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## Determination of specialty food salt origin by using 16S rDNA fingerprinting of bacterial communities.

Jean-Christophe Meile, Clara Donadio, Alain Valla, Didier Montet, Laurent Dufossé

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# Determination of specialty food salt origin by using 16S rDNA fingerprinting of bacterial communities

**S**PECIALTY salt producers intend to access Protected Geographical Indication (PGI) status in order to protect and value coarse salt, ground (fine) salt and salt flower. The PGI associates a product with a region, to confirm its authentic origin. The first PGI obtained by marine salt producers was those of Guérande (France) in 2012. In order to further characterize and associate a salt product with its region of production, we searched for a microbial signature that could be rapidly detected at the molecular level. To this end, bacterial communities of salts from different French Atlantic coast locations were analyzed and compared. The 16S rDNA fingerprinting method used provides an overview of the diversity of bacteria by detecting both cultivable and non-cultivable germs in a given ecosystem. Our results showed that it is possible to statistically link the bacterial ecology of salts and its geographical origin.

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## Material and method

- Raw salt flower (collected at the surface of the salt pan, before landing and bleaching under sun drying) (Fig. 1) and marketed salt flower (after sun drying) from four locations of the French Atlantic Ocean coast (north west of France, Fig. 2).
- Total DNA was extracted from samples by using a DNA purification kit based on magnetic silica beads (Ademtech, France).
- The V3 variable region of 16S rDNA was PCR amplified by using JCM2 (GC<sub>clamp</sub>-40-ACGGGAGGCAGCA, derived from gc338f) and 518r (ATTACCGCGGCTGCTTGG) primers.
- PCR products were analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) by using a Bio-Rad Dcode TM universal mutation detection system.
- Statistical comparisons between DGGE patterns were based on the presence/absence of co-migrating DNA bands in the corresponding samples (1).
- Discriminating DNA bands were further PCR-amplified and sequenced for identification (see Fig. 3).



Figure 1. Manual harvesting of specialty marine salt (fleur de sel) in Saint-Armel solar ponds (France).

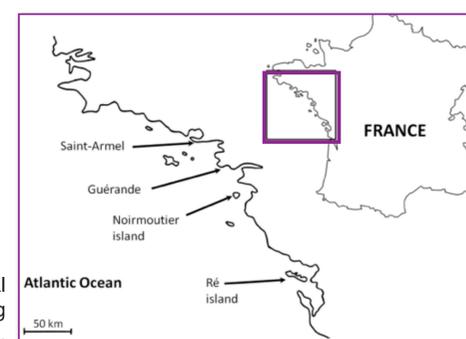
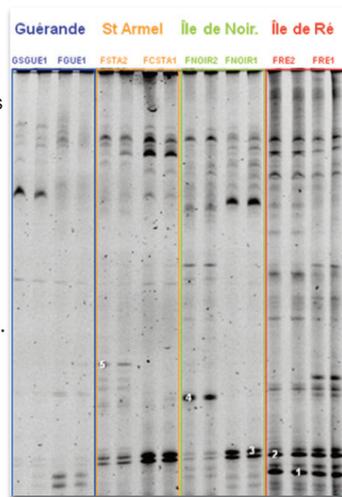


Figure 2. Geographical localisation of sampling sites in France.

## Results

Figure 3. PCR-DGGE band patterns of bacterial 16S rDNA of salt samples of four different geographical origins. Identification of DNA bands: *Pseudomonas halophila* (1); *Gracilomonas tropica* (2); *Balneola vulgaris* (3); *Persicobacter diffluens* (4); *Halanaerobium saccharolyticum* (5).



- DGGE patterns showed variations between salt samples, exhibiting 10-20 discrete bands with various intensities (Fig. 3)
- Factorial analysis (PCA) was used to describe most (>63%) of the variation in the dataset and showed that the bacterial ecology of salt samples tend to vary according to the geographical origins of salts (Fig. 4).
- The five DNA markers sequenced correspond to halophilic or halotolerant bacterial species previously isolated from marine or saline samples (see Fig. 3).

