; the LOQ=3.52 mg/kg. Content of histamine was determined in four types of mould cheeses (Lazur Blue, Brie, Camembert, Gorgonzola) and two types of hard cheeses. The samples were uniformly divided into two groups and stored in a refrigerator at 4 ± 2°C and at room temperature (22 ± 2°C). The assessment of samples stored at room temperature was stopped after 42 days.

*Results*: In soft cheeses, histamine was detected at the highest concentration (730.47 mg/k) in Gorgonzola cheese at 42 days of storage at room temperature. The proposed concentration limit of 400 mg/kg histamine in cheeses (Rauscher-Gabernig, E.) was exceeded, only, in Gorgonzola at 28 and 42 days of storage at 22 ± 2°C and in Camembert at 112 days storage at 4°C. In hard cheeses, histamine was detected at the highest concentration (35.84 mg/kg) in Mlekdamer cheese (Swiss type) after 35 days storage at room temperature.

*Significance*: Consumption of hard cheeses is safe for consumers, even after their expiration dates. It makes sense to define acceptable limits for concentrations of histamine in cheese rennet ripening.

## P1-28 Development of a Flow Cytometry Bead-based Immunoassay for the Simultaneous Detection of Food Allergens

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*Introduction:* Food allergy prevalence has increased (up to 7%) over the last decades. Efficient monitoring of allergens in food is required to ensure safety of consumers facing this health problem. Most of current analytical methods for food allergens are based on immunological reactions (ELISA and immunostrips) or PCR. Classical immunoassays are, generally, suitable for detecting, only, one single allergen at a time, or are limited to few targets. In contrast, multiplexed PCR tests are available; but, for some major allergens, like milk or egg, this technique lacks in sufficient performance.

*Purpose:* Flow Cytometry bead-based Immunoassays (FCIA) present all the advantages of immunological tests (sensitivity, specificity, high-throughput) and, additionally, provide great multiplexing capacity. Different immunoassays developed using individually encoded, fluorescent microparticles can be combined in a final test to broaden the compounds detected. The aim of this work was to explore the potential of FCIA for the simultaneous analysis of four major food allergens; milk, egg, peanut, and soya.

*Methods:* Individual competitive bead-suspension assays have been developed for milk (β-lactoglobulin and casein), egg, peanut, and soya. The tests have been combined in a five-plex assay to detect all of the allergens in a single analysis.

*Results:* The performance of the assay was evaluated in diverse food matrices: speculoos, cookies, ice-cream, spices, chocolate, and tomato sauce. Upon extraction of the fortified matrices, the allergens were detected at concentrations as low as 0.2, 0.3, 0.6, 1, and 2 μg $g^{-1}$ (protein content) for β-lactoglobulin, casein, egg, peanut, and soya, respectively, in all food commodities tested.

*Significance:* The results, presented herein, pointed out the interest of FCIA for food allergen determination as applied in routine quality control, by food industries and official entities. Further work is ongoing to expand the application of the test to other allergens and matrices.

## P1-29 Validation of the Flow Cytometry Immuno-assay, BEADYPLEX™, for Multi-class and Multi-Residue Screening for Antibiotics in Fish

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*Introduction:* Rapid and sensitive analytical methods for the detection of antibiotic residues in the food chain are essential to guaranteeing consumer protection and industrial transformation processes. Much of the effort in food analysis is focused on the development of multi-residue methods, which considerably shorten analysis time and reduce global costs. Flow Cytometric Immunoassays (FCIA) combine the detection of receptor-ligand interactions by immunoanalysis with the multi-parametric characterization of individually encoded beads. This feature provides great multiplexing capabilities, making FCIA especially suitable for the monitoring of multiple targets.

*Purpose:* BEADYPLEX™ is a competitive bead suspension FCIA for the multi-class and multi-residue screening of meat for antibiotics from 10 families, widely used in animal farming: tetracyclines, sulfonamides, β-lactams, aminoglycosides, macrolides, fluoroquinolones, lincosamides, phenicols, polymyxins, and pleuromutilins. The objective of this work was to expand the applicability of the method to fish.

*Methods:* The simultaneous detection of up to 80 antimicrobial residues is possible, due to the combination in a single reaction of multiple bead-based immunoassays, which use generic receptors/antibodies. The rapid and solvent-free extraction method has been, previously, proven to be efficient for the application of BEADYPLEX™ to muscle from different species (porcine, poultry, bovine). We conducted the validation of BEADYPLEX™ in high and low-fat content fish, salmon and coley, respectively, following 2010 CRL Guidelines for the validation of screening methods for residues of veterinary medicines, supplementing European Decision 2002/657/EC.

*Results:* The performance of the method in fish was found to be equivalent to that shown in meat, with detection capabilities, for most of the antibiotics within the scope, at or below European regulatory limits.

*Significance:* BEADYPLEX™ is a high-throughput, user-friendly screening method that provides sensitive and specific broad spectrum detection of antibiotics in multiple food matrices and the identification of the antimicrobial family in one test per sample.

## P1-30 Volatilomics-approach through GC/MS and e-Nose for the Detection of Minced Beef Adulteration with Horse Meat

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*Introduction:* It has not been a long time since the horse meat adulteration scandal came to light. This was an alarm for meat inspectors and authorities to fill the gaps in the field of meat adulteration.

*Purpose:* The aim of this work was to investigate the potential of Headspace Solid Phase Microextraction - Gas Chromatography Mass Spectrometry (HS/SPME-GC/MS) and e-nose in the detection of the fraudulent addition of horse adulterants to minced beef.

*Methods:* Portions of minced beef and horse were mixed to obtain five ratio mixtures corresponding to 0%, 10%, 20%, 40%, and 100% horse in beef. Afterwards, six different burgers (75–80 g), for each level of adulteration (30 samples in total), were analyzed using both HS/SPME-GC/MS and a 12 MOS e-nose system. This procedure was repeated three times, in order to take into account the variablity between batches.

*Results:* Meat volatilome for the two species exhibited qualitative, as well as quantitative differences. As far as the GC/MS chromatograms are concerned, they were analyzed with untargeted and targeted approaches. Principal component analysis for both instruments showed that the increasing amount of horse adulteration in beef followed a distinct gradient pattern. Supervised data analysis results following orthogonal-PLS-DA gave very good discrimination between pure and adulterated samples, yielding 88.1% and 94.3%, overall, correct classification for GC/MS and e-nose data, respectively. Random forest (RF) classifier managed to increase the accuracy by 1.7% for both chemometric methods. Qualitatively, dimethyl sulphide, methyl acetate, acetoin, diacetyl, pentanal, and 2,3-octane-dione were positively correlated with beef meat, while γ-butyrolactone, 3-hepten-2-one, cis-4-heptenal, ethyl alcohol, butyric and hexanoic acid were considered important for horse meat.

*Significance:* This study demonstrates the potential of GC/MS and e-nose in the rapid detection of meat adulteration. Furthermore, it showed the superiority of e-nose to GC/MS.

## P1-31 Distribution Temperature Regulation in the Home Delivery Cold Chain Using Quality and Risk Assessment

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*Introduction:* Temperature management in the cold chains has, recently, receive attention. In Taiwan, an increasing need for chilled and frozen food home delivery services has been observed because of growing demand for chilled and frozen food by consumers. However, the weather in Taiwan can reach 37°C in summer. Incorrect temperature settings or lack of temperature control in handling areas are some of reasons behind broken cold chains. Recently, the government began considering 15°C settings in handling areas. However, this decision cannot be made without information about the average risks for consumers.

*Purpose:* This study aimed to evaluate temperature requirements in home delivery, chilled and frozen food chains, by considering food safety risks to chilled foods and quality impacts to frozen foods.

*Methods:* Temperature data in home delivery cold chains were collected using data loggers. Food safety risk assessments of *Bacillus cereus* in ready-to-eat rice balls and *Vibrio parahaemolyticus* in raw oysters were conducted. Risk data on the prevalence and concentration were collected from scientific literature. A Monte Carlo simulation model was created using @ risk. To assess and describe quality changes in frozen white shrimp, a kinetic model using Arrhenius relations was employed.

*Results:* Preliminary results showed that there were serious temperature abuses in the distribution process of home delivery cold chains. In chilled foods, the average temperature in the loading/unloading area was 21.0°C and in transportation the average was 18.8°C. The estimated average risks per serving of ready-to-eat rice balls and raw oysters were $0.5 \times 10^{-4}$, $2.22 \times 10^{-5}$, respectively. Both risk and quality assessment indicated that the most influential interventions were handling temperature and storage temperatures in home delivery cold chains.

*Significance:* These findings could help food authorities determine temperature management policies to prevent broken cold chains and to reduce food safety risks.

## P1-32 Revised EN ISO 22964: Evaluation of Granucult® and Chromocult® Culture Media for Pre-enrichment, Selective Enrichment, and Detection of *Cronobacter* spp.

**Barbara Gerten**, Michael Gampe, Andreas Bubert and Lisa John, Merck KGaA, Darmstadt, Germany

*Introduction:* EN ISO 22964 has been revised into a full EN ISO standard with an extended scope to *Cronobacter* spp. detection in food products for humans and feeding animals and environmental samples. A non-selective pre-enrichment step in Buffered Peptone Water (BPW) is followed by enrichment in selective medium *Cronobacter* Selective Broth (CSB) and plating out and identification on Chromogenic Cronobacter Isolation (CCI) agar.

*Purpose:* Growth promotion and isolation of *Cronobacter* spp. and *Cronobacter*-related species were tested and confirmed with BPW, CSB and CCI agar, as described by EN ISO 22964:2017.

*Methods:* For growth promotion >15 *Cronobacter* spp. strains, >10 *Franconibacter* spp. and 3 *Siccibacter* spp. strains, including type strains and wild isolates from food and environmental samples and *C. sakazakii* reference material, were spiked into the food matrix for evaluating the media within the entire workflow. Performance tests for productivity, selectivity, and specificity were conducted, as given by the EN ISO standard, for the quality assuarance of the culture media.

*Results:* All *Cronobacter* spp. and non-*Cronobacter* strains from pure strains, spiked into reference material, were able to be detected, following the method given by the revised standard: non-selective pre-enrichment in BPW, incubation between 34–38°C for 18 h ± 2h, followed by selective enrichment in CSB, incubation at 41.5°C ± 1°C for 24 ± 2h, and plating (incubation at 41.5°C ± 1°C for 24 ± 2h) and identification on CCI agar. Productivity, selectivity, and specificity of the media affected the performance, as specified by the standard.

*Significance:* For the tested Granucult®, using BPW, CSB, and Chromocult® CCI agar, the results of this study indicate the applicability of the methods and criteria, as given in the revised International Standard EN ISO 22964:2017, “Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp.”

## P1-33* Microbiological Effects of High Hydrostatic Pressure (HHP) between 150 and 600 Mpa on Liquid Egg Products

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*Introduction:* High Hydrostatic Pressure (HHP) is one of the most promising, minimal processing technologies in food technology. HHP can decrease microbiological spoilage of food products and extend shelf life.

*Purpose:* Several preservation methods are viable in food industry for controlling the microbiological safety of liquid egg products (liquid whole egg (LWE), liquid egg yolk (LEY), and liquid egg white (LEW)); but, most of these use heat or preservatives. On the one hand, high temperatures are effective, but techno-functional properties could change. On the other hand, the use of preservatives is disliked by consumers.

*Methods:* In our study LEW, LWE and LEY were treated with HHP between 150 and 600 MPa (50 MPa, stepwise) for 5 minutes. Microbiological contamination of samples was examined to establish efficacy of HHP.

*Results:* Our results show that 450 MPa was enough to decrease microbiota of LWE by more than a four order of magnitude decrease. The same effect was obseved in LEW treated with 450 MPa HHP. But LEY, which was contaminated at a higher level, required, only, a 500 MPa HHP treatment to provide a five order of magnitude decrease in microbiota.

*Significance:* In our experiment, we found out that different pressure ranges of HHP are needed to decontaminate liquid egg products.

## P1-34* High Pressure Processing Effects on *Listeria monocytogenes* and *Listeria innocua*: Evidence for Variability in Inactivation Behaviour

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*Introduction:* Listeriosis is an important public health issue with considerable negative economic impact. Although it is a disease with relative low incidence, reported case fatality rate is the highest (15.6%) of all the foodborne diseases under European Union surveillance. The use of nonthermal technologies, such as High Hydrostatic Pressure (HHP), has emerged as a new preservation method to control, slow, and prevent the growth of foodborne pathogens; thereby, extending shelf life with high energy efficiency and minimal food processing.

*Purpose:* The effect of HHP on the survival of 14 strains of *Listeria monocytogenes*, from food or clinical origins, and two strains of *Listeria innocua* (2030c PHLS and NCTC 11288) was evaluated.

*Methods:* Stationary phase cells were exposed to 300, 400, and 500 MPa at 10°C for 5 min. The pressure-treated samples and controls were serially diluted, without a prior repair incubation period. Dilutions were plated in triplicate in TSAYE and PALCAM agar. Plates were incubated at 37°C for 48 h and colony forming units (CFU)/ml determined.

*Results:* Two *L. monocytogenes* of food origin were the most sensitive strains at 300 and 400 MPa, showing significantly higher log reductions in comparison with other strains ($P < 0.05$). Strains of *L. monocytogenes* resistant to one or more antibiotics exhibited significantly higher levels of survival after high pressure treatment at 400 MPa. No correlation was found between strain origin or thermal tolerance and resistance to HHP. Selective enumeration of *Listeria* cells in PALCAM yielded higher log reduction values compared to enumeration with TSAYE, reflecting cellular damage caused by HHP treatment and being statistically significant at 400 MPa ($P < 0.05$). *Listeria innocua* exhibited significantly higher sensitivity to HHP than observed for some *L. monocytogenes*.

*Significance:* The data obtained underlines the importance of strain selection for studies aiming to evaluate HHP efficacy to ensure safety of HHP-treated foods.

## P1-35 Attitudes and Perceptions of a Unique Knowledge Transfer Project Implemented in the Food-sector of Small and Medium Sized Enterprises (SME) in Wales, UK

Elizabeth C. Redmond and **David Lloyd**, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom

*Introduction:* The European Union/Welsh Government Knowledge-Innovation-Technology-Exchange (KITE) Project (2008–2015) facilitated a proactive partnership between food-sector small and medium-sized enterprises (SME), affiliates (graduates/individuals with industrial-experience) and academic-knowledge-based partners. Measured end-of-project outputs included attainment of 83 second/third party accreditations (including 46% BRC5/6), £103.3 million in new sales, and creation/safeguarding of >1700 jobs (including quality assurance/manufacturing roles) in the food industry. KITE outputs/outcomes have been reported; however, evaluation of the partner experience was also required.

*Purpose:* This study assessed KITE-project partners' attitudes and perceptions of project management, implementation/delivery, technical operations, food safety practices, benefits, impact, and effectiveness.

*Methods:* Qualitative and quantitative data was obtained from self-completed questionnaires (n=119) administered to all project partners (SME Managing-Directors (MD)/Technical-Managers(TM)), which included affiliates, food technologists (FT)/technical-supervisors(TS)). Attitudes and perceptions towards the KITE-project were obtained, predominately, using Likert-like rating scales and open questions.

*Results:* Cumulatively, KITE project partners reported positive attitudes/perceptions of KITE implementation, improved technical compliance, and improved food

safety knowledge of SME management/workforce. SME MDs/TMs reported "better understanding of BRC requirements," "business growth and expansion," "increased customer confidence" and "attainment of BRC accreditation resulting from KITE partnership. In-addition, 100% indicated improvement of workforce food safety practices and understanding of food safety/quality standards. The majority (>96%) reported satisfaction with project delivery, management and outcomes. All affiliate respondents reported valuable work experience and increased food safety/technical knowledge; 94% reported transferring food safety/quality standard knowledge in SME. Although 44% of the affiliates reported challenges/barriers to embedding new technical-compliance-procedures, 81% were also "very-satisfied" in knowledge-partner mentoring to achieve implementation and >75% FT/TS indicated overcoming technical challenges within businesses and increased specific sector industry knowledge.

*Significance:* Findings of this study can be by future food-sector knowledge transfer projects as a successful example showing effective operational delivery and partner satisfaction to maximize project/SME outputs/outcomes. Combined with project outputs, the attitudes/perceptions of KITE partners identified in this study further evidences the success of KITE and the potential for national/international application.

## P1-36 Baseline Assessment of Product Waste Giveaway: Ready-meal Food Sector Case Studies

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*Introduction:* Food product wastage, reported in the food industry (including the ready-meals sector), is due to production/processing methods and visual, physical, microbiological, or compositional reasons. Minimisation of such losses may not only improve technological effectiveness, but may, also, be of substantial financial benefit for food sector businesses and increase potential sustainability/profitability.

*Purpose:* This work was undertaked to conduct an in-depth analysis of processing techniques and process flows for ready-meal food products, in terms of waste production, processing efficiency, and recommendations for waste minimisation and cost savings.

*Methods:* A detailed, quantitative audit was undertaken for multiple product-lines in two ready-meal sector businesses to evaluate in-use process flows of five product lines and review/identify processing waste volumes and associated costs during raw material weighing, cooking of components, final assembly, and packing. Collated data was compared with company product specifications.

*Results:* Overall, detailed data capture and review from the production processes of ready-meal products indicated slight 'waste giveaway' values of up to 1.8% above target product weights; with one product packed 0.4% below the target weight. Auditing a marinated chicken production process revealed up to 16Kg of marinade/1,060Kg was wasted due to direct drip loss from the belt as the chicken entered the travel oven. Reduction of the chicken marinade would reduce the amount of marinade lost during pre-processing. Reducing the batch size, by up to 20%, could save up to £80,000 annually, based on the volume of chicken processed with no impairment of product quality.

*Significance:* Data analysis indicated that processing, in the reviewed ready-meal food products, accrued minimal product waste giveaway; thus, closely achieving target weights. However, analysis, also, revealed that substantial financial savings could be achieved by improving processing efficiency methods; thus, potentially improving business profitability and sustainability.

## P1-37 Identifying the Barriers to Achieving Food Safety Scheme Compliance and Technical Accreditation in the Welsh Food and Drink Sector

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*Introduction:* The Welsh Government has identified that, to enable growth of the food and drink sector, in Wales there is a need to support the food and drink manufacturing and processing businesses (FDMPB) in order to obtain and maintain food safety scheme compliance/technical accreditation (FSSC/TA). Previous research recognised the need to identify the barriers that exised for small and medium-sized enterprises (SME) in Wales to obtaining/maintaining FSSC/TA.

*Purpose:* This work was conducted to identify the barriers that exists for FDMPB in Wales to obtain/maintain FSSC/TA.

Methods: A desk-based review identified and evaluated available schemes (n = 30). Focus-groups and in-depth interviews of FDMPB with FSSC/TA (n = 20), FDMPB without FSSC/TA (n = 17), stakeholders (n =19), scheme auditors (n = 9), and retail representative (n = 3) identified barriers.

*Results:* The review identified accessible information, regarding requirements, operation, and associated costs, that were lacking and varied between schemes; highlighting differences in openness and transparency, particularly among privately operated schemes, which may hinder FDMPB selection of suitable and beneficial schemes relevant to the business. Barriers identified in focus-groups/interviews related to three key categories.: (1) 'Time/cost/resources', were barriers in terms of time to identify suitable schemes, cost of scheme implementation, capital expenditure, and resources to meet requirements for multiple schemes through duplication of obligations. (2) As for 'Knowledge/skills', the shortage of technical graduates was a major discussion point. Difficulties in meeting workforce training needs and the impact of senior management attitudes upon culture and commitment to schemes were, also, identified. (3) Under 'Communication/information,' the miscommunications regarding the need of schemes by buyers was discussed, along with accessing information to select suitable schemess The number of schemes available

exasperated this and discussions indicated the desire for scheme consolidation/harmonisation.

*Significance:* The research has identified barriers that exists for FDMPB in Wales trying to obtain/maintain FSSC/TA. Findings indicate the need for the development of support mechanisms to increase FSSC/TA uptake and accelerate food sector growth in alignment with Welsh Government aspirations.

## P1-38 Food-safety Knowledge, Attitudes, and Training Experiences of Trainee-Dietitians

**Ellen W. Evans** and Elizabeth C. Redmond, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom

*Introduction:* Dietitians provide food-related information/dietary interventions to vulnerable patients and are identified as trusted, credible, and preferred sources for food safety (FS) information. Delivery of FS advice by adequately-trained, registered dietitians can inform vulnerable patients of increased foodborne illness (FBI) risks and enable risk-reducing FS practices. However, gaps in practicing registered dietitians FS knowledge have been identified. Trainee-dieticians can gain the appropriate/adequate knowledge and skills that dieticians need to deliver effective FS advice.

*Purpose:* This study assessed knowledge, attitudes, and training experiences of trainee-dietitians, regarding FBI and risk-reducing FS practices.

*Methods:* Paper-based questionnaires were completed by trainee-dietitians at Cardiff Met. School of Health Sciences (n = 34).

*Results:* Trainee-dietitians reported awareness that immunocompromised patients had increased FBI risk and recognised domestic kitchens as likely locations to obtain FBIs. Positive attitudes were expressed toward the importance of FS and providing FS information. Although 74–97% indicated awareness of common foodborne pathogens, awareness of associated foods/practices were lacking. Cumulative FS knowledge scores were variable, ranging from 30–81% (average: 62%). No significant differences existed between gender/age group ($P < 0.05$). All trainee-dietitians completed a one-day FS programme; however, 50% considered the training insufficient to enable them to adequately inform vulnerable patients. Many reportedly lacked confidence, as training was not reinforced in lectures. Positive attitudes were expressed towards the role of dietitians in reducing FBI risk and the desire to learn more. However, increased FS knowledge may not be a sufficient prerequisite to assume adequate ability to disseminate FS advice to vulnerable patients. Furthermore, conditions/treatments may have differing food-related needs that impact upon the need to implement specific FS practices to mitigate FBI risk.

*Significance:* Although trainee-dietitians indicated awareness, FS knowledge does not equate to ability to disseminate FS advice. Indeed, many reported insufficient training and lacked confidence to deliver FS advice. Findings identify the need for specifically-targeted training, which will enable trainee-dietitians to gain the information and skills needed to vulnerable patients reduce FBI risks.

## P1-39 Evaluation of the Antifungal Activity of Non-starter Lactic Acid Bacteria (NSLAB) Cultures Using a Caciotta Cheese Model System

Maria Barbara Pisano, Silvia Viale, Valentina Mossa, Maura Deplano, Maria Elisabetta Fadda and **Sofia Cosentino,** University of Cagliari, Cagliari, Italy

*Introduction:* Due to their low pH, nutritional profile, and storage conditions, dairy products are very susceptible to the growth of filamentous fungi that can cause alterations in the cheese rind and texture, which leads to significant economic losses. Lactic acid bacteria are well known for their ability to produce a number of fungal inhibitory metabolites, such as organic acids, cyclic dipeptides, proteinaceous compounds, and fatty acids. So, these bacteria could be good candidates for cheese biopreservation.

*Purpose:* In this study, different non-starter lactic acid bacteria (NSLAB) cultures, containing *Lactobacillus* spp. strains previously selected for their in vitro antifungal properties, were used as an adjunct in the laboratory-scale manufacturing of Caciotta cheese, in order to evaluate their ability to inhibit *Penicillium* spp. and *Aspergillus* spp. strains.

*Methods:* Two trials were performed. In each trial, three cheese batches were produced: a control batch containing the commercial starter cultures; a batch containing the starter with one *Lactobacillus* spp. strain; and a batch containing the starter with the multi-strain NSLAB culture. In the first trial, the mold suspensions were applied to the surface of one week old control and experimental cheeses. In the second trial, the mold suspension was inoculated into the milk after the addition of cultures.

*Results:* The addition of selected NSLAB cultures delayed the mycelial growth of both *Penicillium chrysogenum* and *Aspergillus flavus,* as well as that of environmental fungi, on the cheese surface. A stronger antifungal effect was observed in cheeses produced with multi-strain NSLAB cultures and mould inoculated into the milk. This effect resulted in a growth reduction of *P. chrysogenum* by 2.5 – 3.0 log units after 30 days of storage.

*Significance:* Our results indicate that selected NSLAB cultures could have an application as novel preservatives used to extend shelf-life and prevent fungal spoilage of cheese during storage at 8°C.

## P1-40 Fate of Indigenous Verocytotoxin-producing *Escherichia coli* in Uncooked, Raw Milk Cheeses

**Paolo Daminelli**[1], Elena Cosciani-Cunico[1], Elena Dalzini[1], Paola Monastero[1], Guido Finazzi[1] and Marina Nadia Losio[2], [1]IZSLER, Brescia, Italy, [2]IZSLER-National Reference Centre for Emerging Risks in Food Safety, Brescia, Italy

*Introduction:* Verocytotoxin-producing *Escherichia coli* (VTEC) is a foodborne pathogen that can cause severe illness and death. Cattle is a natural reservoir of VTEC; consequently, they may be isolated from raw cow milk and non-pasteurized dairy products, like Uncooked Raw Milk Cheeses (URMC), typically produced in many Italian regions.

*Purpose:* The aim of this study was to assess a survey of VTEC in URMC, produced in the Northern Italy, and to study the fate of indigenous VTEC during seasoning.

*Methods:* From 2013 to 2016, a total of 1,029 curd samples of URMC were analyzed to check for the presence of VTEC using the standard method, ISO/TS 13136:2012. Cheeses produced with positive curds were collected and the fate of the pathogen was studied.

*Results:* VTEC were detected in 28 out of 1,029 curd samples (2.72%, with 1.82% – 3.91% Confidence Interval at 95% significance level). The proportion of positive samples decreased during cheese ripening. After 8 months at 14°C, the water activity ($a_{w}$) decreased to between 0.977 and 0.919 and no VTEC was detected in the samples.

*Significance:* These data confirm that the cheese ecosystem, especially $a_{w}$, affects VTEC survival. This study can help safety authorities improve the risk analysis of URMC.

## P1-41* Detection of Viable *Mycobacterium paratuberculosis* in UK Retail Pasteurised Milk Using Phage-PCR

**Ahmed Saeedi**, Zara Gerrard, Benjamin Swift and Catherine Rees, The University of Nottingham, Nottingham, United Kingdom

*Introduction: Mycobacterium paratuberculosis* (MAP) causes Johne's disease, a chronic enteritis of ruminants, and has been implicated in the development of Crohn's disease. Milk is a major vertical transmission route within herds and approximately 40–60% of herds in developed countries are infected. Commercial pasteurisation processes do not completely inactivate MAP; therefore, pasteurised milk has been highlighted as a key vector for entry of MAP into the human food chain. The slow growth of this organism prevents the use of standard culture; therefore, the use of alternative rapid methods is required to detect its presence.

*Purpose:* This study completed a survey of commercial, pasteurised, semi-skimmed milk using a rapid detection method (phage-PCR).

*Methods:* Samples of retail semi-skimmed milk (385) were purchased from local retailers. Viable Mycobacteria were detected using a previously described phage-PCR method. The presence of MAP in any plaque positive samples was confirmed by PCR amplification of IS*900*.

*Results:* The phage-PCR assay detected viable MAP in 10.3% (38/368) of the pasteurised milk samples. Only 1.1% of the samples contained more than 10 detectable MAP cells; 3.5% of the samples contained more than two cells (1.1% with >10 and 2.4% with 3-9). The majority of samples (6.8%) contained only one or two detectable cells. Within the main survey, MAP was detected in a small number of filtered milk samples. A further survey, focusing on filtered milk alone, found that Mycobacteria can be detected in this type of milk at a high frequency.

*Significance:* Viable MAP were detected at levels consistent with previous surveys of retail milk by culture or PCR. Detection of very low levels (one to two MAP cells) suggest the rapid phage PCR-method is more sensitive than existing methods. Mycobacteria can also be detected in filtered milk, which is potentially introduced in the cream fraction.

## P1-42 Risk-based Import Food Safety Management Activities: Perspectives on Food Importers in Taiwan

**Hsin-I Hsiao**[1], Chu-Yun Ni[2], Ya-Hui Lu[3], Ming-Chen Lu[3] and Tsung-His Wu[4], [1]National Taiwan Ocean University, Keelung, Taiwan (ROC), [2]National Taiwan Ocean University, Keelung, Taiwan, [3]Taiwan Quality Food Association, Taipei, Taiwan, [4]Food and Drug Administration, Taipei, Taiwan

*Introduction:* Taiwan is limited in natural resources; relying mostly upon importation of raw material, such as foods items. Regulated products come from more than 150 countries. Main countries of origin, by weight, are the United States, Brazil, Thailand, Australia, and China. Growth rates of imported foods have been 5–11% per year. Import food management and border control is, thus, very important to Taiwan. To address food safety, food importers should, also, consider establishing a product safety management program to secure imported food safety.

*Purpose:* This study aims to suggest risk-based food safety management activities for food importers. In particular, we would like to analyze the relationship between risk level and food safety management activities used for food importers prior product entry and during entry process.

*Methods:* During 2015–2016, surveys were mailed to importers (grains, canned foods, dairy, seafood, confections, vegetables, fruit, and picked food). Overall, we received 180 responses, resulting in a 14% response rate.

*Results:* Design of food safety management system encompasses control and assurance activities to guarantee food safety. Our preliminary results indicate that level of risk in imported foods is related to the number of food controls and assurances used for food importers. For high risk levels of imported food, it is necessary that the foreign supplier selection is based on international certification and company reputation, determined prior to doing business; sample testing before entry; and product testing by an independent third-party laboratory during entry.

*Significance:* The results can help food importers design their product safety management program. Moreover, it can optimize the efficiency of food control and cost for importers by reducing unnecessary inspection and testing costs.

## P1-43 Risk-based Food Supply Management for Imported Raw Materials: Perspectives on Chain Restaurants in Taiwan

**Tai-Ying Li** and Hsin-I Hsiao, [1]National Taiwan Ocean University, Keelung, Taiwan

*Introduction:* The use of risk ranking within the food safety risk analysis framework is becoming more and more widespread, since it is often considered an efficient starting point for setting priorities and

allocating resources based on risk. Taiwan is limited in natural resources, mostly relying upon importation of raw material. Ensuring a continuous supply of safe imported food materials is a key objective for food companies in Taiwan. Since 2015, a new food law indicated that food businesses, above certain size, must implement self-management in accordance with food safety laws and other sanitation regulations.

*Purpose:* This study aimed to investigate relationships between microbiological safety risk levels and monitoring frequency and test to determine if risk-based supplier control can optimize the efficiency of food control and cost, by reducing unnecessary inspection and testing costs.

*Methods:* Chain restaurants were chosen in this study because of the complexity of raw materials types and sources from different countries. First, interviews with experts were carried out to identify critical criteria for microbial risk ranking of import food materials; and then, a large number of survey were issued to chain restaurants in Taiwan to test relationships between microbiological safety risk levels, monitoring frequency, and food control performances.

*Results:* Interview results indicated food types, storage temperatures, country sources, pH levels, processed degree, and air control are key critical criteria for microbial risk ranking of import food materials. Preliminary survey results show a high level of risk with imported food materials; requiring more frequent monitoring and sampling. Risk-based supplier control can reduce unnecessary inspections and testing costs, and at the same time secure food safety.

*Significance:* This tool can be used by risk managers to rank food, based on their potential food safety risks and design effective and efficient food control policies.

## P1-44 Quality and Safety Management Systems for Food Production and Hygiene Services/ Technology Providers: A Comparative Assessment

**Stefan Kuepper**, Ecolab GmbH, Monheim, Germany

*Introduction:* Cleaning and disinfection (C&D) programs, accurate dispensing technologies, adequate hygiene services, and support are fundamental to ensuring good hygiene practice and pivotal for risk management of biological and chemical hazards for food safety. However, inadequate quality of C&D products, contaminations, and insufficient process control might result in new, additional hazards introduced by the C&D regime itself.

*Purpose:* Whilst food safety management systems, ranging from CODEX Alimentarius, GFSI guidance, laws, and norms to retails standards, are well established in the food industry and applied equally to manufacturers and the food supply chain, hygiene services and technology providers, i.e. the chemical industry, achieve effective quality management by different means. This presentation explores select key differences, potential consequences, and the need for revised standards and convergence.

*Methods:* A comparative assessment of safety and quality management systems, used in the food and the chemical industry, indicated significant differences and potential for improvement and closer alignment between business partners.

*Results:* Neither standards IFS nor BRC, norms ISO 22000/22001-2, FSSC 22000 nor HACCP-steps and principles are applied in or applicable to the hygiene services industry. Additionally, packaging-related regulations EU 1935/2004, 10/2011, 19/2007 and allergen-related regulations 1169/2011 are not applicable. The quality management systems observed include ISO 9001/14000ff; in select cases, ISO 13485 and GMP ISO 15378; and, furthermore, B2B-contractually agreed specifics.

*Significance:* Clearly, in contrast to the hygiene industry's importance to ensuring food safety, this industry does not, seamlessly, integrate into the food industry's supply chain and the gaps exist between applicable legislations, norms, and systems are only bridged via bilateral quality agreements. While this approach seems to provide workable processes, today, future revision of IFS, BRC standards, and ISO norms ought to aim at closing the gap for good; thus, providing for seamless partnerships between industries, higher-standard food integrity, and quality for consumers around the world.

