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INFAMILK



Key external stakeholders:

Food manufacturers, dairy industry, pharmaceutical companies, research communities; public health agencies and health professionals; policymakers.

Practical implications for stakeholders:

- Human milk is considered the optimum feeding regime for newborns and is a source of bacteria for the developing infant gastrointestinal tract. Nonetheless, there remain gaps in knowledge about which micro-organisms are ingested during breast-feeding that may influence infant gut microbiota development, a key influence on subsequent health and development.
- Establishment of the intestinal microbiota in infants plays a major role in human health and development, including protection against pathogens, maturation of the immune system and metabolic welfare of the host and it is imperative to understand how early infant nutrition influences the development of a healthy gut microbiota.
- Breast Milk represents a baseline for the functional performance of infant formulae.

Main results:

The results of this study described the microbial composition of human milk microbiota with a focus on probiotic development for infant nutrition.

Opportunity / Benefit:

Outputs from the project include new opportunities for optimisation of infant milk formula composition, with appropriate new bioactive ingredients such as milk fractions, probiotics and prebiotics to effectively programme the early infant gut microbiota in a manner closer to mothers milk. Expressions of interest from relevant companies are welcome.

Collaborating Institutions:

This 4-year study was led by University College Cork and involves a partnership between CORK University Maternity Hospital and Teagasc, Moorepark.

Teagasc project team: Prof. Catherine Stanton
Prof. Paul Ross
Katriona E. Lyons,

External collaborators: Prof. Tony Ryan,
Prof. Gene Dempsey,
Carol-Anne O' Shea
Prof. Alan Kelly.

1. Project background:

Our understanding of the milk microbiota and its potential functions has been limited due to inability to readily cultivate many bacterial species in milk. This limitation has resulted in potential bias towards specific microorganisms which are easily culturable, and thus have taken the focus of most investigations. Next generation sequencing has enabled detailed insight into the complex and diverse microbial ecosystem of breast milk. These culture independent, high-throughput sequencing methodologies have allowed better understanding of the true microbiome composition. While initially breast milk was considered a sterile fluid and microbes isolated were considered contaminants, it is now widely accepted that breast milk is home to its own unique microbiome. Human milk derived strains can be good potential probiotics, and in this study, we isolated species from milk for subsequent use in infant health and nutrition markets. Although investigations into the breast milk microbiome using metagenomic approaches has been limited, as new methods to characterise the microbiome are developed, these approaches will enable a more comprehensive analysis of the breast milk microbiome and potential function.

2. Questions addressed by the project:

- To optimize methodologies for analysis of human milk microbiome, in order to accurately describe the evolution of human milk microbiome composition over lactation in women from birth to 6 months.
- To characterize the probiotic potential of milk microbes isolated from human milk throughout lactation.

3. The experimental studies:

- An investigation of the effect of storage, temperature and extraction kit on the phylogenetic composition detected in the human milk microbiota
- An investigation of human milk microbiome composition over lactation in women from birth to 6 months.
- Isolation and characterization of microbes from human milk and assessment of the technological performance and probiotic potential of selected strains.

4. Main results:

The main findings of the project were as follows:

- Initially, we investigated how different storage methods, temperatures, preservatives and extraction kits influence the human milk microbiome, compared to fresh samples. Breast milk samples were processed via six different methods; fresh (Method 1), frozen at -80°C (Method 2), treated with RNAlater and stored at 4°C or -80°C (Method 3 and 4), treated with Milk Preservation Solution at room temperature (Method 5 and 6). Methods 1-5 were extracted using PowerFood™ Microbial DNA Isolation kit (Mobio) and Method 6 was extracted using Milk DNA Preservation and Isolation kit (Norgen BioTek). At genus level, the most abundant genera were shared across Methods 1-5. Samples frozen at -80°C had fewest significant changes while samples treated and extracted using Milk Preservation and Isolation kit had the most significant changes when compared to fresh samples. Diversity analysis indicated that variation in microbiota composition was related to the method and extraction kit used. This study highlighted that, when extraction from fresh milk samples is not an option, freezing at -80°C is the next best option to preserve the integrity of the milk microbiome. Furthermore, our results demonstrate that choice of extraction kit had a profound

impact on the microbiota populations detected in milk.

- The research examined the evolution of human milk microbiome composition in lactating women from birth to 6 months. One hundred and twenty three milk samples were collected from lactating women at 1, 4, 8 and 24 weeks following birth (84% were vaginally delivered and 16% were C-section delivered) and analysed for microbiota composition, based on 16S rRNA sequencing. A significant decrease in milk microbiota diversity throughout lactation from birth to 6 months was observed, with the greatest difference between week 8 and week 24 of lactation. Genera were identified that predominated in milk throughout lactation for 6 months and a significant effect of stage of lactation on milk microbiome composition was found.
- Studies have led to the isolation and characterization of microbes from human milk and we have assessed of the technological performance and probiotic potential of selected strains.

5. Opportunity/Benefit:

Human breast milk is highly regarded as the optimum mode of feeding for newborn's due to its ability to provide complete nutrition and capacity to confer health factors to the infant. Until recently breast milk was considered to be a sterile fluid, however, it has now been established that breast milk has its own microbiota, which provides numerous health benefits, many of which can contribute to infant health and development.

6. Dissemination:

Some of the research findings have been published to date, others being patented, to obtain maximum visibility of the results of this project with consideration for intellectual property, and they have been made available to stakeholders, particularly the Irish food industry for applications.

Main publications:

1. Lyons KE, Ryan CA, Dempsey EM, Ross RP and Stanton C. (2020). Breast milk, a source of beneficial microbes and associated benefits for infant health. *Nutrients*, 12, 1039; doi:10.3390/nu12041039.
2. Katriona E. Lyons, Fiona Fouhy, Carol-Anne O' Shea, C. Anthony Ryan, R. Paul Ross, Catherine Stanton (2020) Effect of storage, temperature and extraction kit on the phylogenetic composition detected in the human milk microbiota
3. Mills S, Lane JA, Smith GJ, Grimaldi KA, Ross RP, Stanton C. (2019). Precision nutrition and the microbiome, Part II: Potential opportunities and pathways to commercialisation. *Nutrients* 11, 1468.
4. Mills S, Stanton C, Lane JA, Smith GJ, Ross RP. (2019). Precision nutrition and the microbiome, Part I: Current state of the science. *Nutrients* 11, 923.
5. Lyons KE, Ryan CA, Dempsey EM, Ross RP and Stanton C. (2020). Evolution of the milk microbiome from birth to 6 months (in preparation).
6. Lyons KE (2020). PhD Thesis on the characterisation of human milk microbiome over lactation from birth to 6 months.

7. Compiled by: Professor Catherine Stanton