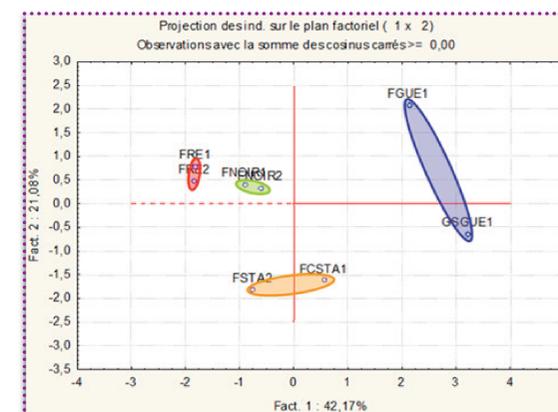


Figure 4. Factorial variance analysis of bacterial (16S rDNA) DGGE patterns of salt samples of four different geographical origins.

## Discussions & perspectives

Residual DNA and/or dormant bacteria appear as a quasi-permanent biological signature or barcode and can be easily used as a reliable traceability marker for salt products, specific of the origin of production, stable and very difficult (if not impossible) to falsify.

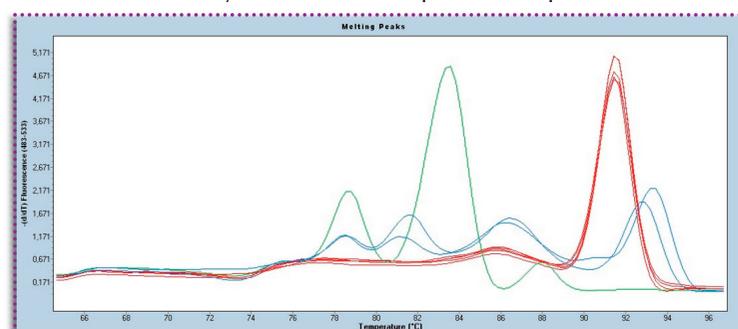
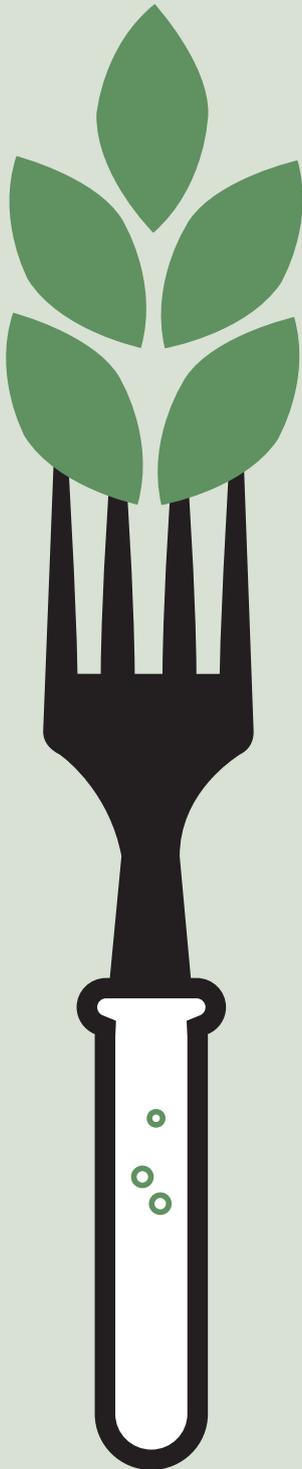


Figure 5. Melting profile of bacterial DNA populations from different salt samples.

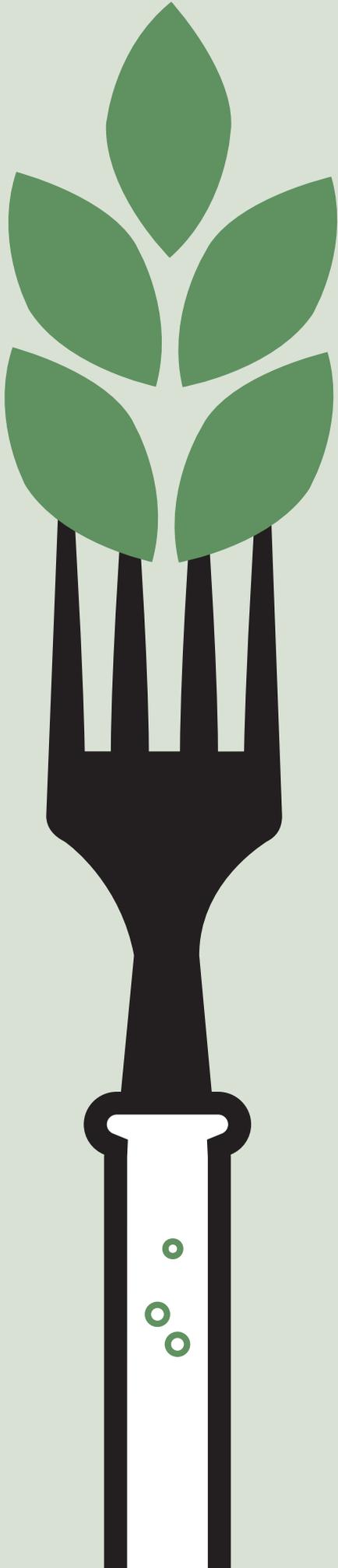
- Global molecular approaches of bacterial ecology could be used as a rapid analytical traceability tool for salt products.
- The analysis will be extended to a comprehensive set of samples from additional geographical locations and various years of harvest to test the robustness of the approach.
- The method will be coupled to rapid DNA profiling methods (2) to deliver a faster, cost-effective method for salt bacterial ecology analyses (see Fig.5).

### References

- (1) Determination of specialty food salt origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: An application on marine salts produced in solar salterns from the French Atlantic Ocean. Dufosse L., Donadio C., Valla A., Meile J.C., Montet D. Food Control, 32, 2, 644-649 (2013)
- (2) High Resolution Melt analysis for rapid comparison of bacterial community composition. Hjelmsø MH, Hansen LH, Bælum J, Feld L, Holben WE, Jacobsen CS. Appl Environ Microbiol. 2014 Mar 7. PMID: 24610853



<b>WELCOME</b>	<b>02</b>
<b>COMMITTEES</b>	<b>04</b>
<b>EVENT PROGRAMME</b>	<b>05</b>
<b>KEYNOTE SPEAKERS</b>	<b>09</b>
<b>DAY ONE – ORAL PRESENTATION ABSTRACTS</b>	<b>16</b>
<b>DAY TWO – ORAL PRESENTATION ABSTRACTS</b>	<b>24</b>
<b>DAY THREE – ORAL PRESENTATION ABSTRACTS</b>	<b>28</b>
<b>POSTER PRESENTATIONS – ABSTRACTS</b>	<b>38</b>
<b>BELFAST</b>	<b>93</b>
<b>SPONSORS AND EXHIBITORS</b>	<b>94</b>
<b>NOTES</b>	<b>99</b>



**WELCOME**  
CONFERENCE  
INFORMATION



**On behalf of the Institute for Global Food Security (Queen's University Belfast), *safefood*, and the organising committees, I am delighted to welcome you to Belfast and the 2nd Food Integrity and Traceability Conference at Queen's.**

The conference programme will provide a balance of science together with its practical application within the agri-food sector. An international audience of scientists, policymakers and industry representatives will share their research, knowledge and expertise, discussing the key topics relating to the challenges facing the world today in maintaining and improving the safety and integrity of the food we eat. Delegates will be informed of key developments toward providing the world's growing population with a sustainable, safe and secure supply of high quality food.

**The conference is organised around three core themes:**

- Reviewing recent progress in delivering safe and authentic food to the consumer
- Identifying the greatest current and emerging threats to the integrity of the agri-food supply
- Delivering new analytical means of verifying the integrity of the agri-food supply chain

Among the highlights of the conference will be a major debate on the issues pertaining to the introduction of GM feeds, crops and foods into Europe. Experts will speak for and against the motion that 'GM crops are a safe and important means of improving food security in Europe'.

The conference will also host an Artisans' Market. Housed directly adjacent to the main conference centre, I invite you to visit the market, to sample just some of the excellent food grown in Northern Ireland and meet the people who produced it.

