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Milk fermentation as a tool to produce foods with health benefits

Key external stakeholders:

Dairy industry, infant formula companies, food and nutritional beverage manufacturers who use dairy ingredients

Practical implications for stakeholders:

There is a growing awareness among consumers that diet as part of a healthy lifestyle can significantly impact on overall health. As a consequence, there is a demand for foods which, in addition to providing basic nutrition have the capacity to actively support enhanced health. A number of such bioactive or functional foods are available in the market and the aim of this project was to establish if fermenting milk with food grade bacteria would result in the development of new dairy foods/ingredients displaying bioactive properties. The research focus was on foods that could address metabolic syndrome and gut health.

- A number of fermented milks were identified which demonstrated bioactivity in *in vitro* bioassay
- All the fermentations were undertaken with food grade Generally Regarded as Safe (GRAS) bacteria using fermentation conditions that are amenable to scaling for commercial production
- For a number of the fermented milks bioactivity was demonstrated to survive spray drying and thus would likely be suitable for development as bio-functional food ingredients

Main results:

The key results were:

- A database and sample bank of over 260 milk fermentates made using Lactic Acid Bacteria (LAB) was established.
- A number of milk fermentates with potential as functional foods for weight management and promotion of a healthy gut microbiome were identified.
- Fermentation and spray-drying were scaled to Pilot Plant level, while maintaining bioactivity indicating that these fermentates could be scaled for commercial production.

Opportunity / Benefit:

This project established a platform for the production of fermented milks from laboratory to pilot scale which can now be exploited by companies interested in developing dairy foods/ingredients in this area. The study identified a number of fermented milks displaying a range of bioactivities *in vitro;* with particular reference to alleviating obesity and promoting a healthy gut microbiome, which can be further investigated to confirm *in vivo* activity and be scaled for commercial production.

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1. Project background:

Fermented foods are regarded by consumers as traditional, safe and healthy products; hence their popularity has increased over the years, especially in Western countries. They became an essential part of our diet thousands of years ago, mainly due to their increased safety with respect to raw or unprocessed foods. Multiple substrates are used to produce fermented foods, from fish, meat and dairy to cereals, fruits and vegetables. Similarly, a huge variety of microbes are used within the fermentation process. As a result, there are many different types of fermented foods that vary from region to region, but their consumption is extended worldwide.

Multiple studies have demonstrated that fermentation of different foods not only increases their shelf-life, but also results in the formation of secondary components (or bioactive compounds) with diverse benefits for health. Regular consumption of fermented products (particularly dairy) has been associated with improvements and lower incidence rates of diseases such as obesity, blood cholesterol levels, type 2 diabetes mellitus, cardiovascular disease and hypertension.

The objective of this project was to investigate the use milk fermentation as an approach to produce milk products/ingredients with demonstrable health enhancing properties, the target health areas being obesity and gut microbiota. To support the ultimate commercialisation of key findings the fermentations were undertaken using LAB, a group of organism with GRAS status that are used extensively in the dairy industry, employing fermentation conditions that would support subsequent scaling to commercial level.

2. Questions addressed by the project:

- Can lactic LAB which have GRAS status be used to produce fermented milks with specific benefits for health issues such as obesity and enhanced gut microbiota?
- Can the fermentates be scaled, ultimately to commercial level?

3. The experimental studies:

Reconstituted skim milk (RSM) was selected as the fermentation substrate on the basis that RSM supports the growth of most LAB, is a bulk commodity product the value of which could be enhanced via fermentation and using skim milk powder decouples the fermentation process from milk production and processing seasons. RSM was reconstituted to 10 or 30% solids, the higher solids level assisting with the follow on spray-drying process. Due to their GRAS status 90 strains of LAB consisting of various *Lactococcus, Lactobacillus, Leuconsotoc* and *Pediococcus* species were selected to undertake these fermentations. Fermentation conditions were selected that would support the growth of the LAB strains but which would subsequently be amiable to scaling to commercial level. In addition the bacterial strains were screened for their ability to survive exposure to bile and capacity to produce the enzyme bile salts hydrolase (BSH). Fermentates were characterised by analysing the pH after fermentation, growth of the strain in milk (log increase), degree of protein hydrolysis (OPA), free amino acid composition and peptidic profile.

