Project number: 5939
Funding source: Enterprise Ireland & Industry
Date: November, 2014
Project dates: Jan 2009 – Jun 2013

Mining for milk based bio-actives using microbial fermentations

Key external stakeholders:

Irish Dairy Industry, dairy farmers, consumers

Practical implications for stakeholders:

Added functionality of casein, whey and milk based powders with health benefits beyond those associated with nutrition, increased profitability to the Irish milk sector

Improved health benefits to the consumer

Main results:

The key results were

- Dairy associated microbes with extracellular proteolytic activity were identified
- Fermented casein, whey and skim milk based substrates and water soluble extracts from commercial cheeses, were made into freeze-dried powders, a number of which had bioactivity across a range of health indicator assays
- Optimized fermentation and post-fermentation heat treatments were established that retained bioactivity.

Opportunity / Benefit:

The range of bioactivities associated with the microbial fermented milk products will increase the functionality of milk-based ingredients, adding market value and extending the applications for the dairy industry. The development of products containing the bioactive ingredients will directly benefit public health. This project was a component of FHI, the primary objective of which was to attempt to release peptides from milk proteins that demonstrate bioactivity in the areas of interest to FHI.

Collaborating Institutions:

Dublin City University, University College Dublin, University College Cork, University of Limerick and the companies Carbery, Dairygold, Glanbia and Kerry

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1. Project background:

In 2004 Enterprise Ireland (EI) with a number of Irish dairy industry representatives established a common functional foods research strategy, FHI; the center-piece of which was to "mine" for constituents in milk that could have an impact on defined health areas.

Bioactive peptides are described as "food-derived components (genuine or generated) that in addition to their nutritional value exert a physiological effect on the body". Such peptides can be encrypted within a protein and require proteolysis for their release and activation. Microbes, including lactic acid bacteria, have evolved extracellular proteolytic enzymes that enable them to breakdown the principal milk proteins, casein and whey into smaller peptides that can be transported into the cell for further metabolism. In some cases these peptides have been shown to have bioactivity and in the current study the aim was to identify additional bioactive components and extend the health benefits associated with them.

2. Questions addressed by the project:

- Could Teagasc identify proteolytic microbes, specifically LAB, capable of producing peptides from casein, whey and milk based substrates?
- Would these crude hydrolysates display bioactivity with a variety of health benefits relating to obesity, immunity, cardiovascular disease, glucose metabolism and infection?
- Can the production of bioactive products from bacterial fermentations be commercially optimized?

3. The experimental studies:

Some 300 strains from the Teagasc Moorepark Culture Collection, representing the genera *Lactococcus*, *Leuconostoc*, *Streptococcus,* *Enterococcus*, *Brevibacterium*, *Corynebacterium*, *Lactobacillus*, *Bifidobacterium*, *Bacillus* and *Pediococcus*, were screened for potential proteolytic activity against casein and whey proteins. In addition, a further 120 strains from species with reported proteolytic activity in milk were also sourced and tested. From this analysis 110 strains were further selected for fermentation of either casein, whey of milk based substrates. Freeze-dried powders from 170 fermentations were tested in one or more of six health bioassays, from which 85 potential bioactive powders were identified. From these bioactive powders 25 were further selected to establish if bioactivity could be retained under optimized fermentation conditions and post-fermentation heat treatments, resulting in a further 140 samples for follow-on testing. Moreover, using technologies based on membrane separation, reverse-phase HPLC and iso-electric charge, crude hydrolysates were fractionated with a view to characterizing the bioactive component, and a further 500 samples were generated. Follow-on testing of samples in the various health bioassays have to-date confirmed a number of key findings:

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- Bioactivity could be achieved under commercially acceptable fermentation parameters of 1% inoculation under aerobic conditions for 16 hours at 30°C in substrates that could be heat sterilized pre and post fermentation.
- A bioactive component associated with an anti-infective crude casein-based hydrolysate was identified, through mass-spectroscopy and subsequent synthetic peptide testing, as a nine amino-acid peptide cleaved from the end of the alpha-casein protein.
- A metalloprotease proteolytic enzyme was associated with a crude casein hydrolysate that enhanced the satiety and immune systems and regulated cardiovascular responses. For the immune response, bioactivity could be generated using a cell free enzymatic preparation.
- A non-peptide based metabolite was associated with a *Bacillus licheniformis* strain that enhanced satiety.

4. Main results:

- Extracellular proteolytic activity of Lactic Acid Bacteria was in general poor, resulting in no discernible hydrolysis of casein or whey proteins in dairy-based substrates for the majority of strains.
- Proteolytic bacteria were identified from non-LAB sources and represent a new category of technological advantageous dairy-related bacteria.
- Bacterial fermentations of casein, whey and skim milk-based substrates generated crude hydrolysates that harbored a range of bioactivities across health assays related to obesity, immunity, cardiovascular disease, infection, glucose regulation, and gut-flora modulation.
- Fermentation and bioactive powder production conditions were considered to be commercially viable and within existing dairy milk processing practices required to produce food-grade ingredients.

5. Opportunity/Benefit:

Dairy-based powders containing functional ingredients with targeted health effects will add market value to the Irish dairy ingredient sector and help improve public health.

6. Dissemination:

Main publications:

Marcone S, Haughton K, Simpson P, Belton O, Fitzgerald DJ (2014) Milk-derived bioactive peptides inhibit endothelial-dependent adhesive interactionswith monocytes via PPAR-□ dependent regulation of NF-kappa B. submitted.

McArdle M, Roche H. (2012) The Anti-Inflammatory Potential of Milk Derived Bio-actives. Nutrition Society Postgraduate Meeting Cork, Ireland.

Ben Larbi N., Canavan M. Simpson P, Loscher CE. (2011) Anti-inflammatory benefits of milk derived hydrolysates. Irish Society for Immummology Annual Meeting, Galway, Ireland.

O'Brien L, Schellekens H, Dinan T, Cryan J, Fitzgerald GF, Stanton C. (2011) Enhancement of calcium bioavailability via milk bioactives. Proceedings of the Annual Walsh Fellowship Conference, Ireland

Phelan M, Kerins D (2011) Milk-derived bioactive peptides: selected cardiovascular effects. Food and Function Journal 2: 153-16.

Bruen C, O'Halloran F, Kett A, Chaurin V, Fenelon M, Nilaweera K, Kelly A, McGrath B, McSweeney P, Cashman K, Giblin L. (2010) Effects of dairy protein hydrolysates on satiety. International Congress on Obesity, Stockholm, Sweden.

Popular publications:

7. Compiled by: Paul Simpson and Tom Beresford

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