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Agriculture and Food Development Authority

An Roinn Talmhaíochta, Bia agus Mara
Department of Agriculture, Food and the Marine

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2023 SYMPOSIUM ON

Listeria monocytogenes in Foods

Recent advances and outstanding questions

24th-25th May, 2023

Teagasc Food Research Centre, Ashtown, Co. Dublin

UCD Dublin

UCC
Coláiste na hOllscoile Corcaigh
University College Cork, Ireland

University of Limerick
Ollscoil Luimnigh

Ollscoil na Gaillimhe
University of Galway

www.teagasc.ie/listeriasymposium2023

Welcome Address

Dear Colleagues & Friends,

On behalf of our project partners and the organising committee, I am delighted to welcome you to the Teagasc Food Research Centre, Dublin, to the **2023 Symposium on *Listeria monocytogenes* in Foods: Recent Advances and Outstanding Questions.**

This two-day symposium marks the culmination of a four-year research project on *Listeria monocytogenes* funded by the Department of Agriculture, Food and the Marine. The project, led by a consortium of *Listeria* expertise in Ireland across six research-performing organisations, sought to understand the behaviour of this important pathogen in foods and in the food processing environment through the characterisation of an Irish strain collection, representing a snapshot of the situation relating to *Listeria monocytogenes* across the Irish food sector. Indeed, the activity in this project has further built on over a decade of research by this consortium on *Listeria monocytogenes*.

Over the next two days, you will hear from our project partners in Teagasc, University of Galway, University College Cork, University of Limerick and University College Dublin, who will focus on themes including stress responses, virulence, persistence in the food processing environment, and predictive modelling in food systems. We are also delighted to welcome to the programme members of our Scientific Advisory Board, as well as partners from the EFSA-funded ListeriaPredict project, a wealth of international experts, as well as updates from regulatory agencies and other stakeholders.

The organising committee look forward to welcoming you to Dublin for what promises to be a symposium not to be missed.

Le gach dea-ghuí,

Prof Olivia McAuliffe
Project Coordinator and Organising Committee Chair, Teagasc

Symposium Organising Committee

Prof Olivia McAuliffe, Teagasc
Dr Muireann Egan, Teagasc
Dr Kaye Burgess, Teagasc
Prof Francis Butler, University College Dublin
Prof Conor O'Byrne, University of Galway
Dr Peter Myintzaw, Teagasc
Ms Siobhán Barry, Teagasc

Programme

2Day 1: Wednesday 24th May	
08.30 – 17:00	Meeting registration
09:00 – 09:15	**Welcome and opening remarks** Olivia McAuliffe, Teagasc Food Research Centre, Moorepark, Ireland
Session 1: Molecular Mechanisms Underpinning Responses to Food Preservation Stress and Virulence Chair: Olivia McAuliffe, Teagasc	
09:15 – 09:45	**New insights into food preservation stress responses in *Listeria monocytogenes* using comparative genomics** Conor O'Byrne, University of Galway, Ireland
09:45 – 10:15	**Cyclic di-AMP, an essential signalling nucleotide of central metabolism and osmolyte homeostasis in *Listeria monocytogenes*** Fabian Commichau, University of Hohenheim, Germany
10:15 – 10:45	**Disarming *Listeria monocytogenes* of its virulence factors using medium- and long-chain fatty acids** Birgitte Kallipolitis, University of Southern Denmark
Coffee break	
Session 2: The Fresh Produce Sector: An Emerging Risk Chair: Conor O'Byrne, University of Galway	
11.15 – 11.45	***Listeria monocytogenes* in the processing environment of fruits and vegetables: The known knowns and the known unknowns of environment contamination** Ana Allende, CEBAS-CSIC - Spanish National Research Council, Spain
11.45 – 12.15	**Growth of *Listeria monocytogenes* on spinach and rocket leaves is affected by cultivation conditions and the vegetable phytobiome** Achim Schmalenberger, University of Limerick, Ireland
12:15 – 12:45	**Supporting the fresh produce sector to prevent *Listeria monocytogenes* contamination** Kaye Burgess, Teagasc Food Research Centre, Ashtown, Ireland

12:45-13:00	**<u>Flash Presentations: Session 1</u>** **Growth potential of *Listeria monocytogenes* on ready-to-eat fresh produce in ambient conditions** Elena-Alexandra Alexa, Teagasc Food Research Centre, Ashtown, Ireland **Survival of *Listeria monocytogenes* in fermented pepperoni with modified formulations and process parameters** Shannon Heapes, Teagasc Food Research Centre, Ashtown, Ireland **Population structure and macroevolution of *Listeria monocytogenes* CC121 in the UK** Ana Victoria Gutiérrez, Quadram Institute Bioscience, Norwich Research Park, Norwich, UK
Lunch	
Session 3: Growth Behaviour of *Listeria monocytogenes*: Predictive Modelling Chair: Cormac Gahan, University College Cork	
14:00 – 14:30	**Predictive modelling of *Listeria monocytogenes:* integrative models and variability** Jesus Frias, Technological University Dublin, Ireland
14:30– 15:00	**Modelling the growth of *Listeria monocytogenes*: what we have learned from the ListeriaPredict Project** Francis Butler, University College Dublin, Ireland
15:00 – 15:30	**Proteomic profiling of a virulent *Listeria monocytogenes* strain grown under several stress conditions** Federica d'Onofrio, University of Teramo, Italy
Coffee break and poster session	
Session 4: *Listeria monocytogenes*: Ecology Chair: Achim Schmalenberger, University of Limerick	
16:00 – 16:30	**The gastrointestinal phase of *Listeria monocytogenes* infection** Cormac Gahan, University College Cork, Ireland
16:30 – 17:00	**The diverse ecology of *Listeria monocytogenes* - niche adaptation for different lifestyles** Edward Fox, Northumbria University, UK

17:00 – 17:30	**Utilising WGS to predict the heightened risk to food producers of certain *Listeria monocytogenes* strains** Nick Andrews, Dawn Farm Foods, Ireland
17:30 – 17:45	**<u>Flash Presentations: Session 2</u>** **Short chain fatty acids (SCFAs) modulate growth, virulence expression and host-pathogen interactions in *Listeria monocytogenes*** Miguel Villoria Recio, APC Microbiome Ireland, University College Cork, Ireland **Effects of chitin on *Listeria monocytogenes* pathogenicity** Monica Cazzaniga, APC Microbiome Ireland, University College Cork, Ireland **Genomic characterisation of long-time persistent *Listeria monocytogenes* strains in cheese production facilities** Elisabet Marti, Food Microbial Systems, Agroscope, 3003 Bern, Switzerland

Day 2: Thursday 25th May	
Session 5: *Listeria monocytogenes*: Detection, Infection and Control Chair: Kaye Burgess, Teagasc	
09:00 – 09:30	***Listeria monocytogenes*: A regulatory perspective** Mary Lenahan, Food Safety Authority of Ireland
09:30 – 10:00	**Listeriosis – an Irish clinical perspective** Martin Cormican, Galway University Hospital/University of Galway, Ireland
10:00 – 10:30	**Novel antibacterials targeting the transcriptional regulators PrfA and BrtA** Jörgen Johansson, Umeå University, Sweden
10:30 - 10.45	**Flash Presentations: Session 3** **An epidemiological review of listeriosis in Galway, Mayo and Roscommon (2010 to 2023)** Donna Kilmartin, Department of Public Health, Merlin Park University Hospital, Galway, Ireland **Reported foodborne outbreaks related to *Listeria monocytogenes* in Belgium between 2016 and 2023** Marie Polet, SCIENSANO, NRL for *Listeria monocytogenes*, Brussels, Belgium **Employing next-generation sequencing to elucidate the Welsh food Listerial landscape** Joshua A Macleod, Cardiff Metropolitan University, Wales
Coffee break and poster session	
Session 6: Growth Behaviour of *Listeria monocytogenes*: RTE Foods and the Processing Environment Chair: Francis Butler, University College Dublin	
11:15– 11:45	***Listeria monocytogenes* in cheese - a model for determining the level of undissociated lactic acid in cheese and predicting complete growth inhibition** Marjon Wells-Bennik, NIZO, Netherlands.
11:45 – 12:15	**Growth of *Listeria* in plant-based milk alternatives** Michael Callanan, Munster Technological University, Ireland

12:15 – 12:45	**Growth behaviour of *Listeria monocytogenes* in semi-soft rind-ripened artisanal cheese at cold chain and abuse temperatures** Peter Myintzaw, Teagasc Food Research Centre, Moorepark, Ireland
12:45 – 13:15	***Listeria* biofilms: Challenges and opportunities for their detection and study** Antonio Lourenco, Teagasc Food Research Centre, Moorepark, Ireland
13:15 – 13.35	**Flash Presentations: Session 4** **Controlling *Listeria monocytogenes* risk: Update of online risk assessment tools to help producers of smoked fish** Karen Pearson, Food Standards Scotland, Pilgrim House, Aberdeen, Scotland **Which is the *Listeria monocytogenes* growth risk in RTE plant-based meat analogues?** Anna Jofré, Food Safety and Functionality Programme, IRTA, Finca Camps i Armet, 17121-Monells, Spain **Molecular characterisation of *Listeria monocytogenes* isolates received by the National Reference Laboratory in 2021** Brian Byrne, Food Microbiology Division, Department of Agriculture, Food and Marine (DAFM), Ireland
Closing Remarks and Late Lunch	

Presentations

New insights into food preservation stress responses in *Listeria monocytogenes* using comparative genomics

Conor O'Byrne

University of Galway, Ireland

Abstract

One of the principal strategies used by producers of processed ready-to-eat foods is to control the acidity of the product to prevent the growth of pathogenic bacteria and to extend product shelf life. Understanding the response of pathogens to acid stress is therefore a key step in developing improved preservation regimes and enhancing food safety. In this work we sought to combine comparative genomics with phenotypic studies to investigate the genetic factors that influence the response of *Listeria monocytogenes* to acid stress. Screening a diverse collection of 168 strains led to the identification of several strains with significantly reduced acid resistance. A detailed characterisation of one of these strains led to finding that a manganese transporter called MntH plays a critical role in growth at low pH. It further revealed the presence of a previously unknown transcriptional regulator, which we call GadR, that plays a central role in regulating the adaptive acid tolerance response of *L. monocytogenes* and contributes significantly to acid resistance in conditions that mimic the mammalian stomach. Together these insights advance our understanding of how this pathogen responds to acid stress and can potentially lead to new control strategies.

