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

14F845 – Application of Novel food processing and microanalytical technologies to Identify and Control Spores, in Dried Food Ingredients, and of Biofilms in Food Processing Environments – a Systems microbiology approach to ensuring Quality and Safety

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

This project, to develop bacillus decontamination strategies for dried food ingredients, is split into two key areas- understanding the microbiome of dried food ingredients and developing control strategies for bacillus species, their sores and biofilms. After developing a strong sampling plan task 2 provided the first description of the bacterial composition of a baked goods production site, using next-generation sequencing (NGS), and demonstrated how this community of bacteria is influenced by climatic conditions. Task 4 determined the phenotypic and genotypic characterisation of > 500 isolates from an Irish artisan bakery environment using conventional microbiology techniques (gram staining, motility tests, growth profiles, and biochemical analysis), matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS), antimicrobial susceptibility, biofilm-forming assays, and whole genome sequencing (WGS). Interestingly, all the Bacillus isolates demonstrated multidrug resistance including resistance to the drugs of last resort (vancomycin, carbapenems, and 3rd generation cephalosporins. In terms of biofilm formation, biomass production was high in 24% of isolates when grown under stressed conditions at 45 °C up to 48 h. The isolates displayed the ability to form biofilms in a range of growth conditions, with persistence and dispersal capabilities. Genotypic characterisation indicated that the isolates represent a diverse repertoire of antimicrobial resistance and biofilm-forming determinants relevant to food safety. Using type-strains and isolates from the bakery environments, new technologies were optimised to inactivate bacillus spores and vegetative cells. These technologies included Plasma decontamination (task 5), UV-LED (task 6) and acoustic decontamination (task 7). Working collaboratively with project leads, A microbial risk heat map was prepared for each bakery environment using Mathematical modelling (task 8) and a multi-hurdle bacillus decontamination system using plasma, UV and ultrasound technologies was developed. Outputs included research papers, workshops, 4 PhD Thesis, 1 MENGSc Thesis and 14 published Scientific papers.

KEYWORDS

Food Safety, Novel Technology, Spore Decontamination

ACRONYM	NOSPORES-DFI
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Section 1 - Research Approach & Results

Start Date

01 May 2015

End Date

28 February 2021

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 4: Technology validated in lab

NRPE Priority area

Processing technologies and novel materials

Total DAFM Award

€879,348.32

Total Project Expenditure

€816,217.53

Rationale for undertaking the Research

Dried Food Ingredients (DFI) are widely used in domestic, culinary, and commercial ready-to-eat product contexts globally. This product category is often contaminated with spore formers, mould, and some enteric pathogens. Control of these bacteria in DFI, and biofilms on food processing surfaces is a considerable challenge to the food industry. This project sought to determine, the baseline microbiome of two baked products production sites, including key DFI. Bacterial phyla, which will be described, using dedicated sampling plans including temporal and geographical comparisons. Microbial population sorting using conventional techniques and metagenomic analysis was used to provide deep insights into the population-level dynamics of this environment. Developing novel food processing technologies is a key development to provide new sanitation and HACCP control steps for spore control. Using Risk Analysis to interrogate data streams will allow the industry to predict high and moderate-risk process areas/ ingredients and create improved strategies for microbiological control in the production environment. Because such in depth assessment of the baked goods industry has not been undertaken here-to-fore in Ireland, the research outputs will have a significant impact on the food safety improvements of DFI and consequent brand protection.

Methodology

This project, to develop bacillus decontamination strategies for dried food ingredients, is split into two key areas- understanding the microbiome of dried food ingredients and developing control strategies for bacillus species, their spores, and biofilms.

Microbiome characterisation was undertaken using Next Generation Sequencing of 16sRNA. Classical culture-based characterisation was run in parallel to obtain environmental isolates so that we could determine the nature of the viable microbes in the baking environment, this was done using traditional selective enrichment, Gram-Staining, and API classification, as well as FDA approved MALDI-Vitek identification.