The conference will also provide ample opportunity for interaction with colleagues from around the world both during the poster displays, workshops and roundtable discussions, together with social events. The conference dinner will take place in Titanic Belfast, an impressive building located right beside where the famous ship was constructed.

I am confident that the conference will be both an informative and an enjoyable experience and I look forward to seeing you all.

**Professor Chris Elliott**

Chair of the Organising Committee

### COMMITTEES

#### Organising Committee

**Chair:** Chris Elliott

**Members:** Ling Bai, Katrina Campbell, Andrew Castles, Simon Haughey, Michael Hills, Owen Kavanagh, Terry McGrath, James McIntosh, Mark Mooney, Joyce Watterson

#### International Scientific Committee

Paul Brereton, United Kingdom  
Drew Cannavan, Austria  
Martin Danaher, Ireland  
Stacey Degrasse, United States  
Chris Elliott, United Kingdom

Gary Kearney, Ireland  
Lujia Han, China  
Jana Hajšlová, Czech Republic  
James Lindsay, United States  
Michel Nielen, Netherlands



### The Institute for Global Food Security

Queen's University Belfast has a proud academic tradition stretching back over 160 years from its establishment by Queen Victoria in 1845 as one of the three Queen's colleges in Ireland. It received its Royal Charter from King Edward VII in 1908, becoming an independent university in its own right.

Throughout the 20th century, Queen's has continued to expand and develop. It has become one of the most respected universities in the British Isles, and its research tradition has gained it an international reputation. Queen's was accepted to join the elite Russell Group of UK universities committed to maintaining the highest standards of research, education and knowledge transfer.

The Institute for Global Food Security based at Queen's University Belfast conducts cutting edge research, developing robust techniques to safeguard the food supply. Working with colleagues across the globe, the Institute aims to play an important role in delivering food security, not only locally but on a global scale, to provide the world's growing population with a sustainable, safe and secure supply of high quality food.



### safefood

**safefood** is the all-island consumer body established in 1999 to promote best practice in food safety and healthy eating across the food chain in the Island of Ireland. **safefood** provides independent research, delivers consumer campaigns and collaborates with partners to contribute towards public health and well-being.

In 2011, **safefood** established seven new Knowledge Networks that are creating linkages between food safety professionals on the island of Ireland. The Networks are a forum for knowledge and information exchange on specific microbiological and chemical food safety topics. The networks foster interaction and collaboration, and assist professionals within the food sector meet new and emerging challenges with an overall aim of ensuring that consumers can continue to have confidence in the food that they eat.

## EVENT PROGRAMME



MONDAY NIGHT REGISTRATION		
<b>18:30–21:00</b>	<b>Pre-event registration (light refreshments served)</b>	Great Hall, Lanyon Building

DAY ONE 8 <sup>th</sup> APRIL 2014		
08:00–09:00	Registration	
09:00–09:15	<b>Welcome address</b> Professor Chris Elliott, Queen's University Belfast	Whitla Hall
<b>09:15–15:00</b>	<b>Theme – Current and emerging threats to the integrity of the agri-food supply</b>	Whitla Hall
09:15–09:45	<b>The major food safety issues in China</b> Professor Junshi Chen, China National Center for Food Safety Risk Assessment	
09:45–10:05	<b>The problem with rice</b> Professor Andy Meharg, Queen's University Belfast	
10:05–10:30	<b>Break</b>	
10:30–11:00	<b>Deadly fraud – food allergen substitution in the food chain</b> Mr Michael Walker, LGC	
10:50–11:10	<b>Debates on genetically modified crops in the context of sustainable development</b> Dr Ksenia Gerasimova, University of Cambridge	
11:10–11:40	<b>Formation of the Institute of Food Safety, Integrity and Protection</b> Mr Graham Jukes OBE, Chartered Institute of Environmental Health	
11:40–12:10	<b>Aflatoxins in feed and milk</b> Dr Carsten Fauhl-Hassek, BfR	
12.10–13.30	<b>Lunch, manufacturer exhibition, Northern Ireland food artisan market</b>	Marquee (front lawn)
	<b>Poster exhibition</b>	Whitla Hall
12:30–13:30	<b>Workshop</b> Bringing Ion Mobility to the Masses (Waters)	Bell Lecture Theatre (Physics Building)
13:30–13:50	<b>Exposure assessment of cattle to plants producing toxins via roughages</b> Dr Leo van Raamsdonk, RIKILT	
13:50–14:10	<b>A new analytical approach for the detection of palm oil in vegetable oil blends</b> Dr Tassos Koidis, Queen's University Belfast	
14:10–14:30	<b>How to measure nanoparticles size distribution in food and consumer products</b> Dr Luigi Calzolari, JRC	
14:30–15:00	<b>The strategy of DG SANCO against fraud</b> Mr Ladislav Miko, DG SANCO	

## EVENT PROGRAMME

DAY ONE 8 <sup>th</sup> APRIL 2014 (cont)		
15:00–15:20	<b>Break, manufacturer exhibition, Northern Ireland food artisan market</b>	Marquee (front lawn)
	<b>Poster exhibition</b>	Whitla Hall
<b>15:20–17:20</b>	<b>Theme – Recent progress in delivering safe and authentic food to the consumer</b>	Whitla Hall
15:20–15:50	<b>A global perspective on food fraud prevention</b> Dr John Spink, Michigan State University	
15:50–16:20	<b>Process traceability from farm to fork – technical, commercial and legislative feasibility</b> Professor Robert Newman, University of Wolverhampton	
16:20–16:40	<b>Research initiatives to support the authenticity of European food</b> Mr Paul Brereton, FERA	
16:40–17:00	<b>State-of-the-art, challenges and authentication of amazonian agro-food products: outlines of a one-year perspective study</b> Dr Vincent Baeten, CRAW	
17:00–17:20	<b>Food fraud – monitor, deter, detect, disrupt and prevent</b> Mr Peter Whelan, FSAI	

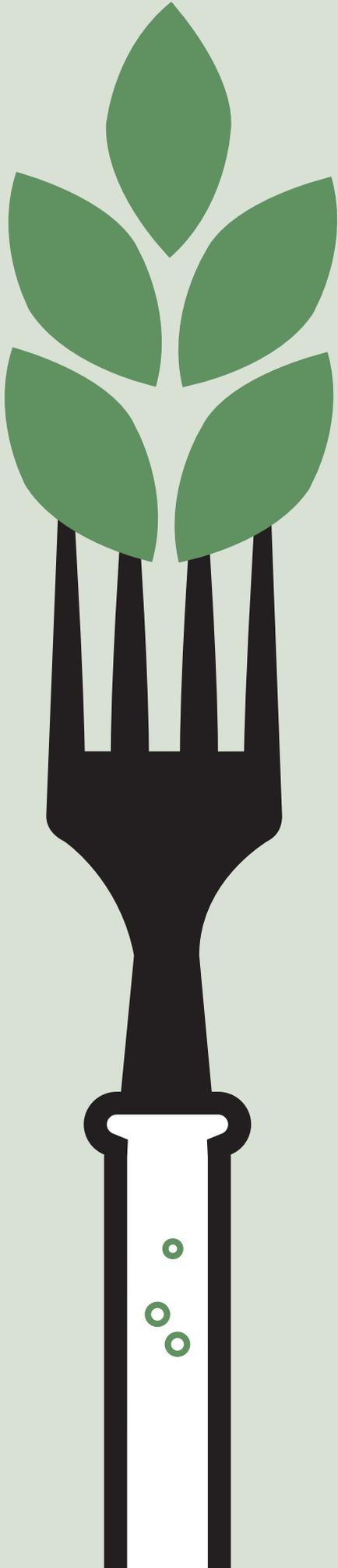
DAY TWO 9 <sup>th</sup> APRIL 2014		
09:00–09:10	<b>Welcome</b> Professor Chris Elliott, QUB	
09:10–12:00	<b>QSAFFE Raman Platform workshop</b>	David Keir Building, Room 0G/336
09:10–12:00	<b>Early stage researcher presentations</b>	Whitla Hall
09:10–09:20	<b>The effect of product risk perceptions and risk attitudes on Canadian consumers' preferences for traceability method</b> Anahita Hosseini Matin, University of Alberta	
09:20–09:30	<b>Detection of game meat adulteration in foods by real-time PCR assays</b> Barbara Druml, Austrian Agency for Health and Food Safety	
09:30–09:40	<b>Comparative analysis of the microbial ecology of organic and conventional foods</b> Céline Bigot, CIRAD	
09:40–09:50	<b>Development and application of an exact mass LC-HRMS/MS library for the screening of mycotoxins and other fungal metabolites in food and feed</b> Elisabeth Varga, University of Natural Resources and Life Sciences, Vienna (BOKU)	
09:50–10:00	<b>Setting standards for the development of a sustainable groundnut value chain in Malawi</b> Gretta Fitzgerald, University College Cork	
10:00–10:10	<b>Recombinant antibody fragments for mycotoxin analytics</b> Henri Arola, VTT Technical Research Centre of Finland	
10:10–10:20	<b>Detecting adulterations of nutmeg with non-volatile DIMS analysis</b> Isabelle Silvis, Wageningen University	
10:20–10:50	<b>Break</b>	
10:50–11:00	<b>Modelling of topoisomerase I poison activity by alternariol and derivatives. A fast in silico procedure to discover new potential poisoning mycotoxins</b> Luca Dellafiora, University of Parma	
11:00–11:10	<b>Development and validation of a QuEChERS based liquid chromatography tandem mass spectrometry method for the determination of multiple mycotoxins in spices</b> Pratheeba Yogendrarajah, Ghent University	
11:10–11:20	<b>Non-targeted proteomic plasma profiling to screen for glucocorticoid administration in bovines</b> Ruth Kinkead, Queen's University Belfast	
11:20–11:30	<b>Phycotoxin analysis of European water samples</b> Sara McNamee, Queen's University Belfast	
12:00–13:30	<b>Lunch and manufacturer exhibition</b>	Marquee (front lawn)
	<b>Poster exhibition</b>	Whitla Hall