Biography

Director of the Bacterial Stress Response Group, Senior Lecturer in Microbiology at the University of Galway. The research focus in my group is on the molecular mechanisms that food-borne bacterial pathogens use to sense and respond to harsh conditions in their environment. Pathogens encounter major physicochemical changes as they transition from food into the host, particularly in relation to pH, osmolarity, oxygen concentration, light and temperature. Understanding how pathogenic bacteria detect and respond to these changes is critical if we are to devise sensible strategies to prevent their entry into the food chain and to prevent infections from arising in the human population. In my laboratory we study two important food-borne pathogens, *Listeria monocytogenes* and *Escherichia coli*.

Cyclic di-AMP, an essential signaling nucleotide of central metabolism and osmolyte homeostasis in *Listeria monocytogenes*

Fabian M. Commichau

University of Hohenheim, Germany

Abstract

Cyclic di-AMP is an emerging second messenger that is synthesised by many archaea and bacteria, including the Gram-positive pathogenic bacterium *Listeria monocytogenes*. *L. monocytogenes* played a crucial role in elucidating the essential function of c-di-AMP, thereby becoming a model system for studying c-di-AMP metabolism and the influence of the nucleotide on cell physiology. c-di-AMP is synthesized by a diadenylate cyclase and degraded by two phosphodiesterases. To date, eight c-di-AMP receptor proteins have been identified in *L. monocytogenes*, including one that indirectly controls the uptake of osmotically active peptides and thus the cellular turgor. The functions of two c-di-AMP-receptor proteins still need to be elucidated. Here, I provide an overview of c-di-AMP signalling in *L. monocytogenes* and highlight the main differences compared to the other established model systems in which c-di-AMP metabolism is investigated. Moreover, I discuss the most important questions that need to be answered to fully understand the role of c-di-AMP in osmoregulation and in the control of central metabolism.

Biography

Fabian Commichau studied biology at the RWTH Aachen, Germany, and obtained a Ph.D. in microbiology at the University of Göttingen. After postdoc stays at the University of Göttingen and the University of Basel he was working for 2 years in industry at DSM Nutritional Products Ltd., Kaiseraugst. In 2011 he became a group leader at the Dept. of General Microbiology at the University of Göttingen and obtained his habilitation. From 2019 until 2022 he held the professorship for Synthetic Microbiology at the BTU Cottbus-Senftenberg and since 2022 he has been a professor for Molecular Microbiology at the University of Hohenheim.

Disarming *Listeria monocytogenes* of its virulence factors using medium- and long-chain fatty acids

Birgitte H. Kallipolitis

University of Southern Denmark, Denmark

Abstract

Naturally occurring free fatty acids (FFAs) have long been known for their antimicrobial activity against the foodborne pathogen *Listeria monocytogenes*. More recently, we found that specific FFAs act as signaling compounds affecting the production of virulence factors in *L. monocytogenes*. The regulatory protein PrfA activates transcription of virulence genes required for the intracellular lifestyle of *L. monocytogenes*. Short-term exposure to low doses of specific medium- and long-chain FFAs led to an instant shut-down of PrfA-dependent transcription of virulence genes. Specific FFAs interfere with the DNA binding activity of PrfA, suggesting that FFAs act as anti-virulence agents by selective inhibition of the key virulence regulator in *L. monocytogenes*. Notably, *L. monocytogenes* may become resistant towards the antimicrobial activity of FFAs, however, such mutant variants are still sensitive towards the anti-virulence activity of FFAs. Altogether, our findings support that specific FFAs may be useful candidates as anti-virulence agents acting to disarm *L. monocytogenes* of its virulence factors.

Biography

Birgitte H. Kallipolitis holds a position as Professor of Molecular Microbiology at University of Southern Denmark and serves as Head of Research Unit for Molecular Microbiology. Her research group aims to uncover how pathogenic bacteria sense and respond to hostile conditions encountered in the external environment and during infection of a host organism. In ongoing projects, the Kallipolitis-group explores the anti-infective activities of medium- and long-chain free fatty acids against *Listeria monocytogenes*.

Listeria monocytogenes in the processing environment of fruits and vegetables: The known knowns and the known unknowns of environment contamination.

Ana Allende

CEBAS-CSIC (Spanish National Research Council), Spain

Abstract

Outbreaks of listeriosis continue to occur across the globe associated with previously reported foods, but also with many previously unreported food vehicles, including fresh and minimally processed fruits and vegetables (e.g. frozen vegetables). Environmental contamination sources have been widely studied but most of the research has not been focused on fresh produce. Zoellner et al., (2018) conducted an exhaustive search to identify all available research, industry and regulatory documents on *Listeria* environmental monitoring (EM) in food processing facilities. Only about 5% of the relevant references were focused on fresh produce, highlighting a research gap. Recently, some attempts have been made to determine the surveillance of *Listeria* spp. in fresh produce processing facilities. The last three years, the MxQ Research Lab have focused on generating practical knowledge and solutions for the implementation of EM programs in fresh produce processing facilities as different industrial practices and processing environments may account for different contamination patterns. This presentation summarises the results obtained in three fresh-cut processing facilities which aim to complement the current knowledge on *Listeria* spp. environmental monitoring (EM) to prevent contamination risks.

Biography

Dr. Ana Allende from CEBAS-CSIC (Spanish National Research Council) in Spain is a Senior Researcher with focus on safety of fresh produce. She obtained her PhD in Food Science and Technology at the University of Cartagena, Spain. She holds several positions in (inter)-national institutions including vice-chair of the BIOHAZ panel at the European Food Safety Authority (EFSA), vice-director of the CEBAS-CSIC, and Member of the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) Roster of Experts. She has published more than 230 research articles in peer-reviewed international journals focused on the safety of fresh produce with about 8500 cites. Her current H index is 53.

Growth of *Listeria monocytogenes* on spinach and rocket leaves is affected by cultivation conditions and the vegetable phytobiome

Achim Schmalenberger

University of Limerick, Ireland

Abstract

Consumption of ready-to-eat leafy vegetables has increased in popularity due to their anticipated health benefits, but their consumption also poses a potential health risk in the form of foodborne pathogens. *Listeria monocytogenes* is a ubiquitous pathogen that has been regularly found on leafy vegetables such as lettuce, spinach and rocket. Past challenge tests provided contradicting evidence of *L. monocytogenes*'s ability to grow on spinach and rocket. However, our latest challenge test provided strong evidence of growth on both vegetables and this growth is greatly affected by a range of environmental variables. These go beyond plant species and includes plant variety, cultivation practice (open field vs. tunnel) or even the time of the year the product is cultivated (climatic conditions). Evidence is now emerging that the phyllosphere bacteriome may also affect the growth potential of *L. monocytogenes*. Phyllosphere bacteria that have the potential to support growth of *L. monocytogenes* include the *Pseudomonadaceae*. In contrast, bacterial families such as *Carnobacteriaceae* and *Pectobacteriaceae* were found to coincide with reduced growth of *L. monocytogenes.* It appears that growth conditions of *L. monocytogenes* are affected by a multitude of conditions that need to be considered for future challenge studies.

Biography

Dr. Schmalenberger graduated from the University of Hannover and completed his PhD at the Federal Agricultural Research Centre and the Technological University Braunschweig, where he investigated the microbial communities of transgenic crops. He continued his postdoctoral research at the University of Bayreuth, the University of Manchester (UK) and the University of Sheffield (UK) where he focussed his attention on the abilities of soil microbes to mobilise otherwise plant unavailable nutrients. Dr. Schmalenberger took up a lecturer position at the University of Limerick in 2010 where he expanded his research, studying *Listeria monocytogenes* growth conditions on fruit and vegetables.

Supporting the fresh produce sector to prevent *Listeria monocytogenes* contamination

Kaye Burgess

Teagasc Food Research Centre, Ashtown, Ireland

Abstract

Listeria monocytogenes is a foodborne pathogen of significant public health concern. It is a saprophytic microorganism widely distributed in the natural environment and can be found in soil, water, vegetation and the faeces of some animals. With such reservoirs it therefore poses a risk of contaminating fruit and vegetables. This is a particular concern as many fruit and vegetables are considered ready to eat and are consumed without cooking. Hence it is essential that steps are put in place right across the fresh produce production chain to minimise crop contamination with *L. monocytogenes*. Pre-harvest, the use of good agricultural practices help reduce the likelihood of contamination during harvesting, handling, and processing of produce. At the post-harvest stage various options can be utilised to prevent or minimise *L. monocytogenes* contamination and proliferation on fresh produce. Coupled with these, the use of regular testing for *L. monocytogenes* contamination, of both crops and the production and processing environment, also plays an important role in an effective food safety risk management system. Understanding the risks and taking proactive steps at each stage of production are key to minimising *L. monocytogenes* contamination of fruit and vegetables.

Biography

Dr Kaye Burgess is Senior Research Officer in the Food Safety Department in Teagasc Food Research Centre, Ashtown in Dublin. Kaye's research focus is on understanding the behaviour and virulence of foodborne pathogens and antimicrobial resistant microorganisms along the farm to fork chain, using a One Health approach. She currently coordinates and participates in a number of DAFM, EPA and Teagasc funded projects, including a number of projects on microbial food safety in horticulture. She is also a funded investigator as part of the One Health European Joint Programme. She has previously served as a host mentor for the Enterprise Ireland-Marie Skłodowska Curie CareerFit programme and led the food production focused work package in the EU funded project Aquavalens which was focused on the quality of drinking water and water used in food production.

Predictive modelling of *Listeria monocytogenes*: Integrative models and variability

Jesus M. Frias, Victoria Caballero, Francesco S. Giordano, Declan Bolton and Kaye Burgess

Environmental Sustainability and Health Institute, Technological University Dublin, Dublin, Ireland
School of Food Science and Environmental Health, Technological University Dublin, Dublin, Ireland
Teagasc Food Research Centre, Ashtown, Dublin, D15 DY05, Ireland

Abstract

Listeria monocytogenes is a concern for the Irish food industry, with a baseline EFSA study identifying positive results in meat and RTE products and a 2016 study in Ireland reporting up to 4.7% positive results in dairy, meat, seafood and vegetable products and processing facilities.

Predictive microbiology is one of the research tools that is used to assess the behaviour of this microorganism in foods and contribute to decision making on the safety of food systems. In order to do this, mathematical models are built using information already available in research and are generally further calibrated using challenge experiments. This then allows to extrapolate to pathogen concentrations and proportions encountered in foods.

Calibration of the model involves estimating parameters from nonlinear models commonly composed of differential equations, as conditions during the processing and cold chain of products are dynamic. Parameter estimation has a unique place in translating difficult to acquire experimental data into mathematical models that can be shared.

Two case studies of model building are presented in this context: 1) the modelling of the dynamics of *L. monocytogenes* populations during the manufacture and storage of fermented products where available models from literature can be used an integrated to describe a complex processing and 2) the model building of *L. monocytogenes* in biofilm form in an RTE product, where statistical models of variance are used to characterise product and microbial variation.

Biography

Jesus Maria Frias Celayeta holds a BSc of Food Science and Technology from the Basque Country University and a PhD from the Catholic University of Portugal. He is presently Professor and Academic Leader of the Environmental Sustainability and Health Institute at the Technological University Dublin where he leads one of the five research infrastructures supporting PhD training and research in TU Dublin. Prof Frias is a member of the Institute of Food Technologists, the Institute of Food Science and Technology Ireland and the Iseki Food Association, He's the Editor in Chief of the International Journal of Food Studies from the Iseki Food Association. His research focuses on making an impact on the safety quality, shelf life and waste in fresh horticultural products, with applied mathematical models. He also collaborates where model building and kinetics may be a relevant part of the research challenge.

Modelling the growth of *Listeria monocytogenes*: what we have learned from the ListeriaPredict project

Francis Butler and Kevin Hunt

University College Dublin, Ireland

Abstract

The ListeriaPredict Project is an EFSA funded Partnering Programme with an objective to enhance capacity across Europe in applying predictive microbiology techniques to shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods. There has been substantial activity within the European Union to provide both detailed and practical information on how to conduct shelf-life studies on *L. monocytogenes* in ready-to-eat foods to ensure conformance to the microbiological criteria. Predictive microbiology has a central role in interpreting the results of challenge tests and extending their application. The ListeriaPredict Project has four European partners and has carried out a series of training workshops on emerging predictive microbiology techniques. One interesting emerging area is using dynamic temperature conditions as part of shelf life studies. Within the project, experimental data on the growth of *L. monocytogenes* was generated at both static and varying temperature conditions. Dynamic modelling was used to successfully predict the growth during the varying temperature conditions. The project also examined the possibility of predicting the growth parameters directly from the dynamic temperature conditions.

Biography

Professor Francis Butler, an engineer by training, joined UCD as an academic in Biosystems Engineering in 1990 having previously worked in production management in the Irish dairy industry. He was appointed a Professor in 2007 and was the Head of School, UCD School of Biosystems Engineering from 2011 - 2014. He was Head of Subject, Biosystems and Food Engineering until September 2022. His teaching responsibilities at undergraduate and graduate level include modules in food chain integrity, global cold chain safety and bioprocess engineering principles. He was the programme coordinator for the UCD MSc Food Safety and Risk Analysis and also the UCD coordinator for the International Master in Food Management and Safety, a joint initiative between UCD, The University of Lorraine, France, and the Universitat Politècnica de València, Spain. Professor Butler is a Principal Investigator in the UCD Institute for Food and Health and the UCD Centre for Food Safety. Professor Butler main research interests are in food safety with a particular focus on food chain integrity and quantitative risk assessment of microbiological and chemical hazards in foods.

Proteomic profiling of a virulent *Listeria monocytogenes* strain grown under several stress conditions

Federica D'Onofrio[1], Luigi Iannetti[2], Maria Schirone[1], Ivanka Krasteva[2], Francesco Pomilio[2], Manuela Tittarelli [2], Francis Butler [3], Mirella Luciani [2]

[1]Faculty of Bioscience and Technology, Università degli Studi di Teramo, Teramo, Italy
[2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy
[3]University College Dublin, School of Biosystems and Food Engineering, Agriculture and Food Science, Belfield, Dublin 4

Abstract

The aim of the following study is to investigate the immunogenic proteins (IP) encoded by *Listeria monocytog*enes when exposed to environmental stress. To give a better understanding of the role of proteins expressed under stressful condition, *L. monocytogenes* cells were cultivated at 37°C, pH 7.0, NaCl 0.5% - optimal condition (C1) and 12°C, pH 5.5, NaCl 7% C4, respectively and collected at late exponential growth phase. The proteins were extracted by enzymatic reagents, precipitated, solubilised, and quantified by BCA method. The proteins identification was performed by shot-gun nLC-MS/MS approach, filtering and selecting only those proteins identified with at least 2 peptides against the *L. monocytogenes* Uniprot database. The IP prediction was obtained by mean of sublocalization and immunogenic software. Gene enrichment analysis was performed by STRING v11.05.
To face stress environmental condition, *L. monocytogenes* seems to encode for proteins related to stress responses, antibiotic resistance *(Lmo0553* and *lmo1466)* and for proteins associated to cell wall (lmo1090, lmo0933, lmo2550, lmo0497): the latters might be an impact on host-pathogen interaction as InlA and Iap proteins, well known virulence factors involved in the host cell adhesion. Moreover, these factors are absent in optimal condition, in which instead was identified a sugar uptake cluster (Lmo0097, lmo0098, lmo0781, lmo0782, FruA) positively regulated by σB virulence and Agr gene, which is associated to quorum sensing system, cell survival, adhesion, invasion and infectivity of host cells.

Biography

Ms D'Onofrio has a Masters degree in Food Science and Technologies from the University of Study of Teramo - Faculty of Bioscience and Technology for Food Agriculture and Environment. She is currently completing her PhD studies at the University of Study of Teramo - Faculty of Bioscience and Technology for Food Agriculture and Environment. Her research studies are in collaboration with the "Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise", Teramo, Food Hygiene Department, who are the national reference laboratory for *Listeria monocytogenes*. Her work focusses on the analysis of *Listeria monocytogenes* virulence factors by proteomic and transcriptomic techniques. She is currently working as an exchange researcher at University College of Dublin - UCD Centre for Food Safety.

The gastrointestinal phase of *Listeria monocytogenes* infection

Cormac G.M. Gahan

APC Microbiome Ireland, School of Microbiology and School of Pharmacy, University College Cork, Ireland

Abstract

Listeria monocytogenes is a pathogen that must survive significant challenges during the infectious cycle. During the gastrointestinal phase of infection the pathogen must overcome exposure to low pH, bile acids, elevated osmolarity and the presence of competing commensal bacteria (the gut microbiota) prior to invading the host mucosal barrier. Whilst we have an extensive genomic and physiological understanding of *L. monocytogenes*, questions remain about how the pathogen interacts with the host microbiota, the role of diet in susceptibility to infection and the role of stress adaptation systems on stress resistance in situ in the host GI tract. This presentation will discuss some recent findings which examine the role of the Western diet in susceptibility to infection, how individual differences between *L. monocytogenes* strains/clonal complexes may govern virulence characteristics and how such findings may be exploited for future therapeutic/preventative approaches to lessen disease risk.

Biography

Prof. Cormac Gahan is a member of faculty in the School of Microbiology and a PI within APC Microbiome Ireland in University College Cork. Prof. Gahan has an interest in how the gut microbiome contributes to the health of the host; through the metabolism of bile acids and the influence of the gut microbiota on colonisation resistance against gut pathogens including *Listeria monocytogenes*. Prof. Gahan currently co-ordinates the EU H2020 funded ITN training Network 'COL_RES' which examines colonisation resistance against a variety of pathogens. His work has been published in numerous journals in the field including Gut Microbes, Microbiome, Proceedings of the National Academy of Sciences and Plos Pathogens.

The diverse ecology of *Listeria monocytogenes* - niche adaptation for different lifestyles

Edward Fox

Northumbria University, UK

Abstract

The ecology and lifestyle of *Listeria monocytogenes* is diverse, ranging from environmental saprophyte to pathogen of humans and animals. This is supported by a diverse genetic complement, and biological function, which has established the organism within these diverse niches. Genomic advances have provided new insights into the genetic mechanisms underlying both the pathogenesis of the bacterium, as well as the mechanisms used to adapt to environmental niches. One such niche is the food processing environment (FPE), where colonisation of the FPE by *L. monocytogenes* undermines food safety and can increase the risk of contamination of foods, leading to outbreaks. In this presentation, the genetics of *L. monocytogenes* will be explored in relation to strains associated with clinical or food environments, and the genetic mechanisms, and their utilisation by the bacterium to colonise the FPE, will be explored. This includes cell signalling and biofilm formation, transcriptional regulation, and genetic markers relevant to environmental stress tolerance and virulence.

Biography

Dr Edward Fox is a Senior Lecturer at Northumbria University, working within the Food and Nutrition Program of the Department of Applied Sciences. He received his BSc (Hons) in Biotechnology from Dublin City University, and undertook his PhD at the Teagasc Moorepark Food Research Centre in Ireland, in the area of microbial food safety. Following a Newman Fellowship at University College Dublin's Centre for Food Safety, he held a Senior Research Scientist position at the Australian government's scientific research organisations, CSIRO, before moving to Northumbria. His research examines the ecology and control of foodborne pathogens through food supply chains, with a particular focus on bacterial species such as *Listeria monocytogenes* and *Staphylococcus aureus*. His work utilises omics approaches to understand the genetic mechanisms that contribute to the ability of these organisms to persist in food chains, and genetic mechanisms of significance to associated public health, such as virulence and antimicrobial resistance.

Utilising WGS to predict the heightened risk to food producers of certain *Listeria monocytogenes* strains

Nick Andrews

Dawn Farm Foods, Ireland

Abstract

Whole genome sequencing (WGS) offers the potential to characterise *Listeria monocytogenes* isolates recovered from foods and food processing environments in terms of the associated risk to brand reputation and public health. This is an application of precision food safety, and to investigate this potential, statistical modelling techniques were applied at successively deeper levels of discrimination to a representative collection of draft genomes to identify the association of clinical adaptation and ecophysiology genetic features with infection and environmental persistence phenotypes. A number of features were identified and strongly associated with virulence and hypervirulence, along with others with environmental adaptation. A quantitative risk assessment model is proposed, highlighting the increased risk of particular isolates which are both well adapted to persist in food processing environments, and are fully capable of causing human infection and outbreaks.

Biography

Nick Andrews is Head of Food Safety and Quality for Dawn Farm Foods, Europe's largest multi-species producer of cooked and fermented meat and flexitarian ingredients. A Chartered Engineer, he holds a primary degree in science from Liverpool University and has worked in various roles in manufacturing industry for over 30 years. Nick heads a team of food safety and science professionals in assuring the production of safe ready-to-eat foods and safeguarding the brands of Dawn's clients. A long-term research collaborator with UCD-CFS, he has a particular interest in the scientific management of food risks and precision food safety.

Listeria monocytogenes: A regulatory perspective

Mary Lenahan

Food Safety Authority of Ireland

Abstract

Compliance with food safety legislation in relation to *Listeria monocytogenes* is complex and can be difficult for food business operators to understand. Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs differentiates between ready-to-eat food able to support (food category 1.2) and unable to support (food category 1.3) the growth of *L. monocytogenes*. Different criterion limits for *L. monocytogenes* apply depending on which category the food belongs to and whether the food business operator has scientific studies sufficient to show how *L. monocytogenes* will grow in their product during its shelf-life. The microbiological criterion limits set in food categories 1.2 and 1.3 are based on data that ≤ 100 cfu/g *L. monocytogenes* at the point of consumption is low risk for causing listeriosis in consumers with healthy immune systems. However, the risk of listeriosis from consuming food with lower numbers of *L. monocytogenes* below this limit is much higher for young children, older adults, those with compromised immune systems or are pregnant. The Food Safety Authority of Ireland work with the official agencies and food businesses to provide clear and evidence-based advice in relation to *L. monocytogenes* to promote food safety and build compliance with food law.

Biography

Dr Mary Lenahan has been working with the Biological Safety team in the Food Safety Authority of Ireland (FSAI) for over 7 years. In her current role as Acting Senior Technical Executive, she provides technical support and guidance to food businesses and official agency staff regarding microbiological issues such as food legislation, shelf-life of foods and risk assessment of microbiological hazards. Her previous experience includes working with both the private and public sectors on various research projects concerning food safety, and sales to microbiology labs in the Irish food and veterinary industry. Mary has a BSc in Nutritional Sciences from University College Cork and a PhD in Microbiological Food Safety attained from the University College Dublin in association with the Teagasc Food Research Centre (Ashtown).

Listeriosis- an Irish clinical perspective

Martin Cormican

Galway University Hospital and University of Galway, Ireland

Abstract

Invasive infection with *Listeria monocytogenes* (*Lm*) is uncommon. The proportion of the population at risk has changed with an aging population and increased use of long-term immune-suppression. The impact of *Lm* on clinical practice is disproportionate to the number of cases. Invasive infection is associated with a high risk of death. The illness, meningitis or blood stream infection, generally cannot be differentiated reliably from other serious infections based on clinical features. *Lm* is intrinsically resistant to third generation cephalosporins. Third generation cephalosporins are a key to the initial treatment of suspected bacterial meningitis. Therefore, *Lm* must be considered and must be treated up-front in a large number of seriously ill patients in whom it is not the cause of disease. Treatment directed towards *Lm* may be continued for two days or more until diagnostic tests provide assurance that it is not the cause of infection. Nucleic Acid Amplification Tests (NAATs) and the MALDI-ToF for culture identification have reduced the time required to identify Lm or an alternative pathogen. Invasive *Lm* is a notifiable infectious disease in Ireland. When *Lm* is identified in a clinical laboratory, prompt submission of the isolate to the reference laboratory is recommended to support linking of cases to other cases and potentially to sources of infection.

Biography

Martin Cormican graduated from the Medical School of the University of Galway in 1986. His postgraduate training was in Ireland, UK and USA. Since 1999 he has been Professor of Bacteriology in the University of Galway and Consultant Microbiologist in Galway University Hospital. He has been director of the National Reference Laboratory service for *Salmonella*, *Shigella* and *Listeria monocytogenes* since the service was established in 2000.

Novel antibacterials targeting the transcriptional regulators PrfA and BrtA

Jörgen Johansson

Umeå University, Sweden

Abstract

In previous work, we discovered that ring-fused 2-pyridones can decrease virulence factor expression in *Listeria monocytogenes* by binding and inactivating PrfA. Here, we tested PS900, a highly substituted 2-pyridone that was recently discovered as bactericidal to other Gram-positive pathogenic bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis*. We show that PS900 can interact with PrfA and reduce expression of virulence factors. Unlike previous ring-fused 2-pyridones shown to inactivate PrfA, PS900 had an additional antibacterial activity and was found to potentiate sensitivity towards cholic acid. Two PS900-tolerant mutants able to grow in presence of PS900 carried mutations in the *brtA* gene, encoding the BrtA repressor. In WT bacteria, cholic acid binds and inactivates BrtA, thereby alleviating expression of the multidrug transporter MdrT. Interestingly, we found that PS900 also binds to BrtA and that this interaction causes BrtA to dissociate from its binding site in front of the *mdrT* gene. In addition, we observed that PS900 potentiated the effect of different osmolytes. We suggest that the increased potency of cholic acid and osmolytes to kill bacteria in presence of PS900 is due to the ability of the latter to inhibit general efflux, through a yet unknown mechanism. We suggest that thiazolino 2-pyridones constitute an attractive scaffold when designing new types of antibacterial agents.

Biography

Prof. Jörgen Johansson defended his PhD at Umeå University, Sweden in 2000, working with gene-regulation in *E. coli*. The same year he started as a postdoc at Institut Pasteur, working in the group of Prof. Pascale Cossart with RNA-mediated virulence regulation in *Listeria monocytogenes*. In 2003, Prof. Johansson returned to Umeå University to start his own group continuing working with *Listeria*. His research interests spans RNA-mediated (virulence) regulation, bacterial stress-sensing and development of new types of antibacterial drugs, targeting bacterial viability and/or virulence. In 2016, Prof. Johansson performed a 3-month sabbatical in the group of Prof. Conor O'Byrne at the National University of Ireland, Galway working with bacterial stress-sensing.

Listeria monocytogenes in cheese - a model for determining the level of undissociated lactic acid in cheese and predicting complete growth inhibition

Marjon Wells-Bennik

NIZO, Netherlands

Abstract

Specific microbiological criteria for *Listeria monocytogenes* in RTE foods have been set in regulation (EC) No 2073/2005. For this food category, it is important to determine whether products can support the growth of *L. monocytogenes*. Challenge tests showed "no growth" with Gouda cheese. Undissociated lactic acid was found to be the most important inhibitor in this cheese. A model was developed to calculate the concentration of undissociated lactic acid in cheese, considering important product parameters (fat content, moisture content, pH, lactic acid and salinity). The growth rate of *L. monocytogenes* in different types of cheese was calculated using a predictive model considering the factors of undissociated lactic acid, temperature, pH and Aw. In addition, experimental growth/no growth data for *L. monocytogenes* for the different cheese types were obtained from the scientific literature. Modelling results were compared with reported growth/no growth and correct growth/no growth predictions were obtained for 9 out of 10 cheeses. The results of our study demonstrate the importance of undissociated lactic acid, temperature, pH and aw for complete inhibition of *L. monocytogenes* in cheese.

Biography

Dr. Marjon Wells-Bennik is Principal Scientist at NIZO and a visiting scientist at Wageningen University. NIZO conducts contract research for leading food and health companies with a mission to contribute to better food and health and sustainable food production. NIZO's food quality and risk assessment team helps customers accelerate innovation and produce safe food with reliable processes thereby protecting image and brands and reducing food waste. Marjon’s extensive expertise and background in food safety and quality was shaped by her PhD at Wageningen University, Postdoc at Harvard University, work at FBR (Wageningen), Quadram (Norwich), and NIZO over the past 18 years.

Growth of *Listeria* in plant-based milk alternatives

Klaudia Bartula, Máire Begley, **Michael Callanan**

Munster Technological University, Ireland

Abstract

An increase in vegan diet preference, lactose intolerance, calorie intake concerns and environmental awareness has led to a rise in the popularity of plant-based alternatives to bovine milk. However, there are still gaps in understanding how known bacterial food pathogens behave in plant-based beverages. The present study is the first to compare the growth of food-pathogen *Listeria monocytogenes* in commercially available ultrahigh temperature processed bovine milk and plant-based milk alternatives (coconut, almond, cashew). Beverage samples were inoculated with a strain cocktail of *Listeria* (approximately 1 x 10^3 CFU/mL) and stored at chilled and ambient temperatures (4°C, 8°C or 20°C). The *Listeria* strains used in the study were capable of proliferating in plant-based beverages at higher rates than in bovine milk at 8 °C and 20 °C. In addition, there was no statistically significant difference ($p > 0.05$) in growth rates between different types of tested beverages at 4 °C and at 8 °C. Similar data was obtained for *Salmonella enterica* which suggests that plant-based beverages may present a significant risk for listeriosis and salmonellosis and post-opening storage recommendations should be carefully considered.

Biography

Dr. Michael Callanan qualified with PhD in Microbiology from UCC in 1996 and spent five years completing postdoctoral studies in Massey University, New Zealand and North Carolina State University, USA, followed by 5 years working on microbial genomics at Teagasc Food Research Centre, Moorepark. In 2007 he moved into industry working for Glanbia Nutritionals in Kilkenny before being recruited to the Nestlé Research Centre (NRC) in Lausanne in 2010. At the NRC, he was responsible for managing several large R&D projects focused on food quality and safety. He has continued to work on food quality and safety since returning to Ireland to teach at Munster Technological University in January 2016.

Growth behaviour of *Listeria monocytogenes* in semi-soft rind-ripened artisanal cheese at cold chain and abuse temperatures

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[2]*UCD School of Biosystems and Food Engineering, University College Dublin, Belfield, Dublin 4, Ireland*

Abstract

Listeria monocytogenes is particularly concerning in cheese because it can proliferate under common control used in the food industry such as refrigeration temperatures, pH and high-salt concentration. The aim was to examine the growth behaviour of *L. monocytogenes* on a semi-soft, rind-ripened cheese made from pasteurised cow's milk at a range of cold chain temperatures and to develop a dynamic predictive model in accessing temperature abuse scenario for this product type. The entire retail size (225 g) of the semi-soft, rind-ripened cheeses were separately challenged with three strains of *L. monocytogenes* and their maximum specific growth rate (μmax) were determined at refrigeration (3.9 °C) and abuse conditions (12.5 °C) respectively over the shelf-life (30 days). The strains were chosen to be representative of dairy isolates namely *Lm*954 (Teagasc Moorepark, Cork, Ireland), 12MOB079LM (EURL) and F2365 (Jalisco cheese outbreak of 1985 in California). All three strains grew at both temperatures during shelf life, the maximum number of 5.44 and 7.48 Log_{10} CFU/g for 3.9 °C and 12.5 °C respectively and strain variation in μmax and Lag phases were observed. This study further highlights the ability of *L. monocytogenes* to proliferate in this product type during the downstream food chain at a realistic temperature and will help in assessing the risks of listeriosis.

Biography

Dr Peter Myintzaw is a postdoctoral food safety microbiologist working in Food Biosciences Department at Teagasc Moorepark, Cork, Ireland. He holds a BSc (Hons) in Nutrition and Health Science from Munster Technological University and an MSc in Food Safety Management from Technological University Dublin. Prior to joining Teagasc, he completed his PhD from MTU on "Harnessing genomic and phenotypic diversity of *Listeria monocytogenes* to improve food safety". He also previously worked as a food analyst at Devenish Nutrition Company, based at University College Dublin. His current postdoctoral scientist work focuses on foodborne pathogens' stress tolerance, challenge studies, food matrices, sterility testing, predictive modelling, pan-genome analyses, and comparative genomics.

Listeria biofilms: challenges and opportunities for their detection and study

Antonio Lourenco

Teagasc Food Research Centre, Moorepark, Ireland

Abstract

Biofilm formation has long been identified as the mechanism through which facilitates the persistence of microorganisms in natural. Through the production of extracellular polymeric substances bacteria the attachment to a substratum become gains an irreversible nature and altered phenotypes are induced, when compared to the planktonic counterparts. *Listeria monocytogenes* has been shown to exhibit a wide range of biofilm formation ability among strains on top of the variability detected between different methods that often rely on direct or indirect quantification of the bacterial cells and usage of surface tests of different mature.
In industrial environments, the detection of the major extracellular polymeric substances composing biofilms (carbohydrates, proteins and eDNA) may be used as means to detect areas particularly susceptible to biofilm formation. Natural biofilms may harbour a wide range of microorganisms that may be characterized through culture dependent methods but also through culture independent methods such as 16S rRNA amplicon sequencing. The environmental microbiota greatly influence the ability of *L. monocytogenes* to form biofilms through symbiotic and antagonistic relationships and therefore may determine its fate in industry.

Biography

Antonio Lourenco graduated in 2005 with B.Eng. in Food Engineering from the Agronomy School at the University of Lisbon, Portugal. In 2009, he graduated from the NOVA University Lisbon with an MSc in Food Technology & Quality and in 2014 with a PhD in Food Engineering from the University of Lisbon. During his PhD, Antonio conducted research stays at the University of Georgia, University of Florida and at The University of Edinburgh. After his PhD, Antonio took on a position as Postdoctoral Fellow at the University of Minnesota. In 2017, Antonio joined Teagasc and was recently a Marie Curie fellow between Teagasc and at the University of Veterinary Medicine Vienna (Austria).

Flash and Poster Presentations

Growth potential of *Listeria monocytogenes* on ready-to-eat fresh produce in ambient conditions

Elena-Alexandra Alexa[1], Michael Arthur[1], Amy McKenna[1, 2], Michael Gaffney[2], Catherine M. Burgess[1]

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[2]*Horticulture Development Department, Teagasc Food Research Centre, Ashtown, Dublin*

Abstract

Food business operators need to know whether the ready to eat foods they produce can support the growth of *Listeria monocytogenes* as it determines their categorisation in regulations. The objective of this study was to determine the growth potential of *L. monocytogenes* on butterhead lettuce, baby leaf spinach and strawberries. Following the technical guidance published by European Union Reference Laboratory (version 4/ 1st of July 2021), challenge tests were conducted at 4°C and 10°C, for which the above mentioned products were spiked with a three strain cocktail of *L. monocytogenes* (EURL12MOB050LM-EUR Lm reference, 6179-food processing, 959-vegetable strains) at ~100 CFU/g and stored in atmospheric conditions over a 5 day period. None of the RTE fresh produce supported the growth of *L. monocytogenes* at 4°C, with numbers increasing gradually at 10°C on baby leaf spinach and butterhead lettuce, but not on strawberries. Enterobacterales, yeasts and moulds, as well as total viable bacterial counts were monitored throughout the study, with higher concentrations at the last sampling point, regardless of the temperature used. Baby leaf spinach showed a higher increase for viable counts and Enterobacterales, compared with butterhead lettuce. Other factors monitored throughout the study such as pH and water activity may have contributed to the decline in *L. monocytogenes* numbers.

Survival of *Listeria monocytogenes* in fermented pepperoni with modified formulations and process parameters

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Abstract

Consumer health considerations are changing and there is pressure on the fermented meat sector to lower the concentrations of added salt and sodium nitrite. This may however lead to enhanced survival of *Listeria monocytogenes* in this ready-to-eat product.
The aim of this study was to evaluate the survival of *L. monocytogenes* in pepperoni made to standard and modified formulations; salt (1.4%-2.5%); sodium nitrite (50ppm-150ppm); and process parameters (final pH 4.8); heating (53.5◦C-64◦C); and target a_w (0.91-0.94) at end of the drying period.
L. monocytogenes (five-strain cocktail) was inoculated (log_{10}6.00 $CFUg^{-1}$) into pepperoni batter. Samples (n=3) were taken at pre-fermentation, post-fermentation, post-heating, mid-point, and at end-point of drying (target a_w). The pepperoni were examined for weight, pH, a_w, and *L. monocytogenes* by direct plate count and enrichment. In the standard formulation, *L. monocytogenes* was reduced from a pre-fermentation level of log_{10}6.14 CFU g^{-1} to log_{10} 1.67CFU g^{-1} at the endpoint.
When sodium nitrite levels were lowered (50ppm), there was greater pathogen survival (log_{10} 2.94 CFU g^{-1} at the endpoint. The inclusion of a heat step (61◦C for 40min) had a significant impact on *L. monocytogenes* with a >6 log reduction from pre-fermentation to end-point. This work will support the development of pepperoni with modified formulation without compromising safety.

Population structure and macroevolution of *Listeria monocytogenes* CC121 in the UK

Ana Victoria Gutiérrez[1], Maria Diaz[1], Michaela Matthews[1], Frieda Jorgensen[2], Heather Aird[2], Anais Painset[2], Nicolle Som[1], Oleksii Omelchenko[1], Rob Kingsley[1], Matthew Gilmour[1]

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[2]UK Health Security Agency, Food, Water and Environmental Microbiology Laboratories and the Gastrointestinal Bacteria Reference Unit, UK.

Abstract

Listeria monocytogenes is a foodborne bacterial pathogen that continues to expand its niche into new food categories following zoonotic and/or environmental persistence. In this study, we established the population structure and macroevolution of *L. monocytogenes* CC121, which is an important contributor of foodborne risk in the United Kingdom. Hybrid genomes were generated on historical (1987–2007) *L. monocytogenes* food isolates from the UK and analysis were enriched with global (Pasteur database) and UKHSA genomes. Using time-scaled phylogenies, hierarchical clustering and pangenome analysis, we identified signature macro-genetic regions unique within hierarchical clusters. We mapped these regions and clusters against key epidemiological information when available, such as the observation of a prophage that, within global CC121 sequences, was uniquely observed in a cluster associated with multi-year persistence in a single food production setting. The hybrid genomes of historical isolates included in the pangenome analysis provide insight into the genetic status and biocide tolerance of non-contemporary isolates. The identification of key genetic regions with epidemiological associations aids in the identification of markers of risk and the overall comparative genomic dataset provides a resource for future studies aimed at understanding the molecular mechanisms underlying persistence, biocide tolerance, virulence and biofilm formation in *L. monocytogenes.*

Short chain fatty acids (SCFAs) modulate growth, virulence expression and host-pathogen interactions in *Listeria monocytogenes*

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[4] School of Pharmacy, University College Cork, Cork, Ireland.

Abstract

Aim: *Listeria monocytogenes* places vulnerable hosts at serious risk despite appropriate use of antibiotics, and alternative solutions to preventing infection are needed. Recent work focused on downregulating virulence expression, but little attention is given to targeting gut barrier function as a therapeutic approach. We investigated the *in vitro* effects of microbial-derived SCFAs as candidates to increase colonisation resistance against listeriosis.
Methods: SCFAs' effects on kinetics and gene expression in *Listeria* were examined using absorbance readings, RNA-Seq, qPCR and bioluminescence. Viability, permeability, gene expression and immune response together with adhesion/invasion, and proliferation assays were used to study infection dynamics in epithelial cells, and macrophages.
Results: Tolerance and virulence gene expression increased with acetate, decreased with butyrate and propionate, and varied in response to oxygen and carbon source availability, and pH in *Listeria*. Permeability and host-pathogen interaction genes were SCFA- and cell type-dependent. Butyrate improved permeability of HT29-MTX-E12 cells and reduced TNF-a/IL6 expression in LPS-induced and *L. monocytogenes*-infected macrophages. Bacterial competency to adhere/invade cells is reduced by propionate-treated bacteria and butyrate-treated cells.
Conclusions: Our data indicate that specific SCFAs may provide a mechanism underpinning colonisation resistance against the pathogen. This work will inform future *in vivo* studies from our group.

Effects of chitin on *Listeria monocytogenes* pathogenicity

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Abstract

Background: The complex polysaccharide chitin contributes to a healthy gut microbial population, modulates the immune response, and attenuates virulence gene expression in *Listeria monocytogenes*. However, its role in host-pathogen interactions remains unknown to date. Here, we investigated chitin effects in the context of *L. monocytogenes* infection.
Methods: Chitin titration was used on a bioluminescent strain to determine virulence-repressing concentrations. The effects of 0.05% and 0.1% chitin suspensions were tested in C2BBe1 and HT29-MTX-E12 epithelial cells for adhesion/invasion assays and permeability (TEER), and in RAW 264.7 macrophages for TNF-a and IL6 cytokine expression using ELISA.
Results: Biologically relevant concentrations of chitin downregulate virulence gene expression, influence adhesion and invasion properties with intestinal epithelial cells, and decrease goblet cell permeability. Chitin also has immunomodulatory properties, reducing LPS-activated IL-6 while inducing a TNF-α response in macrophages. The data suggest that chitin may provide a potential dietary intervention to inhibit *L. monocytogenes* infection in the gut and murine experiments are ongoing to further test this hypothesis.
Conclusion: Our *in vitro* data informs that chitin modulates host-pathogen interactions and is worth using as a potential candidate in dietary intervention to prevent or limit the burden of listeriosis.

Genomic characterisation of long-time persistent *Listeria monocytogenes* strains in cheese production facilities

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Abstract

Over the last years, many studies suggested that the persistence of *Listeria monocytogenes* in food processing plants is an important factor in the transmission of this foodborne pathogen to humans via food chain. The aim of this study was to determine the diversity and the persistence of *L. monocytogenes* in Swiss cheese processing companies applying whole genome sequencing. Overall, 194 isolates collected between 1987 and 2011 in different matrices (water, cheese, milk…) were sequenced and typed based on MLST and cgMLST schemes. Twenty-four different Clonal Complexes (CC) were found in Swiss cheese production facilities, being CC3 (n=70), CC1 (n=47) and CC101 (n=25) the most prevalent. According to the cgMLST scheme, some Complex Types (CT) belonging to CC3, CC101 and CC1 were recurrently isolated in the same companies over a period up to 7 years. The genomic characterisation of these CTs revealed that most of them presented genes conferring resistance to heavy metals such as arsenic (ars) and cadmium (cad) or tolerance to low pH (gad) or to stress (lmo0444-0445). These genes could contribute to the survival of *L. monocytogenes* in harsh environments.

An epidemiological review of Listeriosis in Galway, Mayo and Roscommon (2010 to 2023)

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Department of Public Health Area F, Merlin Park University Hospital, Galway.

Abstract

Listeriosis can be a serious infection, and since 2004 is a notifiable disease.
CIDR was used to review cases from 2010 to present (n=25, 14% of national cases) in Galway, Mayo and Roscommon. The average yearly crude incident rate (CIR) is 0.40 per 100,000; which is above the national average yearly CIR of 0.27 per 100,000. The cases consisted of 5 neonates, 4 pregnant females, and 16 adults (69% ≥65 years). Risk factors were identified in almost all patients (n=24). Death was reported in two patients (both ≥65 years), but it is unknown if listeriosis was a cause of death. Septicaemia was reported in 11 patients, and meningitis was reported in 5 patients. High risk food consumption was reported frequently among patients, however no food items were identified as the source of infection. No clusters were identified during this time period. Typing was performed by NSSRL on isolates from 20 patients, the most common serotype reported being *Listeria monocytogenes* serotype 1/2a (n=10).
Listeria caused severe illness in 65% of patients. High risk food consumption in at risk groups, must be a focus of public health campaigns. In addition, continued monitoring of food production to reduce risk of contamination, is essential.

Reported foodborne outbreaks related to *Listeria monocytogenes* in Belgium between 2016 and 2023

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[2] *SCIENSANO, National Reference Laboratory for Foodborne outbreaks, Brussels, Belgium*

Abstract

Listeria monocytogenes causes listeriosis, a severe disease characterised by a high hospitalisation and case fatality rate. The Belgian Public Health Institute, SCIENSANO, harbours both the NRL for Foodborne outbreaks and for *L. monocytogenes* which analyse foodborne outbreak samples, centralises the data, and report to EFSA.
Between 2016 and 2023, 42 foodborne infections related to *L. monocytogenes* were reported to the NRL with at least six deaths. A suspect food vehicle could be identified in fifty percent of all outbreaks. Among the reported infections, only four collective outbreaks (≥ 2 ill) were reported which led to 9 human cases, 5 hospitalisations, without any death. *L. monocytogenes* 1/2a and *L. monocytogenes* 4b were involved in three of these collective outbreaks. One outbreak in 2022 was categorised as a strong-evidence outbreak. Vegan cheese was identified as the outbreak source using WGS to compare the food and the human isolates.
Finding the source remains a challenge because of the long incubation period of listeriosis (up to 70 days) and its typical sporadic occurrence. Since 2023 a WGS-based surveillance started for both clinical and official food *L. monocytogenes* isolates at national level. This approach will facilitate the linking between some of these sporadic cases and contaminated food products.

Employing next-generation sequencing to elucidate the Welsh food Listerial landscape

Joshua A. Macleod[1], Michael L. Beeton[1], Benjamin E. Johns[2], James Blaxland[1]

[1]*Cardiff Metropolitan University*
[2]*Public Health Wales*

Abstract

Listeria monocytogenes is a notifiable organism in the UK, with 150-200 cases of listeriosis reported per year. With the increasing availability of next-generation sequencing technology, access to multilocus sequence typing (MLST) and phylogenetic analysis allows for an exploration of the dissemination of *L. monocytogenes* and *Listeria* spp. throughout Wales.
The objective of this study was to employ post-sequencing bioinformatic tools on 33 isolates obtained from Public Health Wales to produce a snapshot of the Welsh-Listerial landscape, informing subsequent research.
Isolates obtained from PHW were subject to DNA extraction and library preparation before sequencing. Outputs were de novo assembled using shovill. MLST was conducted to characterise sequence types. Phylogenetic analysis was conducted using RAxML to inform evolutionary relationship of isolates. Subsequently, screening for virulence and antimicrobial resistance genes was conducted using shovill and ABRicate.
Results indicate a dominant sequence type (ST), ST2, within the population and a high heterogeneity of both lineage I and II isolates were identified. Protein annotation suggests intrinsic and acquired tolerances to antimicrobials and biocides well reported already in literature, alongside profiles of LIPIs, stress-tolerances and AMR genes.
This study will inform subsequent research, exploring individual food manufacturing environments and the dissemination of *Listeria* spp. in Wales.

Controlling *Listeria monocytogenes* risk: Update of online risk assessment tools to help producers of smoked fish

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Abstract

As the Scottish regulator for food safety, Food Standards Scotland aims to support food businesses to follow best practice. In close collaboration with technical experts, industry stakeholders, and web developers we recently redesigned and overhauled our online microbiological risk assessment tool for producers of smoked fish.
This free to use and anonymous platform allows smoked fish producers to risk assess their practices and offers advice and recommendations supported by relevant academic literature, guidance and legislation. The tool includes 3 multiple choice assessments, as well as a glossary and resource pages giving background on *Listeria monocytogenes* in smoked fish production, detail of good practice at different process stages and possible actions after an isolation. This tool is a practical approach to provide businesses with targeted advice on how to achieve best practice to reduce microbiological risk in their product. Since the tool was re-released and promoted via industry contacts the hits per month on the platform have increased significantly, with analytic data also indicating longer access time per user. The Safe Smoked Fish Tool can be found by searching for *FSS Safe Smoked Fish Tool*.

Which is the *Listeria monocytogenes* growth risk in RTE plant-based meat analogues?

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Abstract

The consumer's demand for plant-based cooked meat analogues has been increasing in the last years. As ready-to-eat (RTE) products, *Listeria monocytogenes* is a highly relevant hazard, being recently involved in some RASFF alerts affecting plant-based meat analogues. In this framework, the aim of the present study was to benchmark antimicrobial ingredients and additives trends and to evaluate the behaviour of *L. monocytogenes* through challenge testing in different commercial meat analogues. List of ingredients extracted from the Mintel-Global New Products Database (last 3 years) showed that three fourths of them were formulated without antimicrobials and one forth contained organic acids salts and especially vinegar as the most predominant. Experimental results evaluating growth potential of the pathogen in five different analogues of cooked meat products (i.e. cooked ham, mortadella and Frankfurt-type sausages) packaged in air, vacuum and MAP atmospheres and stored up to 28 days at 6.5 ºC showed *L. monocytogenes* growth potential from zero to 6.5 log units, which depended on the presence of antimicrobials in the formulation, the pH, the levels of lactic acid bacteria and the packaging atmosphere. A safety-by-design approach taking into account intrinsic and extrinsic factors is essential for the development of safe meat analogues.

Molecular characterisation of *Listeria monocytogenes* isolates received by the National Reference Laboratory in 2021

Brian Byrne, Kenneth Dygico, Deirdre Prendergast, Rebecca Ham, Dayle Johnson, Daniel Kelly, Juliana Cummins, Saibh Healy, Montserrat Gutierrez

Food Microbiology Division in the Department of Agriculture, Food and Marine (DAFM), Ireland

Abstract

The Food Microbiology Division in the Department of Agriculture, Food and Marine (DAFM) is the National Reference Laboratory (NRL) for *Listeria monocytogenes* under Regulation 2017/625 on official controls performed to ensure the verification of compliance with feed and food law, animal health and welfare rules. The NRL provides a service for the typing and characterisation of *L. monocytogenes* isolates that have been confirmed and cultured in commercial (own check samples) and official (official control samples) laboratories. These isolates have been cultured from different food processing environments and ready-to-eat food products. All submitted isolates (n=3,476) were serotyped using real time PCR and a selection of the submitted isolates (n=779) were sequenced using the Illumina platform and analysed using the BioNumerics pipeline (8.0). Clusters/WGS profiles were defined as isolates showing 7 or less allelic differences between their core genome MLST (cgMLST). Serotype IIa was the most prevalent serotype accounting for 66% (n=2,306). There were 39 different MLST CC groups identified within the 779 isolates sequenced and the most prevalent group was CC121 (29.3%). The NRL WGS database of Irish strains has been valuable in assisting on investigations of foodborne outbreaks, and on identifying persistent and/or new strains within food processing establishments.

Impact of whole genome sequencing in *Listeria* routine surveillance for public health

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Abstract

All *Listeria monocytogenes* (LM) isolates referred to the UK national reference laboratory have been subjected to WGS since December 2015 with some isolates sequenced retrospectively. WGS data are integrated with clinical, epidemiological and food source information into a single database to enhance the detection and investigation of incidents and outbreaks. Single Nucleotide Polymorphism (SNP) distance and phylogenetic analysis are used to group isolates into clusters indicating a common source.

Up to June 2021, 4031 LM were sequenced: 851 from clinical cases and 3180 from food or food environment sources. A total of 83 clusters were identified. Amongst those 57% consisted of only clinical isolates and 43% of at least one clinical isolate and matching food/environment isolates. Using epidemiological data and food history of the cases, evidence of a common food source or food supply chain was identified in 13 out of these 83 clusters. In comparison, during the pre-WGS period (1981 to 2015 covering 34 years), 28 incidents were identified with a confirmed food/food business source of infection.

Despite the immense power of WGS for strain discrimination, there is still a high proportion of cases and defined clusters with no evidence or definite links to the source of infection.

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Genetic diversity and distribution of plasmids in UK *Listeria monocytogenes*

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Abstract

Listeria monocytogenes possesses multiple mechanisms for tolerating environmental stress, allowing strains to colonise food processing environments (FPEs) for years. All *L. monocytogenes* isolates from human cases, food and FPEs that are referred to the gastrointestinal bacteria reference laboratory at the UK Health Security Agency are subject to whole genome sequencing. This ongoing genomic surveillance has identified clusters of closely related food and environmental isolates persisting within FPEs, some of which are associated with cases of disease over that time.

We investigated >4800 genomes for plasmids and genes involved in stress tolerance and resistance to disinfectants. Plasmid presence varies considerably between clonal complexes of *L. monocytogenes*: some (CC121, CC8) display high prevalence but little sequence variation; whilst others (CC7, CC9) have multiple plasmid types and diverse gene content. Long-term clusters of food and environmental isolates are often associated with the presence of plasmids, whilst subsequent clinical isolates deriving from these clusters may show loss of features. Additionally, we identified several novel plasmids.

This project is important as relevant information about the presence of specific genetic features, such as those providing resistance to disinfectants, can be fed back to local authorities to work with food processing companies in eradicating long-term *Listeria* contamination.

Comparative description of incidence demographics and genetic composition of CC1 and CC8 *Listeria monocytogenes* clones

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Abstract

Listeria monocytogenes is a foodborne pathogen causing systemic listeriosis in the elderly, immunocompromised and pregnant women. At the UK Health Security Agency (UKHSA) genome sequencing is used for typing *L. monocytogenes* isolates from clinical cases, food, and food-production environments. The UKHSA surveillance database integrates genomes with associated metadata.

The majority of isolates within Lineage I belong to clonal complex (CC) 1, whereas Lineage II is commonly represented by CC8. Data analysis and phylogenies were utilised for an in-depth comparison of the population structure and genetic composition between CC8 (n=223) and CC1 (n=261).

Human isolates are over-represented in CC1 (CC1=68%, CC8=31%), whereas CC8 have a higher proportion of food and environmental isolates (CC8=62%, CC1=22%). Both clones are characterised by very different food groups. CC1 clones were sampled from ready to eat foods (RTE), fish products, frozen vegetables, dairy and cured meats, and indicates a higher than expected proportion of bovine samples (17%). CC8 is most commonly sampled from RTE, dairy, frozen vegetables and pork, and contains larger genetic clusters linked to specific food groups persisting over a longer period.

This work provides a descriptive analysis of two abundant clonal complexes within Lineage I and Lineage II persisting in the UK.

Deducing clonal complex population structure from gene content

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Abstract

The UK Health Security Agency holds the UK's *Listeria monocytogenes* surveillance database containing whole genome sequencing (WGS) data and associated meta-data from clinical cases, food and food production environments. WGS has proven invaluable in identifying key genes, facilitating *L. monocytogenes* pathogenic success. This work tests for a link between key genes and clone types, through Multiple Correspondence Analysis (MCA) statistical model.