Bacillus species isolated from this research were used to optimise the operating parameters of 5 novel technologies - Acoustic ultrasound, UV LED light, OZONE, and Cold Plasma. Once the individual processes were

optimized for the selected products e.g. Black Pepper, and for bacillus biofilm inactivation, Work began to develop a multi-hurdle approach using the most promising technologies together to give an effective decontamination protocol.

Project Results

This project, to develop bacillus decontamination strategies for dried food ingredients, is split into two key areas- understanding the microbiome of dried food ingredients and developing control strategies for bacillus species, their sores and biofilms. After developing a strong sampling plan task 2 provided the first description of the bacterial composition of a baked goods production site, using next-generation sequencing (NGS), and demonstrated how this community of bacteria is influenced by climatic conditions. Task 4 determined the phenotypic and genotypic characterisation of > 500 isolates from an Irish artisan bakery environment using conventional microbiology techniques (gram staining, motility tests, growth profiles, and biochemical analysis), matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS), antimicrobial susceptibility, biofilm-forming assays, and whole genome sequencing (WGS). Interestingly, all the Bacillus isolates demonstrated multidrug resistance including resistance to the drugs of last resort (vancomycin, carbapenems, and 3rd generation cephalosporins. In terms of biofilm formation, biomass production was high in 24% of isolates when grown under stressed conditions at 45 ºC up to 48 h. The isolates displayed the ability to form biofilms in a range of growth conditions, with persistence and dispersal capabilities. Genotypic characterisation indicated that the isolates represent a diverse repertoire of antimicrobial resistance and biofilm-forming determinants relevant to food safety. Using type-strains and isolates from the bakery environments, new technologies were optimised to inactivate bacillus spores and vegetative cells. These technologies included Plasma decontamination (task 5), UV-LED (task 6) and acoustic decontamination (Task 7). Working collaboratively with project leads, A microbial risk heat map was prepared for each bakery environment using Mathematical modelling (Task 8) and a multi-hurdle bacillus decontamination system using plasma, UV and ultrasound technologies was developed. Outputs included research papers, workshops, 4 PhD Thesis, 1 MENGSc Thesis and 14 published Scientific papers.