The majority of the strains tested grew during fermentation over a 24 hour period as indicated by an increase in cell numbers and a decrease in pH. The degree of hydrolysis varies with strain and while many exhibited <1% hydrolysis as measured by OPA, the most prolific and proteolytic strains exhibited levels of hydrolysis >5%. The data obtained indicated that *L. helveticus* strains grew best and resulted in the highest levels of protein hydrolysis.

The fermentates were tested in *in vitro* bioassay for antioxidant activity, inhibition of pancreatic lipase (PL) and stimulation of satiety hormone production, while the bacterial strains were investigated for their tolerance to bile, BSH activity and production of γ-aminobutyric acid (GABA) and the genomic sequence was established for a number of the most promising strains. In addition, a fermentate demonstrating the capacity to promote the growth of bifidobacteria was scaled to pilot plant level to demonstrate the commercial scalability of the fermentates and for use in an animal trial.

Triacylglycerol is the main source of dietary fat, but this molecule cannot be absorbed in the gut without being hydrolysed into smaller molecules such as monoglycerides and free fatty acids. PL is the main enzyme responsible for this hydrolysis, thus facilitating the absorption of dietary fat. Inhibition of PL has been proposed as a mechanism to reduce dietary fat absorption and thus facilitate weight loss. The fermentates were screened for this activity. Under the experimental conditions used; Orlistat the only approved medication to inhibit PL, resulted in 70% inhibition of PL, while it was observed that some of the fermentates

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resulted in PL inhibition in the region of 50%. The species and strain of bacterium used in addition to the fermentation conditions had a major influence on the levels of PL inhibition observed.

Control of food intake along with exercise is seen as the key to controlling body weight and obesity. Appetite plays a central role in dictating levels of food intake and appetite in turn is controlled by a complex network of signals, many of which are hormone induced. One of the main intestinal hormones involved in appetite suppression is glucagon-like peptide-1 (GLP-1) and it is proposed that stimulation of GLP-1 production leads to a reduction in appetite and thus food intake. The capacity of the fermentates to induce GLP-1 secretion from STC-1 cells in tissue culture was investigated. Approximately 30% of the fermentates tested induced secretion of GLP-1 with the highest activities observed with strains of *L. paracasei, L. rhamnosus, Lc. lactis* and *P. pentosaceus.*

While the health benefits of consuming antioxidants remain controversial many perceive them as beneficial. Data obtained during this study did not indicate significant levels of antioxidant activity resulting from milk fermentation.

PL exerts its activity at the oil-water interface; thus, fat has to be formed into micelles in order for hydrolysis to occur resulting in the formation of monoglycerides and free fatty acids which can then be absorbed into the blood stream. Bile salts play an essential role in stabilising fat micelles which form during gastrointestinal passage. Bile is manufactured in the liver using cholesterol and following fat absorption is reabsorbed in a great proportion in the intestinal lumen and recycled. A reduction in fat absorption could thus be achieved if bile salts were de-conjugated and therefore not available for micelle stabilisation. In addition, de-conjugated bile salts are less soluble and less efficiently absorbed and thus excreted in the faeces, resulting in a requirement for increased *de novo* synthesis of bile from cholesterol in the liver which in turn leads to decreases in total serum cholesterol. BSH catalyses the hydrolysis of conjugated bile salts to free bile salts and has been identified in a range of bacterial species. The bacterial strains used in this study were investigated for their capacity to grow in the presence of ox bile, as a prerequisite to screening them for production of BSH. Approximately 70% of the strains tested exhibited growth in the presence of bile. In general the ability to grow in the presence of ox bile was a species specific trait which was particularly widespread in members of *L. acidophilus, L. brevis, L. casei, L. paracasei, L. curvatus, L. plantarum, L. rhamnosus, L. salivarius, P. acidilactici* and *P. pentosaceus.* Strains demonstrating the capacity to grow in the presence of ox bile were tested for the production of BSH and the data obtained indicated that 60% of the 50 strains tested displayed BSH activity and interestingly many expressed BSH activity in milk without prior exposure to bile. BSH activity was noted to be a species specific trait.