Virulence factors were tested to explore any covariances between lineages and clonal complexes (CC). MCA was performed using the GeneFinder mapping program outcomes, as categorical variables. CC and lineages were supplied as additional variables, but not used for the analysis. The dataset contained isolates from lineage I; CC1(n=237), along lineage II; CC8 (n= 209) and CC9(n=362).

MCA recovered the clonal structure of isolates, with clear groupings in isolates, corresponding to the known CC type. We also recovered the association between isolate clusters, and gene clusters defining each CC. Finally, we identified other isolates and genes without clear population structure, which seemed to correspond to genetic outliers.

Further investigation is needed to determine whether those isolates actually correspond to different phenotypes. This study highlights the power of MCA as a de-novo method to characterise bacteria populations from their gene content.

From old datasets to new insights, using metagenomics to explore the microbiome of food and production facilities: detecting *Listeria monocytogenes* with precision

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Abstract

The use of Next Generation Sequencing protocols includes a deep understanding of the genomes of microorganisms in pure culture using whole genome sequencing and importantly, metagenomics has allowed the extensive comprehension of the microbiota and microbiome of food and food environments. *Listeria monocytogenes* is a ubiquitous foodborne pathogen widely distributed in food production environments and is the target of numerous control and prevention procedures. The detection of this pathogen is based on culture-dependent methods that it doesn't take into consideration the complex dynamics of bacterial interactions and their impact on pathogen detection remains largely unexplored. In this study were analysed metagenomes datasets publicly available of foods and food-producing facilities, including meat, dairy and RTE-food processing plants, fresh vegetables, cheese and fermented foods. The dataset was tested for the identification of high-quality *L. monocytogenes* metagenome-assembled genomes (MAGs) that were compared to previously sequenced isolates, providing a map of clonal complexes and predicted persistent phenotypes. The approach demonstrated the number of unexplored datasets that can be tested retrospectively using novel bioinformatics pipelines and tools and supporting the opportunity of metagenomics as a reliable screening tool for analysing entire microbial communities in environmental settings of food-producing facilities and food matrices for the identification of pathogens at the strain level using metagenome-assembled genomes.

The risk to vulnerable consumers from *Listeria monocytogenes* in ready to eat smoked fish: a qualitative risk assessment

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Abstract

Listeria monocytogenes infections can lead to invasive listeriosis in vulnerable groups, and the case fatality rate in these groups can reach 30%. Ready-to-eat foods pose the greatest risk as there is no heating step at consumption to eliminate any pathogens present. Following a recent outbreak of *L. monocytogenes* in smoked fish in the UK, risk managers requested a risk assessment to ensure advice to vulnerable consumers regarding the consumption of smoked fish was appropriate. A qualitative risk assessment was performed following the Codex Alimentarius framework. The final risk characterisation was determined using the steps of hazard characterisation and exposure assessment. Based on the evidence, the assessment concluded that the likelihood of invasive listeriosis occurring in vulnerable groups from hot-smoked fish was very low (very rare but cannot be excluded) with medium uncertainty. For cold-smoked fish, the likelihood was low (rare, but does occur) with medium uncertainty. The severity of infection with *L. monocytogenes* in vulnerable groups was high (severe illness) with low uncertainty. The uncertainties associated with the likelihood of infection were mainly due to difficulties in estimating the infective dose in vulnerable groups, variations in consumer practices and differences in smoked fish production processes affecting *L. monocytogenes* contamination.

National outbreak of *Listeria monocytogenes* associated with a frozen vegetable producer

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Abstract

Seven cases (onset 2016 – 2021, 2 deaths), geographically spread across the UK were linked with *Listeria monocytogenes* (CC20) in a 25 Single Nucleotide Polymorphisms (SNP) distance cluster.

L. monocytogenes isolates within a distance of 5 or 10 SNPs are usually investigated and in April 2022, isolates from one case and a food sample from a vegetable producer matched within 5 SNPs distance. Sequencing of isolated *L. monocytogenes* from food and environmental samples from this producer allowed the identification of isolates phylogenetically within a 25 SNPs cluster, capturing six more cases from previous years and isolates from food and environmental samples from 10 other food businesses and retailers since 2013 with links to this producer.

Several risk factors were identified: microbiological investigations revealed the large, complex cooling equipment, containing water that cooled food after blanching, to be the source of contamination spreading throughout the factory and to the food products. A programme of immediate and long-term control measures was recommended.

The vegetable producer does not produce ready-to-eat food and therefore, for many years, *Listeria* was not investigated during routine microbiological controls. Furthermore, labelling of the packaged food did not make clear that further cooking of the vegetables was required.

Heterogeneity in biofilm formation capability of *Listeria monocytogenes*' food-associated isolates

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Abstract

The capability of *Listeria monocytogenes* to form biofilm is considered to be one of the major factors contributing to the organism's persistence in the food processing environment (FPE). This study aimed to examine the genetic underpinning of biofilm-forming capacities in *L. monocytogenes* isolated from foods and food processing environments. A collection of 150 *L. monocytogenes* strains isolated from a range of food products, food-processing environments, and clinical sources were available from the Teagasc Culture Collection and were screened for their ability to form biofilm using the crystal violet method. The association of biofilm formation with phytogenic lineage, Clonal complex and serogroup at pangenome level were also investigated using whole genome sequences (WGS). Of the 150 strains evaluated, 16.67 % exhibited strong biofilm formation ($p < 0.05$), in particular isolates sources from seafood, serogroup 1/2a, 1/2b-3b-7 and Clonal Complex (CC) 101. Pan-genome-wide association analysis identified five hundred and twenty-four candidate genes that are associated with strong biofilm formation, many of which (78.05%) were of unknown function (hypothetical). Comparative analysis of the genome sequences of the isolates for a complement of genes previously shown to have a role in biofilm formation (i.e. actA, lmo0435, lmo0673, luxS, inlL, lmo2504, prfA and recO) revealed that all 150 of the isolates carried actA and recO. However, the presence of the remaining genes, namely lmo0435, lmo0673, luxS, inlL, lmo2504 and prfA, were found to be not statistically significant in the ability to form biofilm. The intricate mechanisms behind biofilm formation will be better understood with further research on the genes highlighted by pan-genomes analysis that have unknown functions.

Association of virulence, biofilm and antimicrobial resistance genes with specific clonal complex types of *Listeria monocytogenes*

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Abstract

Precise classification of foodborne pathogen *Listeria monocytogenes* is a necessity in efficient foodborne disease surveillance, outbreak detection, and source tracking throughout the food chain.
The aim was to perform comparative genomic analysis of *L. monocytogenes* isolates to determine specific genetic markers associated with presence of gene previously shown to have a role in virulence, stress tolerance, biofilm formation and antimicrobial resistance.
A total of 150 *L. monocytogenes* isolates Whole Genome Sequences (WGS) from various food products, food processing environments and clinical sources were investigated for variation in presence of specific gene and their possible link to genetic marker namely Clonal Complexes (CC), serogroups as well as phylogenetic relationships.
Pan-genome-wide association analysis by Scoary using Fisher's exacttest identified eleven genes specifically associated with clinical isolates. Screening for the presence of antimicrobial and virulence genes using the ABRicate tool uncovered variation in presence of Listeria Pathogenicity Islands (LIPI) and other known virulence genes. Specifically, the distribution of actA, ecbA, inlF, inlJ, lapB, LIPI-3 and vip, genes across isolates were found to be significantly CC dependent while the presence of ami, inlF, inlJ, LIPI-3 was associated with clinical isolates specifically. In addition, Roary-derived phylogenetic grouping based on Antimicrobial Resistant Genes (AMRs) revealed that thiol transferase (FosX) genes were presence in all of lineage I isolates, presence of lincomycin resistance ABC-F type ribosomal protection protein(lmo0919_fam) were also genetic lineage dependent. More importantly, the genes found to be specific to CC-type were consistent when a validation analysis was performed with fully assembled, high quality complete *L. monocytogenes* genome sequences (n = 247) extracted from the National Center for Biotechnology Information (NCBI) microbial genomes database.
This work highlights the usefulness of MLST based CC typing using WGS as a tool in classifying isolates.

Modelling transfer of *Listeria monocytogenes* from polymicrobial biofilms to smoked salmon

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Abstract

As a foodborne pathogen, *Listeria monocytogenes* raises concern to food producers worldwide. Along with its ability to succeed in harsh environments, it can co-live with other bacteria in polymicrobial biofilms that cause cross-contaminations events. Paradoxically, little is known about the transfer of *L. monocytogenes* from polymicrobial biofilms to food products. The present work aims to model transfer events from two *L. monocytogenes*-positive contamination foci – L96 and L168 – found on surfaces from the seafood and meat industry, respectively.
Polymicrobial biofilms were grown in low-nutrient medium under static conditions for 72 h. To simulate successive contaminations events, up to 25 contacts (with 20 replicates each) were made between each biofilm and smoked salmon slices. Bacterial transfer data were expressed as transfer rates. Kruskal-Wallis and Dunn's test grouped contacts in four groups significantly different ($p = 0.05$). The density distributions of these groups were displayed and fitted to Weibull, Normal and Gamma models. The outcomes give insight about the transfer ability of mixed biofilms containing *L. monocytogenes*, which can help quantify the risk of contamination in the food industry.