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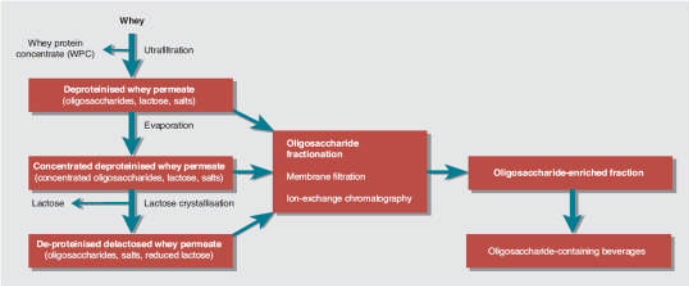
Functional beverages containing health-promoting prebiotic milk oligosaccharides



Key external stakeholders:

IMF manufacturers
Dairy/cheese industry
Dairy farmers

Practical implications for stakeholders:

Oligosaccharides, known to have health promoting properties, are significantly higher in human milk when compared with Bovine milk. In this study, Moorepark researchers in collaboration with UC Davis, sought to extract and enrich oligosaccharides from cows' milk to provide health promoting ingredients for inclusion in infant and adult beverages

The main findings from this research demonstrate that

- In addition to known bovine milk oligosaccharides, detection of 18 new high-molecular weight oligosaccharides was observed in the enriched powders.
- Kg quantities of powders enriched in milk oligosaccharides can be produced using the developed membrane filtration process.
- The oligosaccharide powders produced have been shown *in vitro* to possess prebiotic activity and can prevent invasion of human cells by *Campylobacter jejuni*.
- The oligosaccharides powders also decreased number of potential pathogens *in vivo* in a mouse model.

Main results:

In this study, pilot-scale enrichment of oligosaccharides from whey streams using 1 kDa membranes was successful yielding as high as 17.52% enrichment of oligosaccharides as a % of lactose. In collaboration with UC Davis, this study revealed, for the first time, the presence of several new free oligosaccharides containing up to 10 monomers that correspond in size to the most abundant oligosaccharides present in human milk including some fucosylated structures. A variety of bioactivities were shown to be associated with the bovine oligosaccharides *in vitro* such as increased colonization of human intestinal cells by Bifidobacteria, prebiotic effects and anti-invasive activity against Campylobacter. Of most importance, bovine milk oligosaccharides were found to reduce non-beneficial or pathogenic bacterial populations *in vivo* in the mouse GIT and have no adverse effects on the other health parameters measured.

Opportunity / Benefit:

Whey permeate is either used for fermentation of portable alcohol, lactose crystallization or disposed off at a cost to the industry. Extraction, enrichment or isolation of oligosaccharides with prebiotic and anti-infective activity from whey permeate or from by-products of lactose production could result in the production of value-added ingredients from waste streams, while also reducing disposal costs for companies involved. Moreover, cheese production has recently expanded in Ireland (~170,000 tonnes in 2010; Central Statistics Office, Ireland) and is forecast to continue expanding in the years ahead. Indeed, on the basis of available data the Food Harvest 2020 report envisages that a 50 per cent increase in milk production by 2020 (using the average of the years 2007 to 2009 as a baseline) would be realistic and achievable, and that this will set the foundation for further expansion in subsequent years. This will result in a significant pool of whey for processing and, consequently, a growing source of milk oligosaccharides

Collaborating Institutions

A full list of collaborating institutions is available below

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Teagasc project team:
Dr. Rita Hickey (previously Dr. Raj Mehra (retired) (PI)
Dr. Mariarosaria Marotta
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Sinead Mackey
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External collaborators:
Prof. Bruce German (UC Davis)
Prof. Carlito Lebrilla (UC Davis)
Dr. Daniella Barile (UC Davis)

1. Project background:

Many biological functions have been attributed to human milk oligosaccharides e.g. prebiotic activity, antiadhesion effects, anti-inflammatory properties, glycome modification, brain development, growth-related characteristics of intestinal cells and other uncharacterised effects. However, there are very few commercial products on the market which captilise on these functions. This is mainly due to the fact that the large quantities of milk oligosaccharides required for clinical trials are unavailable. In this respect, oligosaccharides derived from bovine milk which are mostly sialylated and therefore more like human milk, present an attractive source for generation of milk oligosaccharides considering the wide availability of milk and its by-products. Because fluid bovine milk contains only trace amounts of these valuable components, the use of dairy streams, in particular whey permeate, for large-scale extraction is an attractive option. The advantage of using specific whey fractions stems from their wide availability and low cost compared to other dairy streams. Whey permeate is a by-product obtained when cheese whey is passed through an ultrafiltration membrane to concentrate whey protein. Whey proteins are retained by the membrane, whereas smaller molecules such as lactose, oligosaccharides and salts pass through the membrane making up the whey permeate. The objective of this study was to develop a process to take advantage of the oligosaccharide-rich whey streams. To this end, membrane filtration technology was employed to further enrich the oligosaccharides already present. Oligosaccharide enriched powders that were generated during the project were evaluated in terms of their bioactivity by using *in vitro* bioassays and an *in vivo* model.

2. Questions addressed by the project:

- Can a scalable process by developed to enrich milk oligosaccharides present in whey streams?
- What biological properties can be attributed to the presence of bovine oligosaccharides in a beverage matrix?