GABA is a non-protein amino acid that plays a central role as an inhibitory neurotransmitter in mammals. GABA is considered a bioactive compound with multiple health-promoting properties including reducing insulin resistance and combatting obesity. GABA can be found in some foods including fruit and vegetables albeit at low levels. It can also be produced from the amino acid glutamate and some bacteria, including LAB have the necessary enzymes to support this conversion. It has also been established that GABA production by different microbial strains is highly influenced by the culture conditions and the availability of glutamate. It is interesting to note that casein contains a high proportion of glutamate so co-culture of strains with the capacity to produce GABA with proteolytic strains that will release glutamate would be expected to provide conditions that promote GABA production. The bacterial strains used in this study were screened for GABA production during milk fermentation. The five most potent producers were then co-cultured with either of two proteolytic strains under a range of fermentation conditions. The data obtained demonstrated that co-culture and manipulation of fermentation conditions can be used to enhance GABA production to levels consistent with the proposed effective dose.

In order to confirm the potential commercial viability of the proposed fermentates pilot scale production to 300L fermentation scale followed by spray-drying was undertaken, using a bifidogenic fermentate as a model. Fermentation and spray-drying were successfully completed and bioactivity was confirmed in the resulting milk powder.

4. Main results:

- A database and sample bank of over 260 milk fermentates made using 90 strains of *Lactococcus, Lactobacillus, Leuconsotoc* and *Pediococcus* species was established.
- Milk fermentates with the capacity to inhibit PL to levels of 50% in *in vitro* assay were identified. Fermentates made with strains of *L. helveticus* were observed to have the highest PL inhibitory

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activity.

- Fermentates made with specific strains of *L. paracasei, L. rhamnosus, Lc. lactis* and *P. pentosaceus* were identified which had the capacity to induce GLP-1 secretion from STC-1 cells.
- A bank of strains expressing BSH activity were identified, many of which expressed this enzyme in milk without prior exposure to bile.
- Co-culture and manipulation of fermentation conditions was used to maximise GABA production with the highest levels of GABA production being observed in fermentates using specific strains of *Lc. lactis* co-cultured with strains of *L. bulgaricus*
- A number of fermentates were identified which encoded more than one of the bioactivities investigated
- Fermentation and spray-drying were scaled to 300L pilot scale while maintaining bioactivity indicating that these fermentates could be scaled for commercial production.

5. Opportunity/Benefit:

- Several fermented milks with potential benefits linked to control of obesity were identified. These are available for further development with a view to their inclusion as bio-functional ingredients in functional foods.
- A database and sample bank of fermented milks is available for further screening for additional health targets.
- A platform for the production of fermented milks has been established which can be utilised for the rapid production of additional fermentates using novel or proprietary bacterial strains.

6. Dissemination:

Main publications:

Gil-Rodriguez AM, Beresford T. Lipase inhibitory activity of skim milk fermented with different strains of lactic acid bacteria. Submitted for publication.

Gil-Rodriguez AM, Beresford T. Bile salt hydrolase and lipase inhibitory activity in milk fermented with lactic acid bacteria. Submitted for publication.

Gil-Rodriguez AM, Beresford T. *Lipase inhibitory activity and bile salt hydrolase production in milk fermented with lactic acid bacteria*. Poster presