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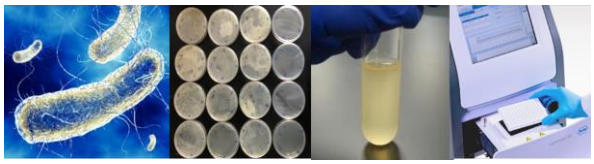
A whole chain approach to the control of bacteria in powdered dairy ingredients, through the development of rapid diagnostic protocols to support food quality & safety challenges in dairy food production; PCR and rapid methods.



Key external stakeholders:

DPTC Dairy Industries, Dairy Industries, Food manufactures, Food researchers

Practical implications for stakeholders:

The outcome/technology or information/recommendation is……

This research was carried out as part of a large national network project, Dairy Processing Technology Centre which is an industry–academic collaborative focused on the technology and information transfer to the main Irish dairy industries. Research by Teagasc in this project focused on assessed PCR capabilities in dairy powders to test for pathogens and spores to improve prevention and controll of microbial safety.

- PCR detection methods optimised for Listeria, Cronobacter, *E. coli,* presumptive *Bacillus cereus* and toxin methods were adapted for dairy products tested.
- Live/dead cell differentiation for Cronobacteria in PCR is verified.
- DNA extraction methods optimised for Listeria, Cronobacter, *E. coli*, *Bacillus cereus* and *Bacillus licheniformis* from dairy powders using both automated and manual methods are valid.
- A survey on spores in skim milk powders found a high prevalence of Bacillus and more specifically *B. licheniformis* that can be used as good indicator organism of spores in skim milk powders.
- PCR method to enumerate *Bacillus licheniformis* in dairy products is feasible
- The potential for growth *of L. monocytogenes* in a new dairy product was determined using the EU challenge test Protocol

Main results:

- PCR methods for Listeria, Cronobacter, *E. coli,* presumptive *Bacillus cereus* and toxin, *Bacillus licheniformis* methods were optimised, compared to standard methods and transferred to Industry.
- Determination of *Listeria Monocytogenes* Numbers at Less than 10 cfu/g is possible using current standard methodology.
- A high prevalence of Bacillus and more specifically *B. licheniformis* was identified as a good indicator organism of spores in skim milk powders.
- Live/dead cell differential is possible using PCR for Cronobacter
- PCR knowledge transfer and training was delivered to Industry and private labs for industry use.

Opportunity / Benefit:

The opportunity for method improvement and PCR capabilities has been demonstrated on testing of dairy powders to improve the prevention and control of safety microorganisms. The study showed that there were methods suitable for each pathogen and spore tested.

Collaborating Institutions:

UL, UCD

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External collaborators: Prof. Francis Butler UCD, Professor Martin Wilkinson (UL)

1. Project background:

Food Safety testing of products is a necessary task of dairy industries, from regulatory, safety and plant hygiene points of view. Completion of product pathogen and spore tests is a bottleneck for industries, as they wait on lengthy standard agar plate-based assays to release their product. Furthermore, if contamination issues arise, identification of sources of contamination and monitoring of production environments is dependent on these agar methods, potentially leading to extended production downtime. Molecular methods offer not only shorter assay times but also higher throughputs generating multi-parametric data on the bacterial species present, viability and vitality, within a single assay. However, these methods are underdeveloped and currently not in use for routine pathogen detection in the industry. The aim of this project is to assess molecular (including whole genome sequencing) for specific pathogens, including spore-forming bacteria, associated with dairy products, facilitating their use by industry, thereby leading to more rapid results.

To research and apply already developed rapid and molecular-based methodologies methods to pathogen detection, including sporeformers, including a process control approach to reduction of pathogens in dairy products and troubleshooting contamination issues. The main objective was to enhance the reputation of the Irish dairy industry by improving rapid and developed molecular-based methodologies to pathogen and spore detection approach to reduction and control of pathogens in dairy products. Molecular-based methods (and some culture-based methods) developed in other projects was first transferred for use in the dairy industry, additional methods were tested and transferred. These methods can be used for determination of pathogens and spore forming bacteria in dairy products.

