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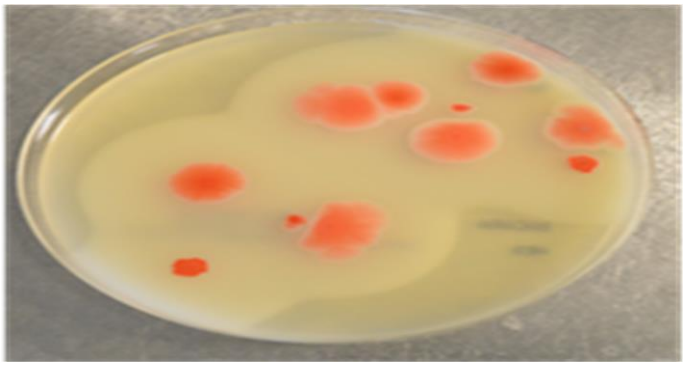
Thermodur-Out:

Eliminating thermodurics to improve the quality of powdered dairy ingredients



Key external stakeholders:

The key stakeholders are the Irish dairy powder processors, food processors that utilise powdered dairy ingredients, Irish Dairy Board, Department of Agriculture, Irish regulatory bodies, researchers within the scientific community and all dairy and dairy derivative consumers.

Practical implications for stakeholders:

The contamination of powdered dairy ingredients with thermoduric (heat resistant) spore-forming bacteria is a major quality issue for the Irish Dairy industry. These bacteria survive pasteurisation, have the ability to form difficult-to-remove layers (biofilms) within pipes and equipment, often resulting in powdered ingredients failing to meet customer requirements and can have serious implications with respect to subsequent processes involving these ingredients. As a consequence of the removal of milk quotas, powdered dairy ingredients are generated on an increasingly greater scale and it is essential that this issue be addressed to ensure that the quality of Irish powdered dairy ingredients is not compromised. It is our hypothesis that the identification of the spore-forming bacteria which are most common in Irish milk processing plants, and in associated powdered ingredients, will enable us to identify the processes which are best suited to preventing the formation of biofilms of spore-forming bacteria and subsequent spore outgrowth.

Main results:

The detection of pathogenic *Bacillus cereus* strains is of utmost importance in the dairy industry to prevent emetic and diarrhoeal syndromes. Even though the presence of *B. cereus* at their infective doses can be detected using chromogenic media (e.g. BACARA™), which are adequately reliable and sensitive, differentiation between the toxins is not possible without using a molecular method. Culture independent methods such as real-time PCR are more beneficial as, compared to culturing techniques, they are more rapid, facilitate higher throughput and allow for the differentiation between toxic and atoxic strains of *B. cereus* strains. Chromogenic media are not able to differentiate toxic from atoxic strains or detect all *B. cereus* strains. None of the commercially available kits, either immunological or PCR-based, are able to detect the four toxins simultaneously. Hence, development of a multiplex assay based on fluorogenic probes potentially allows for reliable and sensitive detection as well as differentiation of each toxin, to at least its infective level. In the dairy industry, this assay can serve as a tool to specifically detect the presence of toxic *B. cereus* strains in milk and milk products. Timely detection of the pathogen will result in adequate control measures being applied, hence reducing spoilage and foodborne illnesses, which in turn will reduce economic losses and increase product safety.

Opportunity / Benefit:

Food: Assure and improve the microbial quality and safety status of Irish dairy products.
Food industry: Control over thermoduric *Bacillus* and other heat resistant spore forming bacteria in the food chain will result in less waste and net losses to the key stakeholders.
Food Industry Development: To provide Technology Development support for food SMEs and start up food businesses.

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Collaborating Institutions:

Teagasc (Moorepark)

Teagasc project team: Prof. Paul Cotter
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External collaborators:

1. Project background:

As a consequence of its highly nutritious nature, milk can quickly become contaminated *via* different sources. One of the most common contaminants is highly heat-resistant spores of spore-forming bacteria. These are a particular cause for concern as they are able to survive industrial pasteurisation. Thus, they pose a major quality issue for dairy companies. Species such as *Bacillus* and other related genera are known to cause spoilage through proteolytic or lipolytic activities and, therefore, timely detection and appropriate control measures are essential to prevent major economic losses. Of further concern is the fact that *B. cereus* is one of the most common pathogenic aerobic spore-forming bacteria isolated from milk and is known to cause emetic and diarrhoeal syndromes in humans. As it is an important cause of food poisoning, detection of the pathogen is vital before it reaches consumers. *B. cereus* can be detected using culture-dependent or culture-independent assays and a selection of methods are assessed in this study.

2. Questions addressed by the project:

- Which culture medium is optimal for the detection of bacillus cereus
- How the traditional culture dependent methods compares to culture independent methods in the identification of *B. cereus* in milk powders.
- Can real-time PCR methods be used to accurately detect the presence of toxins secreted by *B. cereus.*

3. The experimental studies:

- Culture of typed *B. cereus* strains and also milk powders spiked with *B. cereus* on three different commercially available media and their subsequent comparison to determine which performs optimally.
- Evaluation of the commercially available Duopath® cereus enterotoxins kit to investigate rapidity and sensitivity in the detection of *B cereus*
- Appraisal of a PCR based method to detect the *groEL* gene
- Development of a novel RT-PCR multiplex assay to detect *B. cereus* toxins from spiked milk samples

4. Main results:

The real- time PCR method accurately and sensitively detects the presence of each of the four toxins produced by *B cereus*. For this novel multiplex assay, specific primer pairs and probes were designed to target the four toxin gene components, i.e. *hblD*, *nheA*, *ces* and *cytK2*, present in potentially pathogenic *B. cereus* strains. The assay was trialled with both known toxin positive *B. cereus* strains as well as artificially contaminated milk powder samples, and proved both sensitive and quantitative with detection limits of 104 CFU/ml.

The development of a multiplex assay which enables the dairy industry to rapidly detect the presence of thermoduric spore formers in their products is a huge leap forward for the industry.

5. Opportunity/Benefit:

The rapid detection of the presence of thermoduric, spore forming bacteria poses an enormous benefit to the

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whole dairy industry, reducing the presence of spoilage organisms and also enabling identification of the processes which are best suited to preventing the initial formation of biofilms of spore-forming bacteria and subsequent spore outgrowth.

6. Dissemination:

Main publications:

The Prevalence and Control of Bacillus and Related Spore-Forming Bacteria in the Dairy Industry.
Gopal N, Hill C, Ross PR, Beresford TP, Fenelon MA, Cotter PD. Front Microbiol. (2015) Dec 21;6:1418

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