## P1-45 Ochratoxin A (OTA) Production by *Aspergillus fresenii* and *Aspergillus sulphureus* on Niger Seeds

**Dawit Gizachew,** Purdue University Northwest, Hammond, IN, USA

*Introduction:* Mycotoxin contamination of foods and feeds poses a high risk for human and animal health. Ochratoxin A (OTA) is a ubiquitous mycotoxin produced by *Aspergillus* and *Penicillium fungi*. It exhibits nephrotoxicity, teratogenicity, mutagenicity, and immunotoxicity in both humans and animals. Niger (*Guizotia abyssinica)* is an oil seed that is used for extracting cooking oil, an animal feed in countries like Ethiopia and India, and an export to Europe and North America. It has, also, been implicated in high level of mycotoxin contamination. To our knowledge, there have been no studies on the production of OTA on Niger seeds. In this study, the environmental conditions that support OTA production have been investigated.

*Purpose:* This study was conducted to determine the effects of water activity, temperature, and incubation time on OTA production by *Aspergillus fresenii* and *Aspergillus sulphureus* on on Niger seeds.

*Methods:* Fungi were grown in Czapek Yeast Extract agar (CYA), individually, at 30°C for 5 days. Ten µl spore suspensions were used to inoculate two grams of sterilized seeds placed in each Petri dish (12 plates/species), adjusted to different water activity ($a_w$) values (0.90, 0.94, and 0.98). Plates were incubated at 20, 25, 30, and 35°C for 5 or 10 days. After extraction of the seeds from each plate, using 70% methanol in water, the OTA was quantified by enzyme linked immunosorbent assay (ELISA). Limit of quantitation was 0.4 µg/kg.

*Results:* Both fungi growth and OTA production were highly influenced by water activity and temperature. The highest OTA production (166 µg/kg) was observed at 35°C, 10 days incubation, and 0.98 $a_w$. Linear regressions showed that temperature was a statistically significant predictor of OTA values ($P$ = 0.022). The coefficient for temperature was 3.93 (95% confidence interval).

*Significance:* Our studies will help understand the conditions that are important to minimizing contamination of Niger seeds with OTA.

### P1-46 Mycotoxin Contamination in Different Food Commodities in Bangladesh

**Matiur Rahman**[1], Shahnila Ferdoushi[2] and Farida Yasmin[3], [1]National Food Safety Laboratory, Dhaka, Bangladesh, [2]National Food Safety Laboratory, Mohakhali, Bangladesh, [3]National Food Safety Laboratory, Mohakhali,Dhaka, Bangladesh

*Introduction:* Mycotoxins can appear in the food chain as a result of fungal contamination. *Aspergillus flavus* and *Aspergillus parasiticus* are very common moulds that can colonize grains, maize, nuts, spices, and other food commodities before harvest, post-harvest, or during storage. Aflatoxins in foods are demonstrated chemical hazards and are the main cause of food export rejections from tropical countries to Europe/USA.

*Purpose:* For the first time in Bangladesh, different food commodities collected from Dhaka markets were screened for aflatoxin B1, B2, G1, and G2.

*Methods:* The commodities screened included turmeric powder, chili powder, green nut, peanut, corn, wheat flour, rice, chira, fish feed, cattle feed, bread, and several types of dal, used by most households. For the analysis, food samples were extracted with phosphate buffer and cleaned-up through immunoafinity columns. Aflatoxins were determined by high performance liquid chromatography coupled to a fluorescence detector with a cobra cell on-line derivatisation unit (HPLC-FLD). The method was verified in terms of specificity, precision and recovery prior to analysis.

*Results:* This study revealed that 37% of analysed foodstuffs contained detectable quantities of afltatoxins. Mainly, aflatoxins were detected in rice, chilli, fish feed, and corn. Levels of aflatoxins were, however, below Codex MRLs (15 ppb). Only one sample of corn contained 40.26 ppb aflatoxin, exceeding Codex MRLs. Bread, chira, aloe vera, mung dal, anchor dal, wheat, and wheat flour were free from aflatoxins.

*Significance:* The implementation of adequate pre-harvest and post-harvest practices and storage conditions can prevent the contamination of foodstuffs with moulds and prevent the presence of aflatoxins. The adoption of these measures in Bangladesh will improve overall public health and bring new opportunities for international trade.

## Thursday, 30 March – 10.00 – 15.30

### P2 Poster Session 2 – General Microbiology; Laboratory and Detection Methods; Low-water Activity Foods; Meat, Poultry and Eggs; Microbial Food Spoilage; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Packaging; Preharvest Food Safety; Produce; Retail and Food Service Safety; Sanitation and Hygiene; Viruses and Parasites

### P2-01 Insight into the Incidence of Human Botulism in Belgium

**Laurence Delbrassinne** and Sarah Denayer, WIV-ISP, Brussels, Belgium

*Introduction: Clostridium botulinum* may pose a serious hazard in foodstuffs, since it forms highly resistant spores and produces potent botulinum neurotoxins (BoNT), which can lead to a severe neuroparalytic disease in humans and animals. The National Reference Centre/Laboratory for Botulism in Belgium is in charge for the surveillance of human botulism and the laboratory confirmation of *C. botulinum* (foodborne) outbreaks.

*Purpose:* The aim of the study was to give an overview of the situation of human botulism in Belgium, over the past 29 years, and to elaborate on *C. botulinum* foodborne outbreaks.

*Methods:* The NRC collects clinical data on human botulism cases and food investigations. A confirmed case means that clinical (symptomatology) and laboratory criteria (detection of active botulinum toxin or BoNT-producing Clostridia in clinical samples) are in accordance. Mouse bioassays were performed for the detection of BoNT in (cultured) samples and realtime PCR assays for the detection of BoNT genes.

*Results:* Incidence of human botulism in Belgium is low; only 14 cases of human botulism and 1 case of infant botulism were confirmed between 1988 and 2013 (average: 1 case every 2 years). Between 2014 and 2016, four additional cases were confirmed. There is a clear preponderance of botulism type B cases (16 type B, 1 type E, 1 type A, and 2 unknown); all due to the ingestion of contaminated artisanal foods. During the last decade, no contamination of commercially produced foods, available on the market, were detected through monitoring surveillance, except for some honey samples.

*Significance:* Botulism is a rare but severe/fatal disease and the surveillance of cases is indispensable for identification and effective control of potential outbreaks. Appropriate actions must be carried out, promptly, in order to ensure such vital health protection activities. Furthermore, the risk posed by this organism is not negligible in relation to home preserved food, as well as in regard to modern food processing.

## P2-02 Food Safety and Bovine Spongiform Encephalopathy (BSE) Management in Europe: The Experience from Active Surveillance to the Breeding for Scrapie Genetic Resistance in Sicily, Italy

Sergio Migliore[1], Benedetta Amato[2], Vincenzo Di Marco Lo Presti[2] and **Maria Vitale**[1], [1]Istituto Zooprofilattico Sperimentale of Sicily, Palermo, Italy, [2]Istituto Zooprofilattico Sperimentale of Sicily, Barcellona P.G., Italy

*Introduction:* Consumers' trust is important for food stakeholders, as the Bovine Spongiform Encephalopathy (BSE) crisis confirmed in the ninety. Rapid diagnosis on slaughtered animals, elimination of specific risk material, and the banning of animal flour from ruminant feeding resulted in a substantial reduction of the problem after 10 years. In addition, the establishment of the European Food Safety Authority (EFSA) assured public risk assessment analysis for food safety in Europe. In Sicily, two clinical BSE cases in animals imported from UK had been reported in 1994; and the first Italian human case of vCJD was detected in 2001.

*Purpose:* The experience on Transmissible Spongiform Encephalopathy (TSE) surveillance in Sicily is reported to highlight some beneficial outcomes related to BSE crisis management.

*Methods:* Rapid diagnostic tests for TSE was performed, through the years, by Western blot (The Prionics®-Check WESTERN), ELISA (TeSeE Purification and Detection Biorad), chemiluminescent Elisa (Enfer TSE ELISA–Abbott USA) or, more recently, by IDEXX EIA, following manufacturer's instructions.

*Results:* In cattle, five positive cases of BSE in Sicilian born animals were detected during active surveillance. More than 60 scrapie outbreaks have been detected, so far, in small ruminants. Breeding for genetic selection resistance is now performed in the island.

*Significance:* Several public controls are in place to assure food safety in Europe, since the BSE crisis. In Sicily, the surveillance for BSE and scrapie has resulted in a more accurate animal identification system and a better epidemiology knowledge of TSE spread. The results show that Sicily is an endemic area for scrapie. This has reinforced the plans for breeding for genetic resistance, with active involvement of the breeders to accomplish the goal of a full eradication of TSE.

## P2-03 Evidence of Transmission of *Listeria monocytogenes* in Seeds and Placentome of Red *Capsicum annuum*

**Chimya Gundiri** and Boniface Dumpe, University of Nottingham, Nottingham, United Kingdom

*Introduction:* The saprophytic lifestyle of *Listeria monocytogenes* in growing plants has remained a relatively, unexplored area. Salad vegetables are a recognised vehicle for transmission of *L. monocytogenes* and the route of contamination is often assumed to be via surface water. An investigation of the source of contamination of chopped red bell peppers (*Capsicum annum*) within a local food processing factory found that the most likely site associated with *L. monocytogenes* contamination was the waste bins containing the seeds and placentome.

*Purpose:* The goals of this study were to survey commercial, fresh peppers in order to provide more evidence that the primary site of contamination of *Capsicum annum* was the seeds/placentome and to understand the mechanism of entry of the bacteria.

*Methods: Listeria monocytogenes* strains were grown at 22°C in TSB broth. Chemotaxic responses were investigated using agar plugs supplement with 20 mM of plant sugars. Cells were mixed with an equal amount of soft agar (0.6 %, [w/v]) to a final cell density of ~$10^5$ CFU/ml. Chemo-attraction was detected by movement of the bacteria towards the sugar source. Extracts of Capsicum flesh were also tested. *Listeria monocytogenes* detection was carried out using the ISO 11290-1-1996 method. Flesh and seeds/placentome were tested, separately.

*Results:* A survey of bell peppers purchased from local shops (n =80) found that 5 peppers imported from The Netherland contained *L. monocytogenes* (5/40); but it was not detected in samples imported from Spain or Morocco (n = 20 of each). The positive samples came from seed/placentome samples. The chemotaxis experiments suggests that the bacteria are repelled by the flesh of the peppers, possibly due the presence of capsaicinoids, but are attracted to the sugars contained within the placentome of the peppers.

*Significance:* Isolating *L. monocytogenes* from the seeds of the bell peppers and not from the flesh suggest that the bacteria can penetrate the fruit.

## P2-04* Effect of Oxygen Availability and pH on Adaptive Acid Tolerance Response of Immobilized *Listeria monocytogenes* in a Structured Growth Media

**Ifigeneia Makariti**, Nikolaos Grivokostopoulos and Panagiotis Skandamis, Agricultural University of Athens, Athens, Greece

*Introduction:* Food microstructure and oxygen availability are known to affect the growth of *Listeria monocytogenes.* Conversely, little is known about the combined effect of oxygen limitation and sub-optimal pH, likely encountered in a food matrix, on the acid resistance of this organism.

*Purpose:* This study was conducted to evaluate the effect of oxygen availability and sub-optimal pH in a structured medium, on the growth of immobilized *L. monocytogenes,* and subsequent resistance under lethal acid stress.

*Methods:* Tryptic Soy Broth supplemented with 0.6% w/V Yeast Extract (TSBYE) solidified with gelatin (10% w/V) served as growth medium. The pH was adjusted (HCl), post-autoclaving, to 6.2 (common pH in foods) and 5.5 (adaptation inducing level). Two *L. monocytogenes* strains ($C_5$; 6179) were surface or pour plated (3 log CFU/mL) corresponding to aerobic and hypoxic conditions, respectively, and incubated at 10°C for 15 days. Anoxic conditions were achieved by adding 0.1% w/V sodium thioglycollate and a paraffin

overlay. Growth, followed by survival at lethal acidity, in TSBYE (pH 2.0, HCl, 37°C) were assessed on day 7 and 15 (n = 2x2). A biphasic inactivation model was used to fit survival data (GinaFiT tool) and calculate time for 4D reduction ($t_{4D}$).

*Results:* Anoxic conditions resulted in slower growth ($P < 0.05$) of *L. monocytogenes* than aerobic and hypoxic environments; or in lower acid resistance, manifested by the immediate population reduction below the enumeration limit. Prolonged habituation of *L. monocytogenes* (15 days), at both pHs, increased acid tolerance. Cells grown under hypoxic or aerobic conditions, at pH 6.2, demonstrated similar ($P \geq 0.05$) acid tolerance responses after 7 ($t_{4D}$=10-25 min) or 15 days ($t_{4D}$=30 min) storage; while habituation at pH 5.5 resulted in acid resistance, only, after 15 days incubation ($t_{4D}$=15-25 min).

*Significance:* Elucidating the role of oxygen limitation conditions, often encountered in structured food, on acid resistance of *L. monocytogenes,* would assist in assessing the capacity of *L. monocytogenes* originated from different food-related niches to withstand gastric acidity and initiate infection.

## P2-05 Evaluation of a Novel Phage Protein Ligand Method with Immunomagnetic Separation and PCR GENE-UP® to Detect and Isolate the Top 7 Shiga-toxin Producing *Escherichia coli* (STEC) Serogroups in Meat and Raw Milk Cheeses

**Delphine Thevenot-Sergentet**, Marion Boubier-Crozier, Sophie Felix and Aurélie Granjon, VetAgro Sup, Marcy L'Etoile, France

*Introduction:* Shiga toxin-producing *Escherichia coli* (STEC) are an important cause of foodborne illness. This pathogen can be responsible for gastrointestinal diseases ranging from diarrhea to haemorrhagic colitis and haemolytic uremic syndrome. As a result, the food industry needs fast, sensitive, and complete methods for STEC detection to ensure a safe food supply.

*Purpose:* A comprehensive method, combining immune concentration (IC) and PCR, has been developed to detect and isolate the top 7 STEC (O157, O26, O103, O111, O145, O45 and O121) in foods at risk. The performance of this method was evaluated with the ISO 13136 horizontal method, in artificially inoculated foods.

*Methods:* This method included two steps: automated IC with VIDAS® ESPT prior to the GENE-UP PCR assays for *stx, eae,* and serogroup markers. If there are positive results for these markers, another IC is used to isolate presumptive colonies on specific plates agar. A total of 40 STEC strains were inoculated onto 22 different meats and raw milk cheeses. For test method evaluation, the sample size was 375g meat or 25g cheese. For the ISO 13136 horizontal method, the sample size was 25g for both matrices.

*Results:* A total of 110 food enrichment were tested. The results obtained for both methods are comparable under the experimental conditions described.

*Significance:* This study has demonstrated that the VIDAS ESPT combined with the GENE-UP PCR is a promising tool for screening and isolating STEC strains from food enrichments. This automated method will provide technical improvement and time savings in routine testing for the food industry.

## P2-06 Rapid and Accurate *Listeria* spp. Discrimination Using MALDI TOF Mass Spectrometry without the Influence of Selective Growth Media

**Marian Awad**, Danièle Sohier and Markus Kostrzewa, Bruker Daltonics, Bremen, Germany

*Introduction:* Selective media are widely used to detect or enumerate *Listeria* spp. and *Listeria monocytogenes*. A confirmation procedure is, then, required to discriminate the closely-related *Listeria* spp., while a quick and accurate result is more than expected in food testing. Several tests are currently available; MALDI-TOF Mass Spectrometry is one of them.

*Purpose:* The ability of MALDI-TOF MS to identify and confirm isolates, directly, from widely used selective agars was evaluated.

*Methods:* The effect of growth media on the generated spectra and its consequences for species identification were determined by testing 22 target strains, i.e., 5 *L. monocytogenes* strains, 5 *Listeria ivanovii* strains, 3 *Listeria grayi* strains, 3 *Listeria welshimeri* strains, 3 *Listeria innocua* strains and 3 *Listeria seeligeri* strains. Three relevant non-target strains of *Bacillus cereus, Bacillus pumilus* and *Enterococcus faecium* were, also, used. The following selective agars were tested: Ottaviani & Agosti, Oxford, Palcam, and Rapid L.mono. The isolates were plotted onto reusable and disposable targets. Three sample preparations were used: HCCA matrix, HCCA combined with formic acid (70%), and an extraction procedure. Mass Spectra Profiles (MSPs) were acquired and analyzed with the MALDI Biotyper Complete Solution.

*Results:* A total of 375 spectra were generated. All the test isolates were correctly identified; whatever the tested selective agars, targets, or sample preparations. No bias was observed. A subtyping module was also available for use in *Listeria* species identification. It allowed the use of the simplest sample preparation protocol consisting of the overlaid HCCA matrix, only.

*Significance:* There was no influence of selective growth media on the identification of *Listeria* species, and thus, confirmation of *L monocytogenes*. No culture step on a non-selective agar was required prior to confirmation, and the simplest sample preparation can be used.

## P2-07 Database Extension for Species Identification in Fermented Products by MALDI-TOF MS: A Challenge or a Never-ending Story?

**Marian Awad**, Danièle Sohier, Simone Becker and Markus Kostrzewa, Bruker Daltonics, Bremen, Germany

*Introduction:* Lactic Acid Bacteria (LAB) and yeasts have helped to preserve foods and beverages for thousands of years by creating an environment unsuitable for food pathogens to survive. LAB and yeasts are used, worldwide, on an industrial scale; but, also, in thousands of local production units, creating thousands of local microbiomes. The

description of new species is indeed a never ending-story… But, sometimes, some isolates cause food or beverage spoilage. This definitely makes database implementation for LAB and yeasts identification a challenge; or maybe, as well, a never-ending story!

*Purpose:* Opening strategies for developing the database are to focus on fermentation processes, one after another. This helps in creating a road map; starting, first, with processes used at industrial scale, then moving to local productions to enlarge the database, as much as possible.

*Methods:* In the step-by-step database implementation program, isolates from dairy, meat, and malt fermentation processes and strains collections were characterized, using both 16S rDNA sequencing and Maldi-TOF Mass Spectrometry (MS). To set up a Reference MS Profile (MSP) for each isolate, a minimum of 20 MSPs were obtained. Whenever possible, three or more different isolates were analyzed per species.

*Results:* The database contains, currently, 272 different MSPs representing 104 species of lactobacillaceae and 730 MSPs representing 193 species of yeasts. Blind isolates were analyzed and were accurately identified and distinguished from over 7,311 other microorganisms present in the overall database.

*Significance:* The integration of Maldi-Tof MS combined to a fit-for-purpose database into existing fermentation and ripening processes helped to improve quality assurance practices, providing accurate identification and short time to result. This high-throughput, routine platform is a promising technology that can be used to dissect microbiomes from local fermented products and to identify candidates for new starter cultures with specific functionalities.

## P2-08 Pre-PCR Enrichment and Screening of *Campylobacter* in Food Samples by Redox Potential Measurement

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*Introduction*: The major sources of human *Campylobacter i*nfection are poultry meat and raw milk; therefore, efficient and rapid monitoring of *Campylobacter* in the milk and meat processing and retail industry is necessary. The ISO 10272-1:2006 standard method requires at least 136 h for confirmation.

*Purpose*: The aim of the work was to develop a rapid method of screening the *Campylobacter* negative samples by redox potential monitoring during the enrichment phase, before the real-time PCR examination. The practical applicability of this rapid method was demonstrated by the examination of raw milk and broiler meat samples.

*Methods*: A total of 190 raw milk and 375 broiler meat samples obtained from the local market were examined by redox potential measurement. Samples of 25 ml or g were homogenized in 225 ml Bolton Broth with Bolton Selective Supplement, for selective enrichment at 41.5°C. Samples showing the characteristic redox potential change during monitoring were presumed to be *Campylobacter*-positive samples. Real-time PCR was used for species identification o*f Campylobacter* positive samples. The results were compared to those achieved by the ISO standard method.

*Results*: The limit of detection of *Campylobacter* was 1 CFU/sample. In the case of negative samples, the results were be achieved in 38 h by redox potential monitoring. At 5 x $10^2$ CFU/g, which is the infective dose of *Campylobacter*, only 12.5 h was needed. There have been neither false positive nor false negative test results.

*Significance*: Use of redox potential monitoring as an enrichment method significantly decreases the time, cost, and labour requirement of detecting *Campylobacter* spp. in food. This method is fully compatible with the ISO standard, providing results more rapidly and cost-effectively.

## P2-09 Application of Digital PCR to Perform Species Identification in Food

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*Introduction:* The setting-up of new methods for species identification in food is a very important topic to avoid frauds. In fact, to verify foodstuffs compliance to labels and to discriminate accidental contamination from voluntary addition, it is necessary to quantify ingredients. To date, European legislation does not indicate how to achieve this goal, but several studies are in progress to develop and standardize new quantitative methods.

*Purpose:* The aim of this work was to verify the applicability of Digital PCR for species identification in food; in particular, to quantify chicken after artificial contamination in raw meat.

*Methods:* Digital PCR (Quant Studio 3D®) was applied to evaluate three topics: 1) Linearity between the spiking percentage of chicken (100% to 0.1%) and related observed genomic copies. 2) Correspondence between different targets (chicken, turkey, beef, pork) and a housekeeping gene (miostatin) to develop a ratio comparable with known target percentage. 3) Repeatability of 1% chicken in different meat categories.

*Results:* Linearity between target percentage in spiked material and related genomic copies value was verified ($R^2$=0.9996). However, due to DNA extracts moot quantifications, several troubles were observed in attributing the exact copies value to each spiking level. Miostatin was confirmed as the housekeeping gene for chicken, while a discrepancy between target and miostatin copies was observed for the other tested species. Finally, chicken genomic copies in swine and bovine raw meat, were not comparable for each sample.

*Significance:* These data suggest that the digital PCR system could be applied in species identification. However, further studies will be necessary to investigate the possibility of different housekeeping genes and to validate repeatability studies in order to verify the extraction efficiency.

## P2-10 Evaluation of Methodologies for the Isolation of Bioprotectives Cultures of Lactic Acid Bacteria from Aquaculture, Mediterranean Fish Species

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*Introduction:* The development of biopreservation techniques in fishery products has increased, in recent years, with the main objectives being increased the shelf life and reducing the addition of chemical preservatives. Lactic acid bacteria are the main microbial group able to produce inhibition of pathogenic species. However, isolation methods of inhibitory strains should be further studied to propose alternatives for optimizing their efficiency.

*Purpose:* The aim of this work was to develop a suitable method for the obtaining bioproptective cultures of lactic acid bacteria in marine, aquaculture fish species.

*Methods:* For this purpose, different lots of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) were collected from an estuary located in Ayamonte (Huelva). The inhibitory capacity was tested against a cocktail of strains.

*Results: T*he present work showed that enrichment of samples in MRS and in buffered peptone water produced an improved recovery of bacteria with inhibitory potential. Moreover, the results showed a stronger and more heterogeneous inhibition against *Salmonella* sp. than for *L. monocytogenes;* since halo diameters showed statistically significant differences. This could be attributed to the increased susceptibility of *Salmonella* sp. against the growth of lactic acid bacteria, producing greater variability in cell viability.

*Significance:* This study can be a starting point for future work in searching for improved conditions for the isolation and identification of microbial cultures with high inhibitory potential; thus, providing this outcome to food industries.

## P2-11 Preparation of Sublethal Injured Microorganisms for Validation Purposes

**Sabine Lindner** and Charlotte Lindhardt, Merck KGaA, Darmstadt, Germany

*Introduction:* Most bacteria get damaged during food processing. When validating test systems for the detection of bacteria in food, injured bacteria should be used. For time saving reasons a long-term storage of injured bacteria is desirable.

*Purpose:* The purpose of this study was the creation of a protocol for the generation of sublethal injured microorganisms, which reproduces the conditions found in the food itself. These injured organisms should be stable during storage.

*Methods:* A 2 ml aliquot of 24h or 4h culture of *Salmonella* spp., *Staphylococcus* spp.*, Escherichia coli, Listeria monocytogenes* or *Cronobacter* spp. were mixed with 40 grams of lactose or milk powder and submitted to drying in an incubator at 41°C for 24 h. The dried bacteria were stored in a desiccator at room temperature on silica gel or at 4°C and at -20°C. For heat stress, 10 ml of a bacterial culture was stressed in a water bath at 55-60°C. The cells were mixed with nutrient medium and glycerol and frozen at -20°C for long-term storage.

*Results:* Bacteria dried in lactose could be stored at room temperature for over a year with a slow decrease in bacterial count over time. Bacteria dried in milk powder showed a higher stability but cell count slowly decreased. Frozen heat-shock cultures could be stored for over three months without a decrease in cell count. Injury rate and stability differed depending on the bacterium.

*Significance:* A protocol for the in-house preparation of injured organisms and the possibility to store them afterwards for a longer time period was successfully developed. These organism can then be used for performance testing of nutrient media or test devices for microorganisms in food.

## P2-12 Performance Assessment of the SalmoPresto PE Assay Kit According to the ISO 16140-2 Standard for *Salmonella* spp. Detection in Egg Products

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*Introduction:* Rapid microbiological methods for the detection of *Salmonella* are based on advanced technologies, such as PCR or ELISA . Recently, a new detection method based on surface-plasmon-resonance called PlasmIA was described, allowing the detection of *Salmonella* during enrichment. This method was developed to offer a complete solution called SalmoPresto PE, for *Salmonella* detection in egg-products, with minimum handling and a 24h result.

*Purpose:* This study aimed to assess the performance of the SalmoPresto PE system for the direct detection of *Salmonella* in egg-products during enrichment. The selectivity, sensitivity, and relative detection level of the method were assessed according to the ISO 16140-2:2016 standard.

*Methods:* The SalmoPresto PE protocol consisted of diluting a 25g sample in 225mL pre-warmed buffered-peptone-water (BPW) supplemented with novobiocin (10mg/L) and incubating it for 16-20h at 37°C. A 10µL aliquot of the enriched sample was taken from the bag and diluted into 990µL of a selective broth directly into the detection kit. Then, the kit is inserted into an optical reader (MonoPresto) which delivered a reading within 10 hours (for negative results). For the purpose of this study, the SalmoPresto RT250 protocol was compared to the ISO6579 reference method in an unpaired study design. A total of 100 *Salmonella* strains and 30 non-*Salmonella* strains were analysed to assess the specificity. To assess the relative

trueness, 62 egg-product samples were analysed; while 30 samples, with different contamination levels including a fractional positive contamination level, were analysed to assess the relative limit of detection (RLOD).

*Results:* The SalmoPresto PE method detected all the *Salmonella* strains tested and showed cross reaction with a few *Citrobacter diversus* strains. The trueness and RLOD results fulfilled the acceptance criteria, showing that the method is sensitive and reliable.

*Significance:* The SalmoPresto PE method offers a new type of detection method, which provided a reliable result in less than 24 hours, with a very easy to use protocol and limited technician time.

## P2-13 ISO16140-2 Validation of Salma One Day® for the Detection of *Salmonella* spp. in Food Samples

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*Introduction:* The use of chromogenic substrates for *Salmonella* detection in food samples has been described in different commercial methods. Most of the media are based on C8-esterase activity with a high selectivity, but were reported to miss some strains such as *S.* Enteritidis Dublin, or *S.* Enteritidis Paratyphi which express a weak enzymatic activity and/or may be stressed by selective agent.

*Purpose:* The objective of this study was to compare the SALMA ONE DAY® Method to the EN/ISO/6579 standard fordetection of *Salmonella* in food samples.

*Methods:* A total of 522 samples from 6 food categories (including naturally contaminated) were tested by both methods, according to the ISO16140-2 standard. The alternative method used a single enrichment in Buffered Peptone Water (16-24h at 41.5°C) and streaking onto a new medium.

*Results:* The study showed the ability of this new method to detect *Salmonella* (n>100) and to discriminate Non-*Salmonella* strains. Conventional chromogenic medium or XLD demonstrated some difficulties in recovering atypical strains, such as serotypes Seftenberg or Dublin. Of the 522 food samples, 225 were found positive and 249 were negative by both methods. Relative accuracy, sensitivity, and specificity measurements, along with an interlaboratory study (n=15 labs) reported that both methods were comparable, according to the ISO16140-2 standard.

*Significance:* bioMerieux has developed a new medium for the detection of *Salmonella* in food samples. The high specificity was achieved, thanks to the combination of esterase substrates with a specific base. SALMA ONE DAY®method was validated according to ISO16140-2 standard and enabled a faster and easier detection of *Salmonella* spp. in food samples compared to the EN/ISO/6579 method.

## P2-14 Optimization for Recovery with Actero *Salmonella* Enrichment Media and Real-time PCR Detection of Stressed *Salmonella* in Low-water Activity Foods and Spices

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*Introduction:* Due to the ability of *Salmonella* to survive in dry conditions, for extended periods of time, the control of this foodborne pathogen in low moisture foods and their production environments represents a significant challenge for all food manufacturers.

*Purpose:* The main goal of this work was to optimize recovery of low numbers of stressed *Salmonella* in low water activity foods and spices for application with real-time PCR detection method.

*Methods:* Whole black pepper (25 g), wheat flour (25 g), and dried whole egg (100 g) samples where artificially contaminated with different sublethally stressed *Salmonella* strains and stabilized for 14 days prior to testing. Actero™ *Salmonella* Enrichment Media was used, alone (75 mL, black pepper), or supplemented with 50 mg/L green malachite (175 mL, wheat flour), or 5% non-fat dried milk (600 mL, dried whole egg) to optimize recovery of stressed *Salmonella* in a single enrichment step followed by the detection with the DuPont™ BAX® System Real-Time PCR Assay for *Salmonella.* For each matrix, 20 low level and five high level inoculum replicates, as well as five control samples, were analyzed according to the alternative protocol to compare to the samples tested by the US FDA BAM 5 or USDA-FSIS MLG 4.08 methods.

*Results:* As short as 14–18 hours of enrichment were sufficient to detect as low as 0.2–7.1 *Salmonella* MPN/sample. According to the Probability of Detection, the candidate method showed equivalent performance to the reference methods. No false positive or false negative results were observed. Furthermore, the candidate method applied to 240 low water activity matrix samples, including 25 g portions of ground black pepper, paprika, rye flour, and soy flour, as well as 375 g composite samples of whole black pepper, demonstrated high accuracy and reliability.

*Significance:* The candidate method showed the sensitivity comparable to the traditional culture-based methods, with a significant reduction in time to result.

## P2-15 Analyzing Food Integrity Using the Maxwell® RSC PureFood GMO and Authentication Kit

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*Introduction:* Real-time PCR based assays continue to gain widespread use in food safety testing because they are faster and more reliable than traditional methods and can, also, detect more specific genetic targets.

*Purpose:* Here we present the use of the Maxwell® RSC PureFood GMO and Authentication kit to purify amplifiable DNA for PCR-based genetically modified organism (GMO) and food authentication testing.

*Methods:* DNA, purified from beef, pork, canola, corn meal, soy, and cereal using Maxwell® RSC PureFood GMO and Authentication, DNeasy® *mericon*® Food, and r-biopharm SureFood® kits, was analyzed for yield, purity, and amplifiability using GoTaq® qPCR. GMO maize was spiked into GMO-free maize at 10, 1, 0.1, 0.01, and 0.001% (w/w). DNA was, then, purified using the Maxwell® RSC PureFood GMO and Authentication kit and the DNeasy®*mericon*® kit. DNA eluates were amplified, using the TaqMan® GMO Maize 35S detection kit, to identify the percent GMO in the samples. For authentication testing, beef was spiked with pork at 10, 1, 0.1, 0.01 and 0.001% (w/w). DNA from samples was purified, using the Maxwell® RSC PureFood GMO and Authentication Kit and the DNeasy® *mericon*® Food kit. DNA eluates were amplified using the RapidFinder™ Pork ID kit to identify swine DNA.

*Results:* All three kits provided amplifiable DNA. For GMO testing, the 35S event was detected down to 0.01% in the PureFood samples and down to 0.1% in the DNeasy® *mericon*® samples. For authentication testing, DNA was amplified with the RapidFinder™ Pork ID kit from all samples, except the 100% beef sample.

*Significance:* These studies, together, demsontsrate the utility of the Maxwell® RSC for automated purification of food DNA for amplification-based GMO and authentication testing.

## P2-16 Occurrence and Identification of *Salmonella* Isolates Present in Sesame Imported to Greece

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*Introduction:* Recent reports rank *Salmonella* as one of top four pathogens associated with foodborne illnesses. Although salmonellosis is often related to animal-origin food products, reports of *Salmonella* outbreaks related to plant-origin and low water activity foods are increasing.

*Purpose:* This study was undertaken to evaluate the presence of *Salmonella* in sesame, intended to be imported into Greece, and to identify the species, subspecies, and serotypes.

*Methods:* A total of 2,101 sesame samples were tested for the presence of *Salmonella* spp. at the accredited laboratory of the Institute of Technology of Agricultural Products, during the period June 2008 – December 2016, in order to get permission to be imported into Greece/EU. The countries of origin of the samples were India (1,416 samples), Soudan (280), Nigeria (176), Ethiopia (114), Burkina Faso (55), Mozambique (40) and Turkey (20). For the detection of *Salmonella* spp., ISO 6579 (4th ed. 2002-07-15/Cor.1:2004) was applied. Further confirmatory and identification tests to species, subspecies, and serovar levels were performed at the National Reference Laboratory for *Salmonella* (Hellenic Ministry of Rural Development and Food), which is accredited for serotyping.

*Results: Salmonella* was detected in 142 out of 2,101 sesame samples. Of the isolates, 133 were identified as *Salmonella enterica* subsp. *enterica*, 6 as *Salmonella enterica* subsp. *salamae*, 2 as *Salmonella enterica* subsp. *diarizonae* and 1 *Salmonella enterica* subsp. *houtenae*. The *S. enterica* subsp. *enterica* isolates designated by their antigenic formula included *Salmonella* Agona, Alkmaar, Amsterdam, Anatum, Bama, Bergen, Bolton, Bredeney, Canada, Cardoner, Dahra, Dallgow, Ekotedo, Fresno, Hannover, Hato, Havana, Heidelberg, Hemingford, Hongkong, Johannesburg, Karamoja, Kasenyi, Kastrup, Kentucky, Kingston, Kinondoni, Kristianstad, Liverpool, Logone, Maastricht, Madjorio, Mbandaka, Monschaui, Montevideo, Mountpleasant, Orion, Paratyphi B var. Java, Poona, Reading, Ruivu, Sanjuan, Scheissheim, Senftenberg, Stanley, Stormont, Telelkebir, Telhashomer, Tennessee, Tilburg, Tilene, and Vejle.

*Significance:* The study indicated that sesame products may constitute a potential hazard for human, especially if they are consumed raw or GMP are not applied.

## P2-17 Effects of On-farm and Pre-slaughtering Stress on Poultry Meat Contamination from *Campylobacter* spp. and Other Foodborne Pathogens: Preliminary Results

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*Introduction:* There is lack of knowledge about the effects of different levels of animal welfare on poultry meat contamination. New control tools are needed by poultry companies; particularly in view of the new criterion for *Campylobacter* that is expected to be included in the European Union food law.

*Purpose:* This study compares different levels of stress associated to on-farm and pre-slaughtering practices and evaluates their influence on pathogens fecal shedding and consequent contamination in broiler carcasses.

*Methods:* Ten farms belonging to an Italian poultry company, all positive for *Campylobacter* infection, were selected for study. The Welfare Quality® protocol was used to cluster farms into two groups, based on 14 Animal Welfare (AW) indicators. At the time of slaughtering, flocks from different AW groups were tested for *Campylobacter*, *Salmonella*, and *Listeria monocytogenes*. Detection and enumeration were carried out using ISO or validated methods.

*Results:* Four flocks from four different farms have been followed to the slaughterhouse. Two flocks were classified as "higher welfare" (HW) and two "lower welfare" (LW). The frequency of *Campylobacter* in cloacal swabs increased after long transports, with statistically significant differences compared to shorter transports. No statistically significant difference in Campylobacter levels between HW and LW flocks was reported in caecal contents (8.45 vs 7.40 $\log_{10}$ CFU/g) or on carcass skin (1,865 vs 858 CFU/g). Widespread *Salmonella* Infantis contamination was found, only, in LW flocks; this could have influenced *Campylobacter* levels. Lower welfare could have favored *Salmonella* contamination.

*Significance:* The use of 'animal welfare-friendly' management methods able to reduce carcass contamination could be an innovative tool for poultry industries to improve the microbiological quality of their products. Considering some statistically significant evidence achieved, so far, our preliminary results point in this direction. This three-year research project should be concluded before 2018. Therefore, more data will be produced to further confirm or reject this theory.

## P2-18 Metagenomic Analysis of the Spoilage Microbiota of MAP Packaged and Air Packaged Cooked Meat

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*Introduction:* Different packaging may well lead to a different dominant flora in cooked spoiled meat, like hams and hot dogs. Several reports on identification of cooked meat spoilers are present in literature, but it is difficult to extract a general picture in terms of variation in dominance, the influence of packaging, region, etc. Application of metagenomics enables us to analyze more samples, with more depth and less effort, eventually leading to a more comprehensive overview.

*Purpose:* In the current study we analyzed sliced, cooked meat and looked at the influence of modified atmosphere packaging (MAP) and air packaging on the microbiota.

*Methods:* Multiple samples (22 in total) of sliced cooked ham and chicken breast, MAP and air packaged, from two different supermarkets, were incubated at 4°C. The samples were analyzed, on the expiry date and two days later, with regard to the CFU, the gas composition, and microflora via 16 S rDNA metagenomics.

*Results:* The samples (11) taken at the expiry date contained between 106 and 107 bacteria and all samples (11 samples) taken two days later contained between 107 and 108. In literature, a CFU count above 107 is usually considered spoiled. We can conclude that all samples were at or just below the spoilage point at the expiry date. Looking at the microflora, some frequent spoilers of VP cooked meat were present in both MAP and air packaged samples, especially *Leuconostoc carnosum*. Compared to vacuum packaged meat, we saw more *Brochotrix* spp. in MAP.

*Significance:* A better understanding of the dominant spoilage flora will lead to better and specific interventions to elongate shelf life.

## P2-19 Advancement of a Laboratory Scale Recombined Butter Production Method Used for Challenge Testing with the Spoilage Yeast, *Candida guilliermondii*, at Refrigeration and Room Temperature

**Irena Soljic**, Azar Abbasian Genaveh and Frank Devlieghere, Ghent University, Ghent, Belgium

*Introduction:* Development of laboratory-scale emulsion producing methods is crucial to production of emulsions for challenge tests that mimic the structure of commercial samples. *Candida guilliermondii* is a food spoilage yeast isolated from yoghurt, butter, buttermilk, olives, olive oil, fish, and beer.

*Purpose:* The purpose of this study was developing a laboratory-scale method to produce emulsions with structures similar to commercial samples. Samples were inoculated with *C. guilliermondii* and placed at 7°C and 22°C to investigate the influence of yeast growth on sample structure.

*Methods:* Anhydrous milk fat (AMF) and nutritious water phase were combined to produce water-in-oil emulsions with 61% and 82% AMF content. NMR analysis showed that homogenization speeds that between 5,000 to 15,000 rpm produced emulsions whose mean droplet size diameter ($D_{4,3} \approx 3$ µm) and distribution matched commercial samples. Triplicate samples were inoculated with $10^2$ CFU *C. guilliermondii*/mL, placed at 7°C and 22°C, and analyzed for yeast growth and changes in $D_{4,3}$ for 21 days. Growth curves were plotted and $D_{4,3}$ differences compared using ANOVA.

*Results:* Emulsions homogenized at 10,000 rpm had the lowest percentage of vulnerable droplets ($D_{4,3}$ > 10 µm), while still supporting challenge testing. Significant differences were observed between $D_{4,3}$ of samples kept at 7°C and 22°C with both 61% and 82% AMF butter ($P < 0,05$); and between samples of both fat percentages kept at either 7° or 22°C ($P < 0,05$). This shows the influence of emulsion composition and temperature on $D_{4,3}$. *Candida guilliermondii* inoculated in samples of 82% AMF, kept at 7°C and 22°C, reached $10^3$ CFU/g after 21 days of incubation and $10^6$ CFU/g in 61% AMF samples after 7 days at 22°C and after 14 days at 7°C, indicating potential coalescence of water droplets after exhaustive growth in reduced fat emulsions.

*Significance:* Development of this laboratory scale emulsion-making process might aid future challenge tests by mimicking similar products and may deepen knowledge about the influence of microbial growth on emulsion structure.

## P2-20 Microbial Metabolome Evolution in Aerobically Stored, Naturally Contaminated Beef Fillets

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*Introduction:* Metabolome formation in food and, especially, in meat during storage is a complex phenomenon depending on various factors.

*Purpose:* The aim of this study was to (i) use a metabolomics approach, in tandem with bioinformatics, to assess the microbiological quality of aerobically stored beef fillets and (ii) identify microbial biomarkers that are produced and metabolized during storage.

*Methods:* Naturally contaminated (NC) and sterile (S) beef fillets were stored, aerobically, at 2, 8, and 15°C. At appropriate time intervals beef samples were analyzed, microbiologically, for the determination of Total Viable Counts (TVC), while the biochemical changes (metabolomics) occurring in these samples were recorded using both GC/MS and HPLC-DAD-RI.

*Results:* Multivariate statistical models (PLS-R, PLS-DA) were developed for each instrumental, analytical technique, as well as in fusion to (i) predict TVC and (ii) classify the samples into quality classes (fresh,

semi-fresh, spoiled). For TVC prediction, the mean values for bias and accuracy factors in both cases were close to 1 and 88% of the predictions were inside the relative error zone of 20%. Lastly, good prediction during classification, with an overall, correct classification rate close to 90% was achieved for the two methods.

Qualitatively, GC/MS analysis revealed that the compounds 2-butanone, 2-pentanone, 2-octanone, 2-nonanone, diacetyl, acetoin, 3- and 2-methyl-1-butanol and esters of acetic, isobutyric, and valeric acid were increased during storage and ,consequently, they could be characterized from microbial origin. The HPLC results showed that malic, lactic, propionic, glucose, and one unknown compound with retention time 29.2 min were correlated with fresh category; while acetic, citric and isobutyric acids were correlated with semi-fresh and spoiled samples, respectively.

*Significance:* Taking into account the above metabolomic approach, meat inspection could be determined through rapid, analytical techniques that give acceptable results within a few hours instead of 24-48 hrs, as required by the conventional ISO method.

## P2-21 Quality and Safety Assessment of Marinated Chicken Products from Greek Retail Outlets

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*Introduction:* Marinated meat products are becoming increasingly popular in meat retail. However, there is little information about their microbiological safety and quality.

*Purpose:* This investigation was conducted to determine the prevalence of four pathogens in marinated chicken products and to evaluate of quality by microbiological, sensory, and chemical analysis.

*Methods:* Eighty (80) samples obtained from several Greek meat markets were analyzed for the presence of *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes,* and *E. coli* O157:H7. The samples were subsequently stored aerobically at 4°C for 5 days. Microbiological analysis, sensory evaluation, and high performance liquid chromatography (HPLC) analysis were carried out for the determination of spoilage microorganisms, sensory quality based on the evaluation of samples odor, and chemical composition (preservatives and organic acids).

*Results:* The prevalence of *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes* and *E. coli* O157:H7 was 56%, 10%, 36%, and 5%, respectively. *Pseudomonas* spp. was the dominant spoilage microbial group, found in 50% of the samples, while in 27% of samples, a co-dominance of *Pseudomonas* spp. and *Brochothrix thermosphacta* was observed. The counts of total aerobes increased to 7.0 log CFU/g at the 2nd or 3rd day in 70% of the samples, while sensory analysis showed that 80% of the samples were characterized as spoiled after 3, 4 or 5 days. The chemical composition of 36% of the samples contained sodium benzoate, potassium sorbate or both, while an increased concentration of acetic, citric, and propionic acid was observed in 13%, 17%, and 20% of the samples, respectively, compared to raw chicken meat.

*Significance:* The obtained data demonstrated the occurrence of foodborne pathogens in marinated chicken products and allowed the acquisition of an overall view about their quality.

## P2-22 Identification of Potential Emerging Risks in the Salmon and Oyster Food Chain: Piloting an Innovative Text Mining Tool

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*Introduction:* It is part of EFSA's remit in food and feed safety to evaluate the application of innovative technologies to support the identification of emerging risks.

*Purpose:* This pilot project aimed to assess the applicability and capability of the Emerging Risk Identification Support System (ERIS), developed by The Netherlands Organisation for Applied Scientific Research (TNO), to identify emerging hazards in the atlantic salmon (*Salmo salar*) and pacific oyster (*Crassostrea gigas*). ERIS applies text mining rules to identify grammatical and contextual relationships in article titles and abstracts, between potential hazards, effects, and exposure.

*Methods:* During the pilot phase, ERIS' ontology was adapted in an iterative and interactive process, according EFSA's needs, followed by a blind trial in order to align the expert's evaluation from EFSA and TNO. In the second phase the text mining tool was applied to abstracts from two different databases MEDLINE®/PubMed® and FSTA®, published January 2015–June 2016. The output was evaluated by experts for identification of potential emerging risks, according to an accepted quality assured protocol, based on a multi-eye principle, including a benchmark for the relationships found against a database containing scientific literature of 10 years (2005–2014).

*Results:* ERIS processed 1,821,576 abstracts and retrieved 707 abstracts (422 for salmon and 285 for oyster). After a first round of expert evaluation, 67 articles for salmon and 47 for oyster were pre-selected. A second round of expert evaluation, comparing the pre-selected abstracts with the benchmark, led to the identification of 28 potential emerging risks; 18 in salmon and 10 in oyster.

*Significance:* Significant resources were invested by EFSA and TNO to improve the precision of the ERIS tool in identifying potential emerging risks. ERIS has been proven to be a valuable tool for automatically select relevant research abstracts, allowing the identification of potential emerging risks from a trusted and manageable data set, after expert evaluation.

## P2-23 Validation of the Predictive Models for the Behaviour of *Salmonella* Enteritidis during Storage of Yoghurt at Different Temperatures

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*Introduction:* Yoghurt is a fermented dairy product usually considered healthy and safe. However, a predictive model recently developed for the temperature range of 4-25ºC showed that *Salmonella* may survive for days in yoghurt during storage.

*Purpose:* Predictive models for the behaviour of *Salmonella* Enteritidis in yoghurt were validated.

*Methods: Salmonella* survival data in yoghurt were obtained at 16ºC by plate counting method and then a fit-for-use, survival model with shoulder and tailing (Geeraerd et all., 2000) using the package nlsMicrobio in R software (version 3.2.2). The parameters, survival rate ($k_{max}$) and shoulder ($S_l$), were compared with the estimates given by the previously, developed predictive models. The validation procedure was performed based on the use of the bias factor (*Bf*) and the accuracy factor (*Af*).

*Results:* The kinetic parameters for the survival curves obtained at 16ºC were found to be 0.17 $h^{-1}$ ($k_{max}$) and 28.64 h ($S_l$). The *Bf* and *Af* values of the secondary models for $k_{max}$ - temperature were calculated as 0.82 and 1.21, respectively. The $S_l$, *Bf* and *Af* values were 0.80 and 1.24, respectively. According to these indices, the model predictive capacity can be considered acceptable, although it sub-estimated both survival rate and shoulder length, resulting in fail-safe predictions.

*Significance:* Validation of models is essential to evaluate the construction and robustness of models. The results confirm that the developed models can be used to predict the behaviour of *Salmonella* Enteritidis during storage of yoghurt at different temperatures.

## P2-24* Can *Listeria monocytogenes* Growth Variability be Explained by the Genotype?

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*Introduction: Listeria monocytogenes* is a microbial pathogen that causes the foodborne listeriosis. This pathogen is able to survive and grow under a wide range of environmental stressful conditions (low temperature, pH and $a_w$) often found in food processing plants or during food storage. Intraspecific strain variability, although limited, has already been demonstrated in literature but reasons explaining these differences are scarce or contradictory between studies.

*Purpose:* The aim of this study was to look for a potential link between the phenotype (growth rate, $\mu_{max}$, under stressful environments) and the genotype (based on Pulsed Field Gel Electrophoresis; PFGE).

*Methods:* The $\mu_{max}$ of 53 strains of *L. monocytogenes* was determined by optical density measurement in different harsh conditions; i.e. cold (8°C), acid (pH 5, 20°C), and low water activity ($a_w$ 0.95, 20°C). A reference strain was used to assess inter-experimental variability. For each condition, clustering of strains was established based on $\mu_{max}$ values and dendrograms were drawn. PFGE profiles (based on AscI/ApaI restriction enzymes) were obtained for all these strains and a phylogentic tree was established according to UGPMA algorithm. Finally all created trees were compared among each other. A statistical analysis of the relationships between the trees/dendrograms was conducted in order to look for a possible link between pheno- and genotype.

*Results:* A relationship could not be observed under the tested conditions between the PFGE based tree and the $\mu_{max}$ values for each condition. Even the comparisons of the three phenotype dendrograms displayed no similarity.

*Significance:* The next step will be a comparison involving a genotype characterization, based on the whole genome sequence. An existing relationship between pheno- and genotype could help predict microbial behavior during food processing and storage; and thus, introduce genomics in risk assessment.

## P2-25 Modelling the Behaviour of *Listeria monocytogenes* during the Making of Cheese from Raw Milk

**Elena Dalzini**, Elena cosciani-Cunico, Paola Monastero, Giorgio Varisco, Marina Nadia Losio and Paolo Daminelli, IZSLER, Brescia, Italy

*Introduction:* The presence of *Listeria monocytogenes,* even if at low levels, in raw milk used for to produce raw milk cheeses represents a safety issue.

*Purpose:* The aims of this study were i) to study the effect of the inoculum level of lactic acid bacteria (LAB) on the behaviour of *L. monocytogenes* during cheese making and ii) to develop of a predictive model to describe this effect, as well as the growth of LAB as a function of temperature.

*Methods:* Raw milk was inoculated with three different LAB concentrations (4, 6, and 8 log CFU $g^{-1}$) and, also, contaminated with *L. monocytogenes* registered strain ATCC 19115. The contaminated milk was used to produce soft cheeses. The obtained data were used to validate a predictive model, which in turn was based on data, partly produced by IZSLER and partly available from the ComBase database (www.combase.cc). The model of Baranyi and Roberts was used to calculate the primary growth parameters, while the model of Ratkowsky was used to describe the growth rate as a function of the temperature. To take the inhibitory effect of the LAB concentration into account, we assumed that no cell division took place after LAB reached a concentration level (Jameson effect).

*Results:* The concentration of *Listeria* did not exceed the level of 8 log CFU $g^{-1}$, which is normally its maximum population density, but stopped at around 5 log CFU $g^{-1}$ when the LAB reached their threshold concentration. The predicted data were in good

agreement with independent observations in cheese, showing a discrepancy between 2.18% and 4.64%.

*Significance:* This model may be a very useful tool to support the monitoring surveys carried out by officers of the Regional Veterinary Authority.

## P2-26* Assessing the Growth of *Listeria monocytogenes* in Mediterranean Fish Products from Marine Aquaculture

**Araceli Bolívar**, Guiomar Denisse Posada-Izquierdo, Antonio Valero, Jean Carlos C.P. Costa, Gonzalo Zurera and Fernando Pérez-Rodríguez, University of Cordoba, Cordoba, Spain

*Introduction:* Predictive microbiology is an important tool, which has immediate applications to improve microbial food safety and quality. Although several mathematical models have been developed to predict microbial behaviour in seafood and fishery products, most of them were performed on ready-to-eat (RTE) products from Nordic or Atlantic species. Therefore, there is a need for the development of a predictive models for Mediterranean fish species that considers the effects of packaging technologies and storage conditions, on both spoilage and pathogenic bacteria.

*Purpose:* The objective of the present study was to evaluate the growth kinetics of *Listeria monocytogenes* in the range 4–20ºC, at different atmospheric conditions, in fish-based juice (FBJ) of gilthead sea bream (*Sparus aurata*).

*Methods:* Validation was performed with experimental growth data from the pathogens in fresh sea bream and sea bass (*Dicentrarchus labrax*) fillets from marine aquaculture. Then, predictions were assessed with external data from scientific literature. Models were compared with predictions from other existing tertiary models for *L. monocytogenes* growth included in the software Food Spoilage and Safety Predictor (FSSP).

*Results:* Validation with experimental data from challenge testing showed that models generated in FBJ slightly over-predicted growth rates, providing fail-safe predictions ($B_f$ = 2.03). A perfect coincidence between predictions and observations was observed in the case of the FSSP's model ($B_f$ = 1.00). The validation process of the FBJ model developed under anaerobic conditions demonstrated that the model was able to adequately described growth rates observed, by other authors, in different fresh fish species ($B_f$, $A_f$= 1.33).

*Significance:* This study provides useful information about *L. monocytogenes* growth in Mediterranean fish species through the development of predictive models, which might be applied in the food industry. It also demonstrates that the use of food-based systems to evaluate microbial behaviour can be a suitable instrument to obtain accurate predictive models.

## P2-27 Modeling the Growth of *Listeria innocua* and Spoilage Bacteria in Cooked Tuna Loins

**Hudaa Neetoo** and Karina Natasha Olivier, University of Mauritius, Reduit, Mauritius

*Introduction:* Tuna loins are widely consumed in Mauritius, due to their low cost and high protein content. However, tuna naturally harbours spoilage bacteria and occasionally the pathogen, *Listeria monocytogenes*. Growth of *L. monocytogenes* and spoilage microbiota are both affected by temperature. During storage, distribution, and retailing, tuna loins are exposed to a wide range of temperatures, which can impact on the quality and safety of the product.

*Purpose:* The purpose of this research was to develop mathematical models to predict the growth kinetics of spoilage microbiota and *L. monocytogenes* in tuna meat under isothermal conditions.

*Methods:* Briefly, cooked tuna loins were cut in pieces (12 g) and inoculated with *Listeria innocua* ATCC 33090, surrogate of *L. monocytogenes*, to a final population density of ca. 2 log CFU/g and stored at 2, 4, 7, 10, 13, or 15ºC for up to 120 days. Uninoculated tuna loins were stored under similar isothermal conditions. At specific time intervals, inoculated and uninoculated samples were removed and the counts of *L. innocua* and total aerobic bacteria were determined by plating on PALCAM and Plate Count Agar,respectively, and incubating the plates for 2 days at 35°C. Growth data were then fitted to the Baranyi and Roberts model and parameters maximum specific growth rate ($\mu_{max}$), asymptotic cell number ($y_{max}$), and lag time ($t_{lag}$) were, subsequently, extracted. Secondary models were then generated by plotting the log of $\mu_{max}$ as a function of their corresponding temperature.

*Results:* Primary models were found to fit the data with a reasonable goodness of fit; $R^2$ values ranging from 0.916 to 0.968. Secondary models displayed a linear relationship between log $\mu_{max}$ of *L. innocua* or aerobes and growth temperature ($R^2$ of 0.912-0.955).

*Significance:* Once validated, the models developed in this study may be useful tools to predict growth responses of pathogenic and spoilage bacteria in tuna products.

## P2-28 Comparative Microbiological and Hygienic Status of Glass, Plastic, and Wooden Chopping Boards

**Hudaa Neetoo**, Mala Ranghoo-Sanmukhiya and Usha Motah, University of Mauritius, Reduit, Mauritius

*Introduction:* Chopping boards (CBs) are processing equipment, widely used in households and food establishments. Chopping boards harboring pathogenic microorganisms can cross-contaminate food products leading to foodborne illnesses.

*Purpose:* This study aimed to characterize the microbiota of CBs and compare the efficacy of domestic treatments to disinfect CBs.

*Methods:* Briefly, used glass, plastic, and wooden CBs were collected from various households and cut into slabs of 25 $cm^2$. Microorganisms were recovered from the slabs using the swabbing method. Swabs were rinsed in sterile buffered saline and the rinsate was plated onto appropriate media to enumerate counts of mesophilic aerobic bacteria (MAB), yeasts and molds (YM), *Escherichia coli* (EC) and *Listeria* spp. Fungal species were identified by sequencing and phylogenetic analyses. To compare the efficacy of different domestic treatments, sterilized glass,

plastic, and wooden CBs were inoculated with *Escherichia coli* ATCC 25922 (EC) or *Listeria innocua* ATCC 33090 (LI) to a final population density of ~ 3 log CFU/cm$^2$ and subjected to one of five treatments (dishwashing detergent, chloroxylenol-based disinfectant (Dettol), hot water (65°C), chlorine-based disinfectant (bleach) and white vinegar). Treatment was followed by rinsing with sterile water. The bactericidal efficacy (BE) of the different treatments was computed as: population density before treatment – population density after treatment.

*Results:* Mean population density of MAB, YM, EC and listeriae recovered from CBs were as follows: 3.6, 2.2, 1.8 and 2.4 log CFU/cm$^2$ (plastic), 3.3, 3.0, 1.6 and 1.5 log CFU/cm$^2$ (wood) and 2.6, 2.3, 1.9 and 1.7 log CFU/cm$^2$ (glass), respectively. Isolated fungi were identified as *Penicillium critinum*, *Peyronellaea glomerata,* and *Cladosporium halotolerans*. Vinegar and bleach were the two most effective treatments and significantly ($P < 0.05$) reduced EC and LI counts by a maximum of 1.5–1.6 and 1.2–1.5 log CFU/cm$^2$, respectively.

## P2-29 Using Whole Genome Sequencing to Provide Insight into the Epidemiology of Resistance and Virulence Genes in *Listeria monocytogenes*

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*Introduction: Listeria monocytogenes* is a ubiquitous organism in the environment and a rare cause of human disease. Although its incidence is at least 100 times lower than those of other foodborne pathogens, such as Campylobacter and *Salmonella*, listeriosis is characterized by a high case-fatality rate which can exceed 30% percent in outbreak situations. Currently, every isolate in a food or clinical settings is considered problematic; even though some isolates are more likely to persist in a food environment and/or cause human disease. Many virulence and resistance genes have been linked to these features, but no large scale investigation has been conducted on the presence of these factors in isolates from different environments. Therefore, our knowledge on the frequency and importance of known virulence and resistance genes in *L. monocytogenes* is limited.

*Purpose:* As more and more whole genome sequence (WGS) data become available from surveillance, this data can, as shown here, can be used to make an extensive study on the epidemiology of known resistance and virulence genes.

*Methods:* Publically available sequence read sets of over 10,000 *L. monocytogenes* isolates were assembled on the BioNumerics Calculation Engine using SPAdes. A reference database was created with all known virulence and resistance genes, as well as genes determining serovars. This database was used to screen all assembled genomes for the presence of these genes, and to predict the serovar.

*Results:* The BioNumerics® 7.6 software and its Calculation Engine offer a powerful platform on which WGS analysis can be performed and validated against traditional typing data, as well as phenotypic data. The genotyping tool provides the possibility to extract virulence- and antibiotic-related genomic signatures from WGS data.

*Significance:* The virulence genes and resistance genes could be easily extracted and compared to the available metadata, providing insight in the presence and distribution of these genes within all publicly available NGS data.

## P2-30 Antibacterial Control Techniques with Antimicrobial Substances Conveyed with Food Films

**Claudio Gallottini**, ITA Corporation, Miami, FL

*Introduction:* Bacterial contamination is a major problem in food processing and food residues in process lines. New chemical technologies and microbiological analyses with biosensors are an innovative, reliable choice for quality control in foods. New biomolecular techniques for food pathogen detection are being developed to improve biosensor characteristics, such as sensitivity and selectivity.

*Purpose:* Biosensors act as analytical devices employing a biological material or biomimic as a recognition molecules integrated within a physicochemical transducer or transducing microsystems. This technique is rapid, economic, effective, and suitable for *in situ* analysis. This work was undertaken to develop low-cost, disposable sensors.

*Methods:* Agents or bacterial toxins or fragment from microbial infection that can assure the presence of specific pathogenic bacteria were evaluated: *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Listeria monocytogenes*. The analysis was performed, in real time, by binding analyte on the reactive surface, using biosensors with the specific reaction to specific antibodies.

Due to the selectivity of graphite-based amperometric detectors for significant substrates, it was the choice for the development of these low-cost, disposable sensors.

*Results:* We have developed a microbial based biosensor to determine the presence of specific pathogenic bacteria. This immunosensor was able to detect 80–100 CFU/ml in a water solution of bacteria detected. The assays were specific and showed signal in the presence of all microorganisms tested, such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Listeria monocytogenes*.

*Significance:* The advantages of the sensors that we developed are that they are: rapid, reliable, specific, and cost effective. Additionally, there is no need for trained workers and the equipment cost is minimal. Biosensors are an alternative to classical analytical methods. They are biochemical analytical methods with the advantages of being easy to handle, portable, quick, and the user does not require special skills.

## P2-31 Food Security and Prevalence of Anemia in Children under Five Years of Age in the Rio Apurímac Ene and Mantaro Valley

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*Introduction:* The prevalence of anemia in the rural communities of Peru's Valle de los Ríos Apurímac, Ene y Mantaro (VRAEM) is 83%. The causal factor is food insecurity.

*Purpose:* This study assessed the relationship between food security and prevalence of anemia in children under five years of age in the Apurímac Ene and Mantaro River Valley.

*Methods:* The study design was cross-sectional, descriptive, and correlational, with a sample of 208 children younger than five years.

*Results:* Mild anemia was observed in 51.44%, moderate anemia in 28.37%, severe anemia 2.88%, and 17.31% did not have anemia. Highly significant relationships were found between: maternal age and prevalence of anemia ($P = 0.01$), maternal marital status and prevalence of anemia ($P = 0.00$), number of members in the family and prevalence of anemia ($P = 0.022$), predominant material in the walls of the house and prevalence of anemia ($P = 0.027$), predominant material of the floors and prevalence of anemia ($P = 0.000$), type of energy used for cooking and prevalence of anemia $P = 0.025$), years fed breast milk and prevalence of anemia ($P = 0.044$), exclusive breastfeeding and prevalence of anemia ($P = 0.02$), knowledge of three foods that aid in the growth and prevalence of anemia $P = 0.044$), vaccinated and prevalence of anemia ($P = 0.01$), more than three fluid depositions per day and prevalence of anemia ($P = 0.015$), when diarrhea gives it equal, more or less fluids and prevalence of anemia ($P = .045$), handwashing and prevalence of anemia ($P = .036$), physiological needs and prevalence of anemia ($P = .045$), health and prevalence of anemia ($P = 0.03$), has a greenhouse and anemia prevalence ($P = 0.00$).

*Significance:* There are significant relationships between food security and prevalence of anemia in children under five years of age in the VRAEM.

## P2-32 The Effect of Biological Treatment of Manure on the Presence of Antibiotic Residues, Antibiotic Resistance Genes, and Zoonotic Pathogens

Tina Van den Meersche[1], **Geertrui Rasschaert**[2], Freddy Haesebrouck[3], Els Van Coillie[2], Els Daeseleire[4] and Marc Heyndrickx[2], [1]Institute for Agricultural and Fisheries Research (ILVO), Melle, Belgium, [2]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Melle, Belgium, [3]Ghent University, Merelbeke, Belgium, [4]Institute for Agriculture Fisheries and Food (ILVO), Melle, Belgium

*Introduction:* In past years, concerns about the occurrence and dissemination of antibiotic residues, antibiotic resistance genes, and zoonotic pathogens, in the environment, have emerged. In Belgium, about 39.3 kilotons of nitrogen from manure are treated, yearly, before deposition on the fields; but, the fate of antibiotic residues, antibiotic resistance genes, and zoonotic pathogens during manure treatment is unknown.

*Purpose:* The purpose of the study is to assess whether biological treatment of manure has an effect on the occurrence and fate of antibiotic residues, antibiotic resistance genes, and zoonotic pathogens.

*Methods:* Samples were taken from different stages of the biological treatment of swine manure on one pig farm, at six different time points with a two-week interval. The quantification of antibiotic residues (ceftiofur, colistin, doxycycline, oxytetracycline, sulfadiazine, trimethoprim and tylosin A) was performed with UHPLC-MS/MS. Tetracycline resistance genes (*tet*(B), *tet*(L), *tet*(M), *tet*(O), *tet*(Q) and *tet*(W)) were quantified using real-time PCR. The presence of zoonotic pathogens (*Salmonella* spp. and *Campylobacter* spp.) and *Escherichia coli*, as indicator bacterium, was assessed using culture techniques.

*Results:* Our results showed a reduction of sulfadiazine and doxycycline after biological treatment of manure. This treatment resulted in at least a 10-fold reduction of the tetracycline resistance genes, with the exception of *tet*(L). Concerning the pathogens, our results show that *Salmonella* Typhimurium can be present in the different stages preceding the biological manure treatment, but it was never detected in the storage lagoon. The *Campylobacter* that was detected in the liquid fraction, only, and confirmed as *Campylobacter coli*. For *E. coli*, a reduction from $10^5$ CFU/g to below the detection limit was observed during biological treatment of swine manure.

*Significance:* The data suggest that biological treatment of manure may be a tool to reduce the amount of antibiotic residues, tetracycline resistance genes, and zoonotic pathogens present in the manure.

## P2-33 Reduction of Virulence and Antibiotic Resistance of *Salmonella* in Commercial Broiler Ceca Samples

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*Introduction:* Livestock may serve as a reservoir for antibiotic resistant bacteria that may transfer to our food system; some of zoonotic concern such as *Salmonella*. These antibiotic resistant bacteria result in a reduced effectiveness of antibiotic compounds in treating illness. Interventions are needed to reduce the reservoir of antibiotic resistance genes in food animals.

*Purpose:* The purpose of this study was to evaluate XPC supplementation as a method to reduce virulence and antimicrobial resistance of *Salmonella* in broilers.

*Methods:* A large field study was conducted to determine the effects of feeding XPC on reducing *Salmonella* in broilers, including an evaluation of *Salmonella* virulence and antibiotic resistance to florfenicol, ceftiofur, and enrofloxacin, in a total of 134 commercial broiler houses. In this study, houses were fed either a diet that contained 1.25 kg/MT of Original XPC (XPC) or the company standard diet (CON).

At the processing plant, one cecum was collected from between 50-100 birds/house (4,675 total cecum tested). Data were analyzed in SAS using the Chi-Square procedure with feeding treatment as the main effect.

*Results:* All isolated *Salmonella* colonies were measured for virulence and antibiotic resistance. Virulence was significantly lowered ($P < 0.0001$) in isolates from XPC fed birds compared to CON (0.17% vs. 1.05%, respectively). *Salmonella* antibiotic resistance was significantly lowered ($P < 0.0001$) in isolates from XPC fed birds compared to CON (florfenicol: 1.95% vs. 12.84%; ceftiofur: 0.48% vs. 9.55%; enrofloxacin: 0.01% vs. 3.96%, respectively).

*Significance:* These data suggest that virulence and antibiotic resistance of *Salmonella* can be reduced by the inclusion of XPC in broiler diets.

## P2-34 Pests as Carriers of Zoonotic Bacteria on Production Farms: A Pilot Study

**Maria Rönnqvist**, Marjaana Hakkinen, Satu Hakola, Satu Olkkola and Pirkko Tuominen, Finnish Food Safety Authority Evira, Helsinki, Finland

*Introduction:* Zoonotic bacteria can be transmitted to humans via food or drink; many of them via animal-derived foods or cross-contamination. Production animals can be carriers, acquiring the infection via different routes, such as from the farm environment or contaminated feed. The occurrence of zoonoses in rodents and other pest animals needs to be studied in order to estimate the contamination risk they pose to foods of animal origin.

*Purpose:* The objective of the pilot study was to test a trapping protocol for rodents and shrews and to examine whether these animals, in Finland, carry *Campylobacter* or *Samonella* bacteria in their intestines.

*Methods:* The pests were trapped near to two cattle farms (Farm 1 and Farm 2) and two houses from urban area (Control 1 and Control 2), during autumn 2016. They were transported to a laboratory where their intestinal contensts were examined for *Campylobacter* and *Salmonella* using standard methods.

*Results:* Yellow-necked mice (*Apodemus flavicollis*), house mice (*Mus musculus*), brown rats (*Rattus norvegicus*), and common shrews (*Sorex araneus*) were caught during the study. In total, *Campylobacter jejuni* was isolated six times, while *Salmonella* Typhimurium was isolated once from a yellow-necked mouse from Farm 2. *Campylobacter* was a common finding from the rodents from farm environments (4/8 trap checks) and from control environments (2/8 trap checks).

*Significance:* The results of the pilot study showed that the rodents in Finland may carry both *Campylobacter* and *Salmonella* in their intestines and that the trapping protocol is suitable for a larger study. As rodents can be a significant route for zoonotic bacteria from the environment to the farms, the impact of these pests as vectors should be studied. Increasing knowledge on the pathways of zoonotic pathogens in food-producing animals may help risk managers target their actions accordingly; to prevent consumers from food-mediated zoonotic infections.

## P2-35 Insect Rearing on Manure: Are Microorganisms and Antibiotics Being Transferred from the Substrate to the Larvae?

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*Introduction:* Insects are believed to be a sustainable protein alternative for feed and food; however, food safety is an important factor. Besides the production method, the insect species, the harvest stage, and the substrate used is known to be important in the biological and chemical hazards of the non-processed insects.

*Purpose:* This study aimed to assess the microbiological and chemical risks from rearing black soldier fly larvae on solid pig manure. Additionally, two washing procedures were evaluated.

*Methods:* Larvae of the black soldier fly were reared at 24°C for 20 days until fully grown, with fresh pig manure as substrate. This manure was spiked with *Listeria* spp. or sulfadiazine, lincomycin, and doxycycline. Fresh manure, harvested larvae, and residual substrate were analysed for dry matter content and microbiology. Larvae were washed with a physiological water (10 washing steps) and with ethanol; both were analysed. Fresh manure and harvested larvae from the chemical hazard trial were, also, analysed for the presence of antibiotics.

*Results:* A microbiological hazard of rearing insects on the solid fraction of fresh pig manure seems to be present for certain bacterial groups. *Escherichia coli* and *Listeria* spp. disappeared after insect farming: they were no longer present in the residual substrate or the larvae. Yeast and fungi slightly decreased in the residual substrate. *Salmonella*, as well as sulfite-reducing anaerobic organisms decreased in both larvae and residual substrate. Washing the larvae did not decrease the number of microorganisms found. For the spiked antibiotics, carry-over percentages from the manure to the larvae was between 0.12% and 0.83%.

*Significance:* A transfer of microorganisms and antibiotics from the substrate to the larval biomass was found. For the growing stage, we could not establish an effective washing procedure. Farming insects for feed or food certainly needs special attention.

## P2-36 Reduction of *Escherichia coli* as a Surrogate for *Salmonella* spp. on the Surface of Grapefruit during Various Packing Line Processes

**Michelle D. Danyluk**[1], Loretta Friedrich[1], Jiuxu Zhang[2] and Mark Ritenour[2], [1]University of Florida, Lake Alfred, FL, [2]University of Florida, Fort Pierce, FL

*Introduction:* The US Produce Safety Rule allows for use of water that does not meet its microbial

standards if corrective measures, including commercial washing to remove microorganisms, are employed.

*Purpose:* This research was initiated to determine the suitability of nonpathogenic *Escherichia coli* as a surrogate for *Salmonella* spp. during citrus washing and to evaluate the removal of *E. coli* from grapefruit on two pilot packing lines.

*Methods:* The equators of whole grapefruits were inoculated, with either *E. coli* or *Salmonella* spp. and, then, dried. Treatments including fruit wetting (water, 200 ppm free chlorine), fruit washing (water, 85ppm peracetic acid (PAA)), PAA with an acidic detergent, an alkaline detergent (AD), and an AD with 2% sodium-o-phenylphenate (SOPP) were applied using a lab scale brush wash system. Individual processes evaluated on the pilot packinglines with *E. coli* only included fruit wetting, brush washing, pre-wax drying, and wax application plus drying. with ADs, sanitizers (chlorine, PAA, SOPP), and waxes (shellac, carnauba+morpholine) were evaluated. Treated fruit were rubbed, by hand, with Dey/Engley neutralizing broth, which was enumerated on selective and non-selective media.

*Results:* Log reductions for *E. coli* populations ranged from 2.7 to 4.9, and *Salmonella* spp. reductions ranged from 2.8 to 4.9. In all lab scale, brush wash systems treatments, bacterial population reductions between *E. coli* and *Salmonella* spp. were not significantly different ($P \leq 0.05$). On pilot packing lines, *E. coli* populations were reduced by various fruit wetting, washing, waxing, drying. The complete packing line processing treatments reduced *Salmonella* spp. by 2.1 to >4.5 log CFU/grapefruit at one packing line system, and by 3.2 to >5.0 log CFU/grapefruit at the other. Treatment of fruit through complete packing line processing, at both locations, reduced *E. coli* populations to levels below the detection limit (<1 log CFU/grapefruit).

*Significance: Escherichia coli* is an appropriate surrogate for *Salmonella* spp., under the tested conditions; citrus packers can use commercial washing as a corrective measure, if low microbial quality water was used.

## P2-37 Withdrawn

## P2-38 Metal Detectable Brush Bristles: Do They Work?

**Deb Smith**, Vikan, Swindon, United Kingdom

*Introduction:* If a food is contaminated by a foreign body, the repercussions for the food business can be expensive and damaging. One source of foreign body contamination is food industry brushware. Recently, brushes with metal detectable bristles have been marketed to the food industry as a way of detecting foreign bodies from this source. But, do they work?

*Purpose:* This study investigated the durability, detectability, functionality, and cleanability of metal detectable brush bristles.

*Methods:* To determine durability, metal detectable and plastic bristles were investigated with regard to their strength and elasticity using a Zwicki 5kN (Zwick Roell). Metal detectable bristles were investigated with regard to their detectability using a Mettler Toledo metal detector, with and without the presence of wet and dry food. Functionality, the ability of metal detectable bristled brushware to clean a surface of a wet and a dry food, was compared with that of a plastic bristled brush, using a robotic cleaning rig (Vikan). And to assess cleanability, metal detectable and plastic bristles were contaminated (Brownes test soil, Steris), and cleaned under the same conditions.

*Results:* Durability testing showed that plastic bristles were 68% stronger and more than twice as elastic as metal detectable bristles. Metal detectable bristles were not detectable in the presence of food. Based on visual inspection, metal detectable bristled brushes were no more effective at cleaning than plastic bristled brushes and microscopic inspection showed that metal detectable bristles were rougher and harder to clean.

*Significance:* Metal detectable bristled brushware offers no advantage, with regard to cleaning efficacy, and are unlikely to minimise the risk of bristle contamination in food. In fact, they may increase it due to their reduced strength and elasticity; and a perception that any metal detectable bristles will be controlled via the metal detector.

## P2-39 An Excel Tool-based on the Modeling of Chlorine Dioxide Disinfection of Irrigation Water Suitable to Determine Optimal Disinfectant Doses

Ana Allende[1], Imca Sampers[2], Maria Gil[3] and **Francisco López-Gálvez**[1], [1]CEBAS-CSIC, Espinardo, Spain, [2]Ghent University, Ghent, Belgium, [3]CEBAS-CSIC, Espinardo, Murcia, Spain

*Introduction:* Irrigation water can be a source of contamination of fresh produce with pathogenic microorganisms at the pre-harvest stage. Irrigation water disinfection is a mitigation strategy that could be applied to reduce the risk of microbial contamination. Tools should be developed to help growers in the selection of optimal disinfectant doses.

*Purpose:* In the present study, different types of irrigation water (reservoir, river, irrigation ditch) were treated with a range of chlorine dioxide ($ClO_2$) doses (0.1-2.5 mg/L).

*Methods:* Inactivation of naturally occurring generic *Escherichia coli* in the water was monitored and its relationship with some physicochemical characteristics of water (pH, chemical oxygen demand, turbidity, conductivity, oxidation-reduction potential, and absorbance of 0.45 µm filtered water at 254 nm (Abs-254)) elucidated.

*Results:* A mathematical model for the prediction of *E. coli* inactivation in irrigation water by $ClO_2$ was developed by linear regression. In the *E. coli* inactivation model (adjusted $R^2$=0.77), initial $ClO_2$ concentration, initial *E. coli* concentration, and Abs-254 were the explanatory variables.

*Significance:* An Excel workbook, developed using this model, allows the selection of optimal initial $ClO_2$ dose, taking into account the goals regarding *E. coli* inactivation and water characteristics such as Abs-254 to meet the microbiological criteria recommended for irrigation water.

*Significance:* Vinegar and bleach can potentially disinfect CBs to minimize risks of microbial cross-contamination.

## P2-40 Persistence of Viruses in Food: The Effect of Acid and Various Solutes on Stability

**Annette Sansom**, Campden BRI, Chipping Campdem, United Kingdom

*Introduction:* Currently, there is limited information on the control of foodborne viruses in foods. At present, the key viruses of concern are Norovirus, Hepatitis A, and Hepatitis E. Due to technical issues, it is not possible to easily culture these viruses in the laboratory and alternative approaches have to be used to determine virus stability and infectivity, including the use of surrogates, such as bacteriophage.

*Purpose:* Two species of bacteriophages, identified for use as surrogates, were assessed for their effect of food safety control measures; pH and water activity ($a_w$) on the infectivity of viruses.

*Methods:* In the pH studies, solutions of nutrient broth adjusted to various pH levels with hydrochloric and citric acid were prepared. Water activity was studied by adjusting solutions to $a_w$ 0.88 in water with sodium chloride, sucrose, or glycerol. Both pH and $a_w$ solutions were inoculated with a cocktail of both MS2 and φX174. The inoculated solutions were stored at 5°C for 6 months (pH broths) or 25°C for 2 months ($a_w$ broths). At set time points, the level of both bacteriophages were enumerated using the double layer agar plaque assay method.

*Results:* Data showed that the viruses were capable of surviving at pH values of 3–7 for at least 60 days. Both viruses had the lowest resistance to pH 2; with φX174 inactivated after 4 days (6.8 log PFU/ml reduction) and MS2 after 18 days (6.9 log PFU/ml reduction). With regards to $a_w$, the bacteriophages showed greater stability in salt solution than in water. Sucrose, and then glycerol, had the largest effect on reducing infectivity for both surrogates. A one log PFU/ml reduction, in the sucrose solution, occurred in 4–5 days for φX174 and 1–2 days for MS2.

*Significance:* Trial results indicated that MS2 and φX174 were capable of persisting under low water activity conditions and at low pH; this demonstrates the dangers of assuming conventional antimicrobial processes are effective against viruses.

## P2-41 Presence of *Alaria alata* in Red Foxes (*Vulpes vulpes*) in North West Italy

**Francesco Chiesa**, Selene Rubiola, Stefania Zanet, Tiziana Civera and Ezio Ferroglio, Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Grugliasco, Italy

*Introduction: Alaria alata* is a common intestinal trematode of red foxes in European countries. However, its presence in Italy has been rarely reported. The complex life cycle of *A. alata* requires a freshwater snail as the first intermediate host and an amphibian as the second one. Reptiles, rodents, wild boars, and other vertebrates can act as paratenic hosts after feeding on infected amphibians. *Alaria alata*is is regarded as a potential zoonotic agent, especially considering the contamination of wild boar meat with viable mesocercariae.

*Purpose:* The aim of the study was to determine the presence of the parasite in northwest Italy.

*Methods:* Between 2013 and 2015, the gastro-intestinal tracts of 100 red foxes, chosen by convenience, were sampled by the Fish and Wildlife Service. Trematodes morphologically consistent with *A. alata* were submitted for molecular identification by amplification and sequencing of the D2 region of the 28S rRNA gene and the mitochondrial COI gene.

*Results:* Of the sampled foxes (n = 100), 20% were found positive for the presence of trematodes morphologically consistent with *A. alata*. However, molecular identification of the species was not successful: the analysis of the sequences of both genes indicated that the parasites found in this study belonged to a different group of digenean species.

*Significance:* Our study demonstrated the presence of a previously unknown digenean parasite, morphologically similar to *Alaria* spp., but genetically quite distinct. Further studies are in place for the taxonomic identification and the description of its biological characteristics, including the pathogenic and zoonotic potential.

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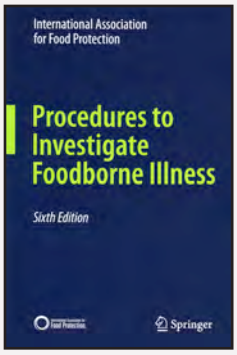


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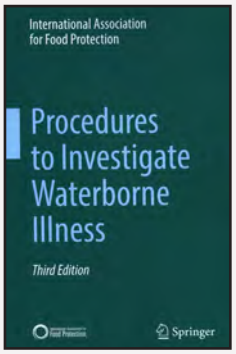


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- Developing Environmental Monitoring Programs for Small and Midsize Processors

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**IAFP Workshops** – 8:00 a.m. – 5:00 p.m.

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# NOTES

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# EXHIBITORS

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# EXHIBITORS

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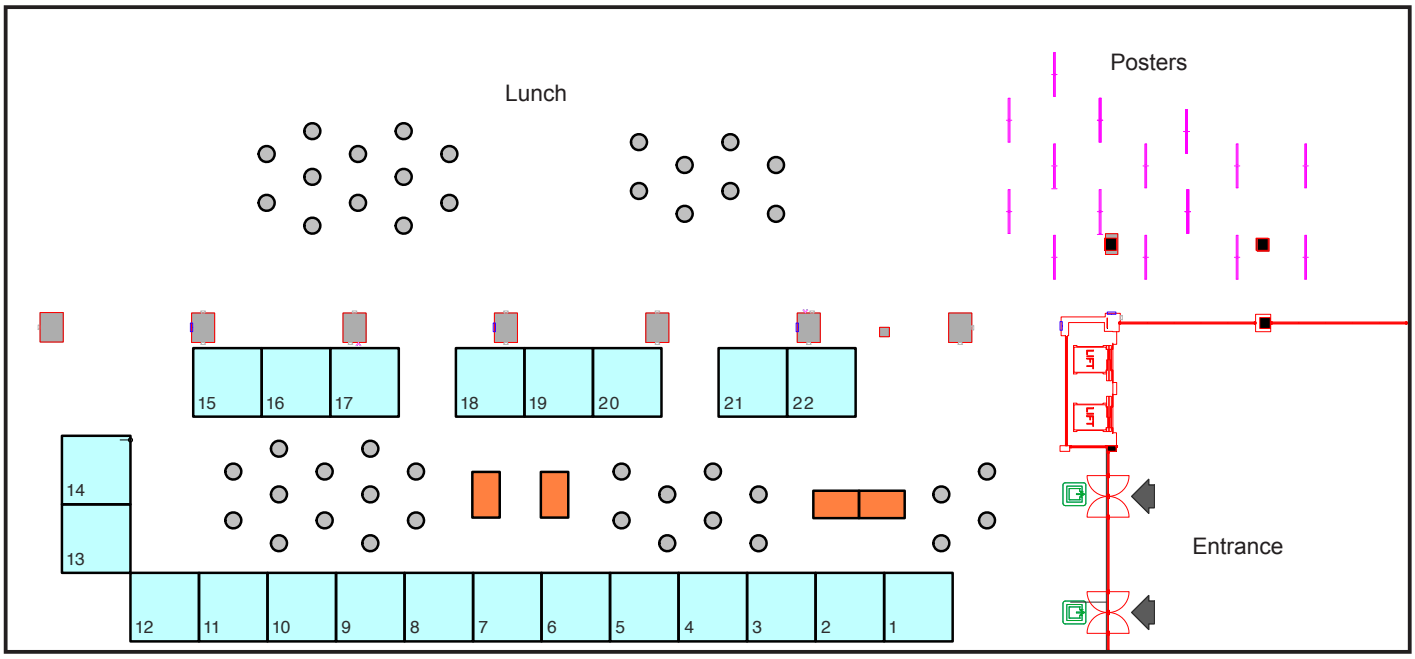


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| 12 | International Committee of Food Microbiology and Hygiene (ICFMH) |
| 13 | GENERON S.p.A. |
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| 17 | Microbiologics |
| 18 | Prestodiag |
| 19–20 | Merck |
| 21 | BIO-RAD Laboratories |
| 22 | PolySkope Labs |

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**Phone: +32 2722 52 64**
**www.3M.be/voedselveiligheid**

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**Phone: +33.182.009.577**
**www.theconsumergoodsforum.com**

The Global Food Safety Initiative (GFSI) brings together key actors of the food ecosystem to collaboratively drive continuous improvement in food safety management systems around the world. With a vision of safe food for consumers everywhere, food industry leaders created GFSI in 2000 to reduce food safety risks and inefficiencies while building trust throughout the supply chain. The GFSI community is composed of experts from the full stakeholder spectrum, across industry and international organisations to governments and academia. GFSI is powered by The Consumer Goods Forum (CGF), a global industry network working to support Better Lives Through Better Business.

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Founded in 1986, ILSI Europe fosters collaboration among the best scientists from industry, academia and the public sector to provide evidence-based scientific solutions and to pave the way forward in nutrition, food safety, consumer behaviour and sustainability. To deliver science of the highest quality and integrity, scientists collaborate and share their unique expertise in expert groups, workshops, symposia and resulting publications. ILSI Europe's activities are mainly funded by its member companies and academic experts contribute through their voluntary work. In addition, ILSI Europe receives funding from the European Union-funded projects and projects initiated by Member States' national authorities.

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**www.icfmh.org**

Since 1953 the ICFMH officially represents the IUMS in all issues related to food microbiology. Its major aim is to contribute to food safety internationally by means of several activities, including: the "FoodMicro" Conference, workshops, publications (e.g., the *International Journal of Food Microbiology*), mobility grants and awards for young scientists, and by supporting and initiating education and training in food microbiology. The ICFMH particularly focuses on the food safety situations in developing countries.

The 26th International ICFMH Conference, FoodMicro 2018, will take place in Berlin (Germany) at University College Dublin, 3–6 September 2018, with the theme "Biodiversity of Foodborne Microbes" (http://www.foodmicro2018.com/). We shall be pleased to welcome you there!

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**http://www.quodata.de**

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The largest international publisher of scientific books, Springer is co-publisher with IAFP of the revised 6th edition of *Procedures to Investigate Foodborne Illness*, the 3rd edition of *Procedures to Investigate Waterborne Illness*, and the *Food Microbiology and Food Safety* book series. Stop by our booth to meet the Food Science Editor, Sabina Ashbaugh, and discover an authoritative range of books and our journal program in food science. All IAFP Members now receive a 25% discount on our books.

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# SPEAKERS

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**Abdelrahman, Wael**, *Diamond V* (P2-33*)
**Afonso, Ana**, *European Food Safety Authority (EFSA)* (P2-22, S15*)
**Ahmed, Dr. Ali Meawad**, *Suez Canal University* (P1-24*)
**Akkermans, Cynthia**, *Feyecon* (T1-03)
**Aklilu, Solomon**, *Canadian Food Inspection Agency* (T6-02)
**Alguacil, Jóse Manuel**, *University of Cordoba* (P2-10)
**Allard, Marc**, *U.S. Food and Drug Administration* (T4-03, T5-06)
**Allende, ANA**, *CEBAS-CSIC* (P2-39)
**Almeida, Carina**, *University of Minho* (T3-02)
**Alvarez-Martin, Pablo**, *Novolyze* (T1-02)
**Amato, Benedetta**, *Istituto Zooprofilattico Sperimentale of Sicily* (P2-02, P1-03)
**Amato, Ettore**, *University of Milan* (T5-06)
**AndrÉ, Stéphane**, *CTCPA* (T7-04)
**Anes, João**, *University College Dublin* (T1-06*)
**Antignac, Jean-Philippe**, *Laberca USC1329, INRA, Oniris* (T6-01)
**Aouadhi, Chedia**, *Pasteur Institute of Tunisia (IPT)* (T7-04)
**Aragão, Gláucia M. F.**, *UFSC - Universidade Federal de Santa Catarina* (T8-01)
**Argyri, Anthoula**, *Hellenic Agricultural Organization DEMETER* (P2-16)
**Arizza, Vincenzo**, *University of Palermo* (P1-03)
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**Baguet, Justine**, *ADRIA Food Technology Institute* (P2-12)
**Bailey, Cheryl**, *EnviroLogix, Inc.* (T3-06)
**Baiwir, Dominique**, *University of Liège* (T3-01)
**Barampouti, Sevasti**, *Agricultural University of Athens* (P1-08)
**Baranyi, Jozsef**, *Imperial College* (T8-01)
**Barnes, Ruth**, *University of Reading* (T1-05*)
**Barraco, Giancarlo**, *University of Perugia* (T3-04, P1-11)
**Barretto, Caroline**, *Nestec Ltd- Nestle Research Center* (T2-02)
**Bascon, Isabel**, *University of Cordoba* (P2-10)
**Becker, Simone**, *Bruker Daltonics* (P2-07)
**Beekmann-Metselaar, Karin**, *Corbion* (P2-18)
**Benevenia, Roberto**, *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna* (P2-09*)
**Berghof-Jäger, Dr. Kornelia**, *BIOTECON Diagnostics* (P1-19, P1-20, P1-18, P1-17)
**Bernard, Muriel**, *ADRIA Food Technology Institute* (P2-12)
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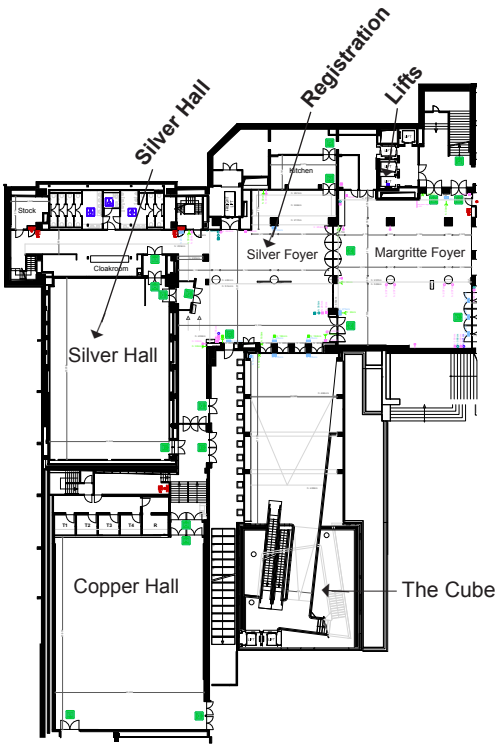


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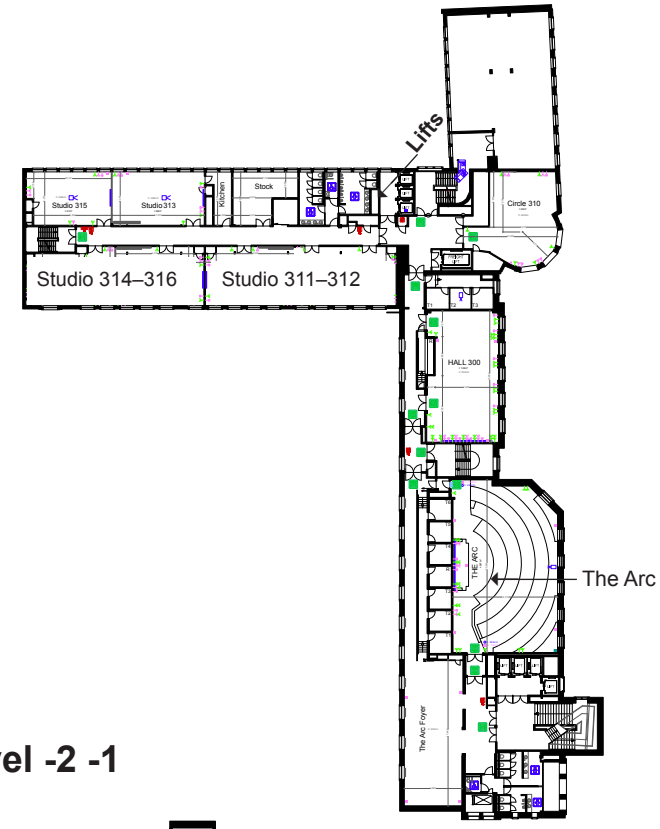


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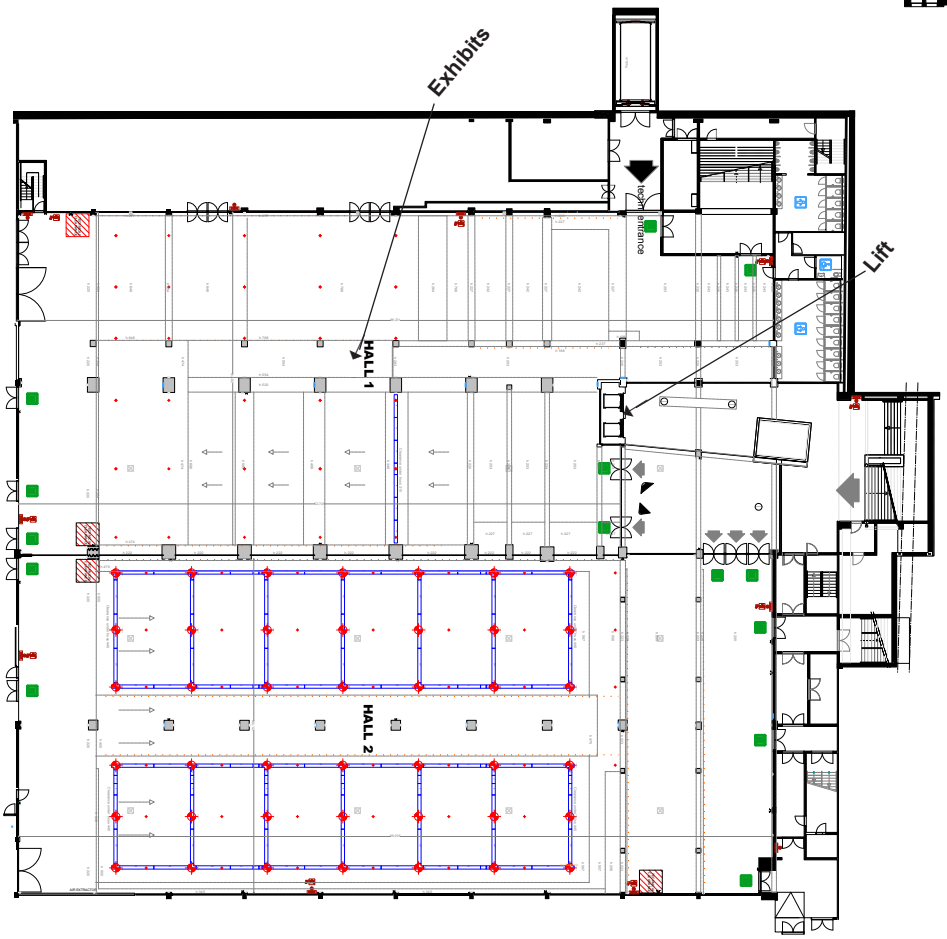

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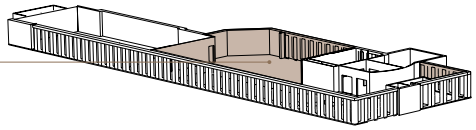


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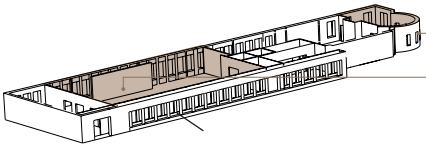


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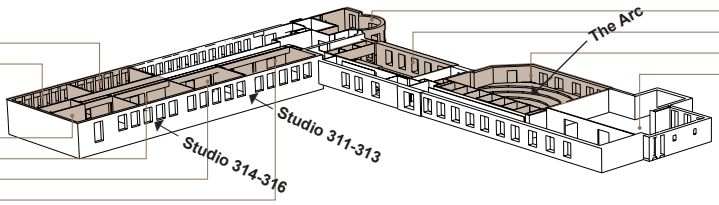


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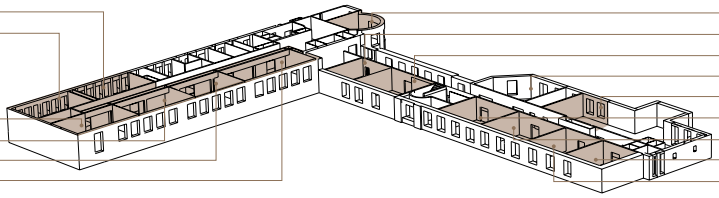


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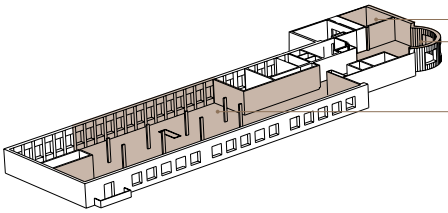


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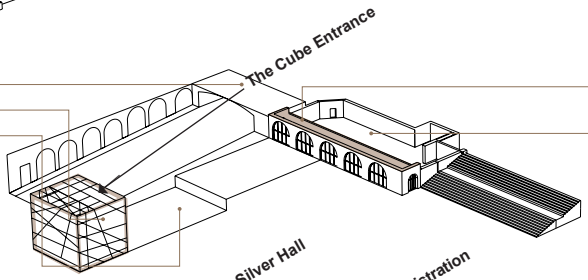


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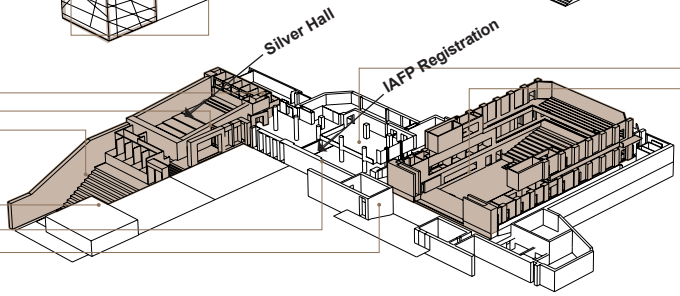


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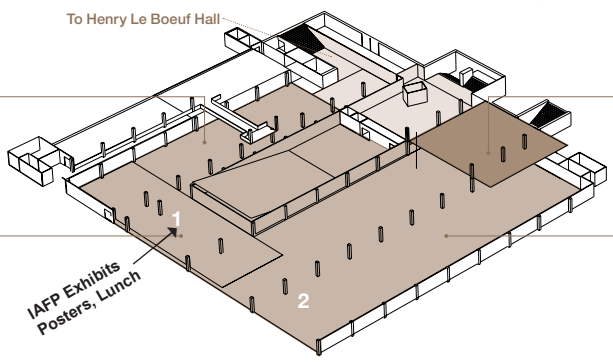

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