3. The experimental studies:

Whey streams were clarified and various membrane materials were characterised at lab-scale for suitability to enrich the oligosaccharides. Once the most suitable membrane material was chosen, pilot scale membrane filtration was used to enrich the oligosaccharides and remove much of the lactose and monosaccharides. At every processing step the stability, concentration and composition of oligosaccharides was analysed. *In vitro* assays using cell lines and microtitre plates were employed to assess the prebiotic and anti-infective properties of the oligosaccharides. The effects of the oligosaccharides in an *in vivo* model were examined in the mouse by investigating the variation in bacterial populations between feed groups using 454 pyrosequencing and short chain fatty acid (SCFA) analysis.

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4. Main results:

A scalable approach for the enrichment of oligosaccharides from whey permeate using membrane filtration was developed. The highest enrichment of oligosaccharides (17.52% as a % of lactose) was achieved using a 1kDa membrane which resulted in the production of Kilogram quantities of enriched powder. The combination of membrane filtration processing and advanced mass spectrometry (UC Davis) allowed the identification of 18 previously unidentified high-molecular weight neutral oligosaccharides. Among those revealed, several large free oligosaccharides containing up to 10 monomers that corresponded in size to the most abundant oligosaccharides present in human milk were identified. This study also demonstrated that bovine oligosaccharides dramatically reduce cellular invasion and translocation of *Campylobacter jejuni* in human intestinal cells in a concentration dependent manner *in vitro*. Furthermore, the milk oligosaccharides in the enriched powder contribute *in vivo* to eliminating bacterial groups from the murine intestinal microflora. Many of the decreased families of bacteria are families that contain pathogens. The SCFA results also demonstrate that the milk oligosaccharides do not act as a food source for non-beneficial or pathogenic bacteria in the murine gut. Few studies exist where milk oligosaccharides have been tested in an *in vivo* model for health benefits. The outcome of this project lends support to the notion that these molecules maybe of benefit if added as an ingredient to infant formula to replace or act synergistically with commercial prebiotics.

5. Opportunity/Benefit:

The technologies to enrich oligosaccharides described here are based on membrane filtration techniques. Such membranes are already well established in the dairy industry and depending on the extent of use of an existing plant, it is anticipated that little additional costs would be required in terms of plant, personnel and training investment. Furthermore, bearing in mind the potential applications of oligosaccharides if produced by such industries, the initial capital and production costs would be spread between different high value-added ingredients for diverse applications.

6. Dissemination:

Research preformed in this project was presented at IDF World Dairy Summit 2007, the Carbohydrate Bioengineering Meeting, Italy, 2009, the 6th International Symposium of the International Milk Genomics Consortium, 2009, Paris, the Teagasc Walsh Fellowship Annual Seminar, 2009, Dublin, the International US Ireland Functional Foods Conference, 2010, Cork, The first International Conference on the Glycobiology of Human Milk Oligosaccharides 2011, Denmark and the 16th International Workshop on Campylobacter, Helicobacter, and Related Organisms, 2011, Canada.

In total, there were 8 peer reviewed scientific publications, 7 presentations at national and international scientific conferences and 3 popular publications.

Main publications

Mehra, R. and Kelly, P. (2006). Milk oligosaccharides: Structural and Technological Aspects. International Dairy Journal, 16, 1334-1340.

Hickey. R. (2009). Harnessing Milk Oligosaccharides for nutraceutical applications. In: Dairy-derived ingredients: Food and Nutraceutical uses. (Ed. M. Corredig), Woodhead Publishing Limited, UK.

Lane, J. A., Mehra, R. K., Carrington, S. D. and Hickey R.M. 2010. The food glycome: A source of protection against pathogen colonization in the gastrointestinal tract. International Journal of Food Microbiology, 142(1-2), 1-13.

Barile, D. Marotta, M. Chu, C. Mehra R., Grimm, R. Lebrilla C. B. and German J.B. (2010). Neutral and acidic oligosaccharides in Holstein-Friesian colostrum during the first 3 days of lactation measured by high performance liquid chromatography on a microfluidic chip and time-of-flight mass spectrometry. Journal of Dairy Science, 93, 3940-3949.

Lane, J. A., Mehra, R. K., Carrington, S.D., and Hickey, R.M. 2011. Development of biosensor-based assays to identify anti-infective oligosaccharides. Analytical Biochemistry 410, (2), 200-205.

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Hickey, R. (2012). The role of oligosaccharides from human milk and other sources in prevention of pathogen adhesion. International Dairy Journal 22: 141-146

Lane, J. A., Kavanaugh, D., Mariño, K., Rudd, P.M. Carrington, S.D., Naughton, J., Clyne, M. and **Hickey***, R.M. (2012) Anti-infective bovine colostrum oligosaccharides: Campylobacter jejuni as a case study. International Journal of Food Microbiology 157,182–188.

Lane, J. A., Marino, K., Slattery, H., Carrington, S. D., Rudd, P. M., and **Hickey***, R.M. (2012) Methodologies for screening of bacteria-carbohydrate interactions: anti-adhesive milk oligosaccharides as a case study. Journal of Microbiological Methods 90(1):53-9.

Popular publications:

Healthy oligosaccharide drinks. *Relay Research first update BE007*, issued April 2006.

Mehra, R. (2007). Milk's hidden gems. *TResearch,* 2(3), 9-11.

Exploiting oligosaccharides from cows' milk. *Relay Research second update BE007*, issued April 2008.

Whey-based streams as a source of functional oligosaccharides *Relay Research Final update,* submitted February 2012

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