Section 2 - Research Outputs

Summary of Project Findings

This research has been innovative as it has identified and characterised the key microbial species, particularly Bacillus Sp., that are commonly found in the baking environment and the dried ingredients used therein. This information has enabled us to tailor the novel technologies to best inactivate their biofilms on the processing surfaces, and the vegetative cells and spores that can contaminate dried ingredients causing quality and safety issues in downstream processing and consumer use. The microbial inactivation data for the new technologies can be used by the scientific community, industry, and policy makers to approve parameter limits for these technologies in food processing with a view to ensuring product quality and consumer safety going forward.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
MSc Students	0	1	1
PhD Students	1	3	4
Post Doctorates	0	1	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	14	1. Tiwari B.K., (2015) Ultrasound: A clean, green extraction technology. TrAC Trends in Analytical Chemistry, Vol 71: 100-109, https://doi.org/10.1016/j.trac.2015.04.013. 2. Charoux, C.G.M., Ojha, K.S., O'Donnell, C.P., Cardoni, A., Brijesh K. Tiwari, B.K. (2017) Applications of airborne ultrasonic technology in the food industry. Journal of Food Engineering, 208, 28-36, https://doi.org/10.1016/j.jfoodeng.2017.03.030. 3. Charoux, CMG., Tiwari, BK and O'Donnell, CP (2017) Ultrasound processing and food quality. IN: Ultrasound: Advances for Food Processing and Preservation, Daniela Bermudez-Aguirre (ED) Elsevier Publishing Ltd. eBook ISBN: 9780128046142. 4. Pérez-Andrés, J. M., Charoux, C. M., Cullen, P., & Tiwari, B. K. (2018) Chemical Modifications of Lipids and Proteins by Nonthermal Food Processing Technologies. Journal of agricultural and food chemistry, 66(20), 5041-5054. 5. Charoux C.M.G., Inguglia E.S., O'Donnell C.P., Tiwari B.K. (2019) Ultrasonic Waves: Inactivation of Foodborne Microorganisms Using Power Ultrasound. IN: Reference Module in Food Science https://doi.org/10.1016/B978-0-08-100596-5.22930-2. 6. Charoux, C., Hinds, L, Free, L., Vijayaraghavan, R.K. (2019) Effect of non-thermal plasma technology on microbial inactivation and total phenolic content of model liquid food and black pepper grains. Lebensmittle-Wissenshaft und Technologie 118(1-4) 108716; DOI:10.1016/j.lwt.2019.108716. 7. Gaurav R, G., Priyadarshini, A., O'Donnell, C., Tiwari B.K., (2019) Emerging food processing technologies and factors impacting their industrial adoption, Critical Reviews in Food Science and Nutrition, 59: 3082-3101. doi:10.1080/10408398.2018.1483890 8. Kulawik, P., Tiwari, B.K., (2019) Recent advancements in the application of non-thermal plasma technology for the seafood industry. Critical reviews in food science and nutrition, 59:3199-3210. doi:10.1080/10408398.2018.1510827. 9. Hinds, L., Akhter, M., O'Donnell, C., Tiwari. B.K. (2019) Principles and mechanisms of ultraviolet light emitting diode technology for food industry applications. Innovative Food Science & Emerging Technologies 56: 102153. https://doi.org/10.1016/j.ifset.2019.04.006. 10. Charoux, C. M. G., Patange, A. D., Hinds, L. M.. Simpson, J.E.C., O'Donnell, C.P., Tiwari, B.K. (2020) Antimicrobial effects of airborne acoustic ultrasound and plasma activated water from cold and thermal plasma systems on biofilms. Nature Research, Scientific Reports 2020:10, 17297, https://doi.org/10.1038/s41598-020-74504-z.

		11. Hinds, L., Charoux, C., Akhter, M., O'Donnell, C., Tiwari. B.K. (2020) Effectiveness of a novel UV light emitting diode based technology for the microbial inactivation of Bacillus subtilis in model food systems. Food Control 114(Supplement C):106910 http://dx.doi.org/10.1016/j.foodcont.2019.106910. 12. Hinds, L.M., Gucluc, G., Kelebek, H., Sellice, S., 0'Donnell C.P.,Tiwari B.K. (2021) Effect of ultraviolet light emitting diode treatments on microbial load, phenolic and volatile profile of black peppercorns. 2021: LWT,152: 112-133 https://doi.org/10.1016/j.lwt.2021.112133. 13. Lamba, S., Mundanda Muthappa, D., Fanning, S., Scannell, A.G.M. (2022) Sporulation and Biofilms as Survival Mechanisms of Bacillus Species in Low-Moisture Food Production Environments. Foodborne Pathogens and Disease; 19:7, 448-462. https://doi.org/10.1089/fpd.2022.0006 14. Mundanda Muthappa, D., Lamba, S., Sivasankaran, S.K., 15. Naithani, A., Rogers, N., Srikumar, S., Macori, G., Scannell, 16. A.G.M., Fanning, S. (2022)16S rRNA Based Profiling of Bacterial Communities Colonizing Bakery-Production Environments. Foodborne Pathogens and Disease, 19: 7, 485-494. https://doi.org /10.1089/fpd.2022.0014. 17. Hinds, L., O'Donnell, C.P, Tiwari, B.K. (2022) Investigating the Effect of Selected Ultraviolet Light Emitting Diode Treatments on the Inactivation of B. subtilis, the Pasting Properties and the Surface Morphology of Tapioca Starch. Starch 2022 https://doiorg.ucd.idm.oclc.org/10.1002/star.20210021
Peer Reviewed Conference Papers	11	1. Charoux, C.M.G., Ojha, K.S., Tiwari, B.K. and O'Donnell, C.P. (2016) Investigation of the effects of airborne acoustic ultrasound on vegetative cells and spores. 2nd international conference on ultrasonic-based applications, Lisbon, Portugal (6 – 8 June 2016). 2. Charoux, C.M.G., Ojha, K.S., Tiwari, B.K. and O'Donnell, C.P (2016) Investigation of the effects of airborne acoustic ultrasound on vegetative cells and spores. Ultrasonics. IUFoST international conference, 2016, Dublin Ireland, (21 – 25 August 2016). 3. Charoux, C.M.G., Tiwari, B.K. and O'Donnell, C.P. (2016) Ultrasound technology for food processing applications. AFSTI Conference 2016, Amristar, India (10-12 November 2016). 4. Tiwari, M., O'Donnell, C.P., Scannell, A.G.M. (2016) Comparative study on microbial ecology of a new and mature Irish Sourdough. 25th International ICFMH conference on Food micro 19th-22nd July 2016, University College Dublin Ireland. 5. Free, L; Charoux; C; Singh Gonga, G; Kelly, S; Tiwari, B.K; Daniels, S. (2016) Atmospheric Pressure Plasma as a non-Thermal Tool for Decontaminating Surfaces in the Food Industry. IUFoST international conference, 2016, Dublin Ireland, (21 – 25 August 2016).