2. Questions addressed by the project:

- Can *Listeria monocytogenes* grow in new dairy products that contain inhibitory ingredients?
- Is live/dead cell differential possible for Cronobacter using PCR?
- What are the most suitable spores for detection in dairy products?

3. The experimental studies:

- The potential for growth *of L. monocytogenes* in a new dairy product was determined using the EU challenge test Protocol with modification to increase sensitivity.
- A live dead study for Cronobacter using ISO 22964:2017 method, along with PCR detection, to determine the sensitivity and interference from dead cells using the iQ-Check® *Cronobacter* spp. kit and the Biotecon Diagnostic Cronobacter Detection LyoKit. The Biotecon kit was used with manual and automated DNA extraction methods.
- A survey from industry partners of milk powders to identity the most common spore-forming bacteria was carried out using classical methods, 16S and whole genome sequencing for identification.

4. Main results:

- Using the pour-plate method in a 140 mm Petri dish, 10 mL of a 1:10 dilution of food allowed determination of numbers as low as 1 cfu/g. Applying this method, *L. monocytogenes* in naturally contaminated food samples were enumerated at numbers as low as 1-9 cfu/g. This method was applied to challenge testing of new dairy products that contained inhibitory ingredients for industry.
- Live dead study for Cronobacter showed PCR kits resulted in positive detection at log 4, 5 and 6. Seven skim milk powders from different dairy manufacturers were tested resulting in the manual DNA extraction method at log 6 cfu/ml, all of the powders resulted in positive detection, while 6 of the powders resulted in positive detection at log 5 cfu/ml. Using the automated Roche MagNA Pure Compact System all 7 powders resulted in positive detection at log 5. When log 4 cfu/ml dead cells were added to the same matrix as used for live cell detection, no cells were detected by PCR in both methods. When log 5 cfu/ml dead cells were added, there was detection. In summary both kits tested were suitable for detection in skim milk samples and there will be no interference from dead cells if the numbers are < log 4 cfu/ml.
- A survey from industry partners of milk powders identified the most common spore-forming bacteria as *Bacillus licheniformis* (representing 80% of the 300 isolates obtained) in 7 of the 8 industries.

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5. Opportunity/Benefit:

The opportunity for method improvement and PCR capabilities has been demonstrated on testing of dairy powders to improve the prevention and control of safety microorganisms. The study showed that there were methods suitable for each pathogen and spore tested.

6. Dissemination:

From this project there were 7 peer review publications, 11 conference papers,5 workshops,37 methods,198 reports to Industry,31 presentations and 3 extension projects

Main publications:

Hunt, K., M. Vacelet, and Kieran Jordan. 2017. "Determination of *Listeria Monocytogenes* Numbers at Less than 10 Cfu/g." Irish Journal of Agricultural and Food Research 56 (1). Teagasc: 25–30. doi:10.1515/ijafr-2017-0004.

Hunt, Karen, Marjorie Blanc, Avelino Álvarez-Ordóñez, and Kieran Jordan. 2018. "Challenge Studies to Determine the Ability of Foods to Support the Growth of Listeria Monocytogenes." Pathogens 7 (4). MDPI AG. Doi: 10.3390/pathogens7040080.

Li, Fang, K. Hunt, Koenraad Van Hoorde, Francis Butler, Kieran Jordan, and John T. Tobin. 2019. "Occurrence and Identification of Spore-Forming Bacteria in Skim-Milk Powders." International Dairy Journal 97 (October). Elsevier Ltd: 176–84. doi:10.1016/j.idairyj.2019.05.004.

Popular publications:

DPTC Knowledge day "A Whole-Chain Approach to the Control of Bacteria in the Dairy Chain" Poster presentation Francis Butler, Lynda Gunn, Karen Hunt, Kieran Jordan, Richard O'Kennedy, Elaine O'Meara, Niamh Phillips, Martin Wilkinson (2/3/2017).

Cronobacter sakazakii ISO 22964:2017 Testing of Milk Powders Using Commercially Available PCR", Karen Hunt, Kieran N Jordan and Charlène Legeay (21/07/2019)

7. Compiled by: Triona O'Brien and Geraldine Duffy

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