## EVENT PROGRAMME

DAY TWO 9 <sup>th</sup> APRIL 2014 (cont)		
12:30–13:30	<b>Workshop 1 – Get published: How to improve the chances of your paper being accepted</b> Ms Rupal Malde, Elsevier Publisher for Food Science	Peter Froggatt Centre Room 02.025
	<b>Workshop 2 – PCR (R-Biopharm Rhone) workshop</b>	Bell Lecture Theatre
<b>13:30–15:00</b>	<b>GM Debate</b>	Whitla Hall
	<b>Chair</b> Ella Mc Sweeney, Radio and television presenter and producer for RTÉ	
	<b>Pro-GM</b> Mr Owen Brennan, Chairman and Chief Executive of Devenish Group Professor Klaus Ammann, Professor Emeritus of Biodiversity at the University of Bern	<b>Anti-GM</b> Dr John Fagan, Chief Executive Officer of Annapurna Global Inc. Dr Michael Antoniou, Lecturer in Molecular Genetics at Kings College London
15:00–15:20	<b>Break</b>	
<b>15:20–17:20</b>	<b>Theme – Recent progress in delivering safe and authentic food to the consumer</b>	Whitla Hall
15:20–15:50	<b>Enforcing the consumer’s right to be stupid</b> Professor Peter Shears, Plymouth Law School and Freelance Journalist/TV Presenter	
15:50–16:20	<b>Food fraud – a government perspective</b> Dr Lucy Foster, DEFRA	
16:20–16:40	<b>A UK based study on the monetary value of animal health and welfare standards, safety and traceability of beef</b> Dr Marco Boeri, Queen’s University Belfast	
16:40–17:00	<b>Infrared spectroscopy applied to a minced beef adulteration</b> Professor Gerry Downey, Teagasc	
17:00–17:20	<b>RFID technology and smart phones will bring the traceability and authentication of food products to the consumer</b> Dr Mira Trebar, University of Ljubljana	
17:20	<b>Close</b>	
19:00–23:00	<b>Drinks reception, followed by conference dinner</b> Titanic Belfast is the most popular tourist attraction in Northern Ireland	Titanic Belfast

DAY THREE 10 <sup>th</sup> APRIL 2014		
09:00–09:10	Welcome	
<b>09:10–14:30</b>	<b>Theme – New analytical means of verifying the integrity of the agri-food supply chain</b>	Whitla Hall
09:10–09:40	<b>Antimicrobial resistance a modern day public health challenge and the role of surveillance as an important tool to support control measures</b> Professor Séamus Fanning, University College Dublin	
09:40–10:00	<b>Cost effective serotyping of salmonella enterica to communicate pidemiological trends across continents</b> Dr Jean Guard, US Department of Agriculture	
10:00–10:20	<b>Break</b>	
10:20–10:50	<b>New HRMS-based fingerprinting strategies enabling traceability/authentication of herbs and spices</b> Professor Dr Jana Hajšlová, Institute of Chemical Technology, Prague	
10:50–11:10	<b>Design and demonstration of a high throughput DNA tracking system for genetic improvement and brand verification in the canadian beef industry</b> Ms Kajal Devani, Canadian Angus Association	
11:10–11:30	<b>Creation of a biological barcode to trace the origin of food using their microbial ecology</b> Dr Didier Montet, Universite Montpellier	
11:30–12:00	<b>Recent developments in analytical methods for detecting emerging hazards</b> Professor Bruno le Bizec, French National Reference Laboratory (LABERCA)	

DAY THREE 10 <sup>th</sup> APRIL 2014 (cont)		
12:00–13:00	<b>Lunch and manufacturer exhibition</b>	Marquee (front lawn)
	<b>Poster exhibition</b>	Whitla Hall
	<b>Workshop 1 – Adv MS based on Orbitrap tech and latest developments in data mining (Thermo)</b>	Bell Lecture Theatre (Physics Building)
	<b>Workshop 2 – Toximet workshop</b>	Lanyon Building, Room 09.074
13:00–13:30	<b>Mycotoxins in cereal based food and feed: From target analysis towards metabolomics</b> Professor Rudolf Krska, University of Natural Resources and Life Sciences, Vienna	
13:30–13:50	<b>Food safety risk assessment using LED spectral imaging</b> Assoc. Professor Jens Michael Carstensen, University of Copenhagen	
13:50–14:10	<b>Mass spectrometry to assess meat authenticity: Use of biomarker peptides to detect pork and horse in meat products</b> Dr Jens Brockmeyer, University of Münster	
14:10–14:30	<b>The use of ion mobility enabled mass spectrometry (IM-MS) for the development and characterisation of robust analytical methods for the quantitation of veterinary drug residues in foods of animal origin</b> Dr Michael McCullagh, Waters Corporation	
14:30–14:45	<b>Break and manufacturer exhibition</b>	Marquee (front lawn)
	<b>Poster exhibition</b>	Whitla Hall
<b>14:45–17:05</b>	<b>Theme – International session on quality and safety of feed and food in Europe</b>	Whitla Hall
14:45–15:05	<b>The need for advanced testing methods in the European animal feed sector: The industry perspective</b> Mr Declan Billington, John Thompson and Sons, Belfast, Northern Ireland	
15:05–15:25	<b>Early detection of contaminants in feed mills by near infrared spectroscopy and chemometrics</b> Dr Juan Antonio Fernández Pierna, Walloon Agricultural Research Centre, Belgium	
15:25–15:45	<b>Analytical traceability of feed materials</b> Dr Carsten Fauhl-Hassek, Federal Institute for Risk Assessment, Germany	
15:45–16:05	<b>Validation of a lamp-based method for detection of <i>salmonella Spp</i> in soya meal</b> Mr Martin D'Agostino, Microbiologist, Food and Environment Research Agency, UK	
16:05–16:25	<b>Transfer of dioxins and PCBs from feed to food</b> Dr Ron Hoogenboom, Programme Leader Food Contaminants, RIKILT Wageningen UR, Wageningen, The Netherlands	
16:25–16:45	<b>The impact of the QSAFFE project: The advisory board perspective</b> Mr Eamonn Whelan, Operations Director, Devenish Nutrition, Northern Ireland	
16:45–17:05	Closing remarks	
17:05	<b>Close of conference</b>	



