Project number: 6641
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New weapons to fight old enemies (novel strategies for the biocontrol of bacterial biofilms in the food industry)

Key external stakeholders:

Dairy industry, milk producers and producers of milk-derived products, dairy farmers and food industry in general.

Practical implications for stakeholders:

Functional metagenomic studies in conjunction with classical microbiology tools allow for the identification and development of novel strategies to ensure effective cleaning in order to prevent biofilm formation in food and dairy industries. In this way, the issue of bacterial persistence in industry environments will be better addressed by combined treatments using inhibitors of biofilm formation in conjunction with the cleaning protocols already in place. Milk and milk-derived products producers and food producers will benefit by using new treatments to eliminate biofilms in industrial environments and consequently increasing the safety and quality of their products

Main results:

Biofilms consist of different types of bacteria and other microorganisms that form layers on/in food production and processing equipment and which can cause food contamination and spoilage. In the dairy and food industry standard cleaning may not get rid of the biofilms and hence new strategies are needed to ensure effective cleaning. The method developed here is to stop quorum sensing, which is one way the microorganisms interact to make more effective biofilms. In this project, cutting-edge molecular methodologies are used in combination with classical microbiology tools to identify novel compounds or beneficial microorganisms that inhibit quorum sensing among various undesirable microorganisms of concern for the dairy industry.

Opportunity / Benefit:

Dairy: Increase the quality, safety and competitiveness of Irish milk industrial environments
Food: Assure and improve the microbial quality and safety status of Irish food
Food Industry Development: To provide Technology Development support for food SMEs and start up food businesses in the Transfer of Research Knowledge Transfer Technologies

Collaborating Institutions:

Teagasc and UCC

Teagasc project team: Dr. Avelino Álvarez-Ordóñez, Laura Coughlan, Prof. Paul Cotter

External collaborators: Prof. Colin Hill, UCC

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1. Project background:

Biofilms are microbial communities characterized by their adhesion to solid surfaces and the production of a matrix of exopolymeric substances, which surround the microorganisms lending structural integrity and a unique biochemical profile to the biofilm. Pathogenic and spoilage bacterial species capable of forming biofilms are a significant problem for food and dairy industries, as their biofilm-forming ability protects them from common cleaning processes and allows them to remain in the environment post-sanitation. In the food and dairy industries, persistent bacteria colonize the inside of mixing tanks, vats and tubing, compromising food safety and quality. Strategies to overcome bacterial persistence through inhibition of biofilm formation or removal of mature biofilms are therefore necessary. Current biofilm control strategies employed in the food industry (cleaning and disinfection, material selection and surface preconditioning, plasma treatment, ultrasonication, etc.), although effective to a certain point, fall short of biofilm control. The application of an strategy focused on targeting molecular determinants regulating biofilm formation to the food and dairy industries would greatly aid efforts to eradicate undesirable bacteria from food processing environments and, ultimately, from food products. These approaches, in contrast to bactericidal approaches, exert less selective pressure which in turn would reduce the likelihood of resistance development. Targeting quorum sensing systems, which regulate gene expression in response to fluctuations in cell-population density governing essential cellular processes including biofilm formation, is a particularly interesting strategy. In this project, functional metagenomic studies in conjunction with classical microbiology tools allowed for the identification and development of novel strategies to prevent biofilm formation through the inhibition of quorum sensing.

2. Questions addressed by the project:

- Could the application of molecular approaches in combination with classical microbiology methods be applied to identify novel compounds or microorganisms to inhibit biofilm formation?

- In order to remove biofilms in food and dairy industries is it possible to develop a new strategy to improve cleaning methods from the industrial environment?

3. The experimental studies:

Construction of metagenomic libraries: a fosmid metagenomic library of ~20,000 clones from total metagenomic DNA isolated from the samples was constructed

High throughput screenings for the identification of isolates/clones with quorum sensing inhibitory activity have been optimized

Luciferase tagged strains of *Cronobacter sakazakii*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* are readily available at the Teagasc culture collection to use them in biofilm formation inhibition screenings.

A crystal violet screening assay to measure biofilm formation ability by bacterial strains has been optimized and used to characterize a collection of strains with the aim of identifying strong biofilm formers

4. Main results:

The colonization of industrial environments by spoilage and pathogenic bacteria forming complex communities of cells (i.e., biofilms) may constitute a continuous source of contamination to foods coming in contact with them. Conventional cleaning and disinfection protocols are not effective to remove biofilms and therefore alternative methodologies are needed. This project is used cutting-edge molecular methodologies to identify novel compounds or microorganisms inhibiting quorum sensing (the molecular pathways that regulate biofilm formation) in various microorganisms of concern for the dairy industry (*Geobacillus stearothermophilus*, *Pseudomonas* spp., *Listeria monocytogenes*, *Cronobacter sakazakii*). Samples from raw milk, dairy products (raw milk cheese) and particular environments (e.g. drains, floors, sinks, trolleys, etc) within collaborating dairy industries have been collected and collections of culturable microorganisms and a metagenomic library have been created. High throughput quorum sensing inhibition screenings using *Chromobacterium violaceum* and *Vibrio harvey* as reporter microorganisms have been optimized and were used to identify isolates with relevant activities. Biofilm forming abilities of the microorganisms of concern were assessed and interesting isolates against the problematic bacteria to inhibit their biofilms have been tested. A number of quorum quenching/biofilm inhibiting strains were identified that will be the subject of further development.

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5. Opportunity/Benefit:

A collection of bacterial isolates and metagenomic libraries from dairy-related environments has been created and can be used for the screening of other interesting biological activities. A number of strains with very interesting activities have been identified and are available for further development.
A high throughput quorum sensing inhibition screening has been optimized and is now an available tool for detection of quorum sensing and/or biofilm formation inhibitors. Luciferase tagged strains are readily available at the Teagasc culture collection to use them in biofilm formation inhibition screenings.

6. Dissemination:

Main publications:

Coughlan LM, Cotter PD, Hill C, Alvarez-Ordóñez, A (2016) New weapons to fight old enemies: novel strategies for the (bio)control of bacterial biofilms in the food industry. Frontiers in Microbiology 7(1641)

Coughlan LM, Cotter PD, Hill C, Alvarez-Ordóñez A (2015). Biotechnological applications of functional metagenomics in the food and pharmaceutical industries. Frontiers in Microbiology, 6, 672

Alvarez-Ordóñez A, Coughlan LM, Briandet R, Cotter PD. Biofilms in Food Processing Environments: Challenges and Opportunities. Annu Rev Food Sci Technol. 2019 Mar 25;10:173-195.

Alexa (Oniciuc) EA, Walsh CJ, Coughlan LM, Awad A, Simon CA, Ruiz L, Crispie F, Cotter PD, Alvarez-Ordóñez A. 2020. Dairy products and dairy-processing environments as a reservoir of antibiotic resistance and quorum-quenching determinants as revealed through functional metagenomics. mSystems 5:e00723-19

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