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DairyBiota

Key external stakeholders:

Milk producers and producers of milk-derived products, users of milk-derived products such as infant milk formula producers and dairy farmers.

Practical implications for stakeholders:

Through the utilization of Next Generation Sequencing (NGS) technologies, we can gain a greater than ever understanding of the microbial composition of milk, investigate more accurately than ever the impact of a variety of factors on these microbial populations and develop rapid, commercially valuable, DNA-based screening approaches that can replace existing milk microbiology tests.

- Milk producers and producers of milk-derived products will benefit through access to rapid and accurate tests that will ensure the safety and quality of their products and decrease the testing-associated delay between the receipt of samples and release of the end product.
- Infant milk formula producers can rapidly determine the quality and safety of the milk powder they purchase.
- The importance of establishing the relationship between farming practices and milk quality will also ultimately be of benefit to dairy farmers as these tests will identity practices which can lead to elevated microbial contaminants and, in turn, lead to the development of approaches to prevent such problems from arising

Main results:

Current temperature and storage duration practices impact the microbiota of raw milk, but these impacts are modest relative to the more considerable differences between mid and late-lactation milk.
Sequence-based microbiota analysis identified possible sources of raw milk contamination, and highlighted the influence of environment and farm management practices on the raw milk microbiota.
Shotgun metagenomic sequencing (a particular type of NGS) has the potential to in particular become a valuable tool for monitoring the microbiology of food production facilities.

Opportunity / Benefit:

Dairy: Increase the quality, productivity, competitiveness, and sustainability of Irish milk production systems to enable an increase in milk production with no adverse environmental impact.
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

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1. Project background:

Raw milk contains a rich microbial population. While many of the microbes present are harmless, and indeed may even have beneficial roles, those which contribute to spoilage and disease are deservedly the focus of most attention. Traditionally, this microbial population (microbiota) was assessed using microbiological growth/propagation techniques. However, it is now appreciated that this approach reveals only a minority of the microbes that are present. Fortunately, the powerful technique of next generation DNA sequence (NGS) determination and analysis enables the detection of microbes that are slow growing, difficult to grow or which are present at a lower percentage than the dominant species. Surprisingly, however, these new culture-independent techniques had yet to be applied extensively to the study of milk before the beginning of Dairybiota. However, we had previously adapted NGS-based technologies to study dairy populations. During Dairybiota, we applied this new technology to the dairy setting in a manner that can be used by stakeholders in a meaningful way (i.e. identify undesirable microbes and determine which farming practices influence the presence/absence of these undesirable microbes) while, in the process, we have established Teagasc as a leader in this field.

2. Questions addressed by the project:

- Could the application of molecular approaches (next generation sequencing and qPCR) be applied to more accurately investigate the factors which influence the microbiota of milk?

- What are the practices that negatively impact on the milk microbiota?

3. The experimental studies:

Further develop state-of-the-art Next Generation Sequencing (NGS)-based technologies to assess the microbial composition of raw milk (and establish Teagasc as world leaders in this field)

Use of culture-independent approaches to assess/evaluate the impact of a variety of factors (including seasonality and storage temperature) on the microbiota of milk

Culture-independent investigations to analyze the composition of the sulphate-reducing *Clostridium* (SRCs) and sulphate-reducing bacteria (SRBs) in milk

4. Main results:

This study shows how NGS can revolutionise our understanding of the microbiome of food and of food production environment. The application of this technology has enabled us to study the influence of seasonality and management practices on the raw milk microbiota. It has identified teat and faeces as the primary sources of bacterial contamination in raw milk and, finally, this work has demonstrated the usefulness of applying HTS in the context of monitoring bacterial species, strains and even phage in the microbiome of dairy foods and dairy production/processing environments. With the advantages of applying HTS to study the dairy production microbiome highlighted throughout this thesis, it is evident that this technology has the potential to be applied to monitor the microbiome of other food production chains too. There are obstacles to implementing HTS to achieve this, such as the current cost of sequencing, storage and computational power requirements for analysing metagenomic datasets and the necessity to validate/gain accreditation for methods for detecting potentially pathogenic and spoilage bacteria in the food production microbiome. However, it is likely that with concerted efforts from food microbiologists, bioinformaticians, technicians and regulatory bodies this type of approach will become the gold standard in microbial surveillance of food production chains in the not too distant future.

5. Opportunity/Benefit:

NGS is now available as a tool for microbiome analysis throughout the dairy production and processing chain. This tool can be availed of through Teagasc. We have also identified regions within the genomes of SRCs and SRBs that can be used as the basis for qPCR assays (rapid detection and quantification)

6. Dissemination:

Main publications:

Doyle, C. J., D. Gleeson, K. Jordan, T. P. Beresford, R. P. Ross, G. F. Fitzgerald and P. D. Cotter (2015). Anaerobic sporeformers and their significance with respect to milk and dairy products. International journal of food microbiology 197: 77-87.

Doyle, C. J., D. Gleeson, P. W. O'Toole and P. D. Cotter (2017). High-throughput metataxonomic characterization of the raw milk microbiota identifies changes reflecting lactation stage and storage conditions. International Journal of Food Microbiology.

Doyle, C. J., D. Gleeson, P. W. O'Toole and P. D. Cotter (2017). Impacts of seasonal housing and teat preparation on raw milk microbiota: a high-throughput sequencing study. Applied and environmental microbiology 83(2): e02694-02616.

Doyle, C. J., P. W. O'Toole and P. D. Cotter (2017). Metagenome-based surveillance and diagnostic approaches to studying the microbial ecology of food production and processing environments. Environmental Microbiology.

7. Compiled by: Therese McNamee and Paul Cotter