		6. Tiwari, M., Scannell, A.G.M. and O'Donnell, C.P. (2016) Effect of ozone in combination with cleaning in place reagents for controlling sessile and planktonic biofilms in dried food process environment. 10th International conference on BIOFILMS REACTORS 2017 University College Dublin, Ireland 9-12th May 2017. 7. Lamba, S., Dechamma M.M., Fanning, S., Scannell, A.G.M. (2017) Seasonal & Geographical Comparison of Microbial Ecology in two Irish Bakeries. 46th Annual Food Science and Technology Conference, Teagasc, Dublin. 6-7 Dec 2017. 8. Charoux, C.M.G., Ojha, K.S., Tiwari, B.K. and O'Donnell, C.P. (2017) Investigation of the effect of a non-thermal plasma jet system on dried food. 46th Annual Food Science and Technology Conference, Teagasc, Dublin. 6-7 Dec 2017. 9. Lamba, S., Dechamma M.M., Fanning, S., Scannell, A.G.M. (2018) Development of a Predictive Growth Model for Seasonal and Geographical Comparison of Microbial Ecology in two Irish Bakeries. FoodMicro2018 Berlin, Germany 3-6 Sep 2018. 10. Lamba, S., Dechamma M.M., Fanning, S., Scannell, A.G.M. (2019) Phenotypic characterization of biofilm-forming Bacillus species identified in the Irish artisan bakery environment. International Association for Food Protection Annual Meeting, Louisville, Kentucky, USA. 21-24 Jul 2019. 11. Lamba, S. (2022). Sporulation and biofilms as survival mechanisms of Bacillus species in low moisture food production environments. In International Association for Food Protection Annual Meeting 2022, 31 Jul - 3 Aug 2022, USA. (Oral Presentation, Symposium organiser, IAFP Speaker Travel Award)
PhD Theses	4	1. Charoux, Clémentine M.G. (2020) Airborne acoustic ultrasound and plasma technologies for decontamination of dried food ingredients and biofilm disruption. PhD thesis UCD, Dublin. 2. Hinds, Laura (2021) Ultraviolit light emiting diode technology for food processing and surface decontamination applications. PhD Thesis, UCD, Dublin. 3. Free, Louis (2022) Reactive oxygen and nitrogen species detection produced by an atmospheric pressure plasma jet. PhD thesis, Dublin City University. 4. Lamba, Sakshi (2023) Exploring the microbial ecology of Irish artisan bakery environments with a focus on the biofilm formation and resistance profiles of Bacillus. PhD thesis, UCD, Dublin.
Masters Theses	1	1. Tiwari, M. (2018) Inactivation of bacterial spores and Biofilms of Industrial Relevance using Ozone and Hurdle Technology. MEngSc Thesis, UCD, Dublin.

Intellectual Property

N/A at this time.

Summary of other Project Outputs

Project Outputs	Details	Total Number
New Processes	Two Novel processes for microbial decontamination: 1. Using LED pulsed light and Airborne acoustics to decontaminate dried food ingredients. 2. Plasmadised water and OZONE to disrupt biofilms on food surfaces.	2
New Industry Collaborations Developed	This Project has allowed us to build research collaborations with 3 SMEs: 2 Artisan Bakery Companies – Walshes Bakehouse and Arbutus Bread 1 Ingredients Company – All in All Ingredients, These companies are also happy to collaborate on future projects	3

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	The development of optimal process parameters is fully documented in the 5 theses published. These data are available to policymakers and to industry to assist the production of industry guidelines to these novel decontamination strategies in real-world applications, and will inform HACCP CCP monitoring and compliance criteria.

Dissemination Activities

Activity	Details
Workshops at which results were presented	NOSPORES-DFI has successfully disseminated its research findings through the website, published peer review articles (n=14), and Industry workshops (n=2). The importance of effective decontamination of dry food ingredients was highlighted and technology transfer from the project to Industry was facilitated through one-to-one meetings and demonstrations of the technologies at the workshops.
Other	Project results were disseminated to the Scientific Community and Policy Makers at 12 international conferences.
Other	Stakeholder Consultations: During the project we undertook 10 individual stakeholder interactions at both Industry Workshops, and via zoom. While these were confidential troubleshooting queries, we were able to give practical solutions on Spore decontamination in the relevant facilities based on the results we found in this project.

No response	School Visits: We were invited to give three Senior cycle workshops in a Dublin 8 Secondary School. At these events we discussed microbiology in general, the importance of Spores to food Quality, and methods to inactivate spores, including biopreservation and novel technologies.

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	This project generated new data on how key novel technologies can be used singly or as part of a multi-system decontamination strategy to control Bacillus spp. And their spores in Dried Food Ingredients. The microbiome of the bakery environment and the dried food ingredients used therein were characterized. A risk assessment of the two bakery sites was developed showing heat maps of the most vulnerable points for bacillus contamination.
Identify any knowledge transfer activities executed within the project.	Within this project we worked closely with our industry partners on the project. We had regular informal project updates during which we communicated the microbial results of the microbiome research. This allowed us to share specific sites, processes and ingredients that may impact the quality and safety of their baked products. We also ran two industry workshops during which we disseminated the progress made in developing novel decontaminating processes to our partners, but also to other food industry stakeholders. While not a dried food environment, we also disseminated the potential for use of plasma, ozone, and LED technologies to the Campylobacter Stakeholder Group meeting which is chaired by Prof. Pat Wall and whose membership includes poultry processors, large supermarket chains, research institutes and policymakers. Additionally, we had individual telephone queries from food Industry contact during which we explored how novel technologies may be applied to a range of food production processing environments.
List any impacts resulting from the knowledge transferred during the project.	The key tangible impact of the knowledge transferred during the project was the implementation of a significant change in our Industry partners' Sanitation and Safety practices. This resulted in real-world benefits for the companies by reducing the potential of cross-contamination between ingredients and baked goods and reducing the microbial load in key process sures by improving the cleaning processes.

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€1,228450	17F275 Controlling Campylobacter Contamination in Poultry Processing - CAMPYDECON

Future Strategies

This consortium is preparing to submit further research proposals for National and EU funding to further develop the decontamination technology to pilot scale and commercial scale-up. This work in ongoing.

Project Publications

1. Tiwari B.K., (2015) Ultrasound: A clean, green extraction technology. TrAC Trends in Analytical Chemistry, Vol 71: 100-109, https://doi.org/10.1016/j.trac.2015.04.013.
2. Charoux, C.G.M., Ojha, K.S., O'Donnell, C.P., Cardoni, A., Brijesh K. Tiwari, B.K. (2017) Applications of airborne ultrasonic technology in the food industry. Journal of Food Engineering, 208, 28-36, https://doi.org/10.1016/j.jfoodeng.2017.03.030.
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