**POSTER  
PRESENTATIONS  
ABSTRACTS**

POSTER 44

**STARTEC: Novel processing technologies in the development of a decision support tool to ensure safe and nutritious Ready-To-Eat foods**

<b>Name</b>	Dr Gonzalo Delgado-Pando
<b>Institution</b>	Institute for Global Food Security, Queen’s University Belfast
<b>Co-authors</b>	Gonzalo Delgado-Pando, Alexandros Stratakos, Tassos Koidis

Ready-to-eat (RTE) foods can be readily consumed with minimum or without any further preparation; their processing is complex—involving thorough decontamination processes— due to their composition of mixed ingredients. Compared with conventional preservation technologies, novel processing technologies can enhance the safety and quality of these complex products by reducing the risk of pathogens and/ or by preserving related health-promoting compounds. These novel technologies can be divided into two categories: thermal and non-thermal. As a non-thermal treatment, High Pressure Processing is a very promising novel methodology that can be used even in the already packaged RTE foods. A new “volumetric” microwave heating technology is an interesting cooking and decontamination method directly applied to foods. Cold Plasma technology is a potential substitute of chlorine washing in fresh vegetable decontamination. Ohmic heating is a heating method applicable to viscous products but also to meat products. Producers of RTE foods have to deal with challenging decisions starting from the ingredients suppliers to the distribution chain. They have to take into account not only the cost factor but also the benefits and food products’ safety and quality. Novel processing technologies can be a valuable yet large investment for several SME food manufacturers, but they need support data to be able to make adequate decisions. Within the FP7 Cooperation funded by the European Commission, the STARTEC project aims to develop an IT decision supporting tool to help food business operators in their risk assessment and future decision making when producing RTE foods with or without novel preservation technologies.

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POSTER 45

**Determination of specialty food salt origin by using 16S rDNA fingerprinting of bacterial communities**

<b>Name</b>	Dr Jean-Christophe Meile
<b>Institution</b>	Cirad (Montpellier, France)
<b>Co-authors</b>	Jean-Christophe Meile, Clara Donadio, Alain Valla, Didier Montet, Laurent Dufossé

The determination of geographical origin is required for the traceability system of food products. One way of tracing the source of a product is to analyse the bacterial communities present on the food samples. For this purpose, molecular techniques (such as PCR-DGGE) employing 16S rDNA profiles were used to detect variations in bacterial community structures of salts from various regions. Statistical analyses of rDNA profiles revealed that distinct microbial communities were detected. The profiles of salt bacteria from different producing areas were different and specific for each location and could be used as a bar code to certify the geographical origin of salts. These profiles could serve as specific markers for a specific location. This method is proposed as a new traceability tool which provides salts with a unique bar code that permits to trace back salts from store shelves to their original location of production.

# OUR FOOD IS OUR FUTURE

ASSET 2014



# FOOD INTEGRITY & TRACEABILITY CONFERENCE

QUEEN'S UNIVERSITY 8TH—10TH APRIL

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## The Institute for Global Food Security and *safefood* are delighted to announce a major international conference on food safety, to be held at Queen's University Belfast (8th–10th April 2014).

The ability to protect the integrity of the food supply chain is a massive challenge but one which is of the utmost importance to protect the consumer.

The conference to be held in Belfast will concentrate on three key themes:

- 1) Reviewing recent progress in delivering safe and authentic food to the consumer
- 2) Identifying the greatest current and emerging threats to the integrity of the agri-food supply
- 3) Delivering new analytical means of verifying the integrity of the agri-food supply chain

As a major part of this conference a debate on the issues pertaining to the introduction of GM feeds, crops and foods into Europe will be held. Experts will speak for and against the motion that 'GM crops are a safe and important means of improving food security in Europe'.



## FOOD NI ARTISANS' MARKET

QUEEN'S UNIVERSITY FRONT LAWN - TUESDAY 8TH APRIL (8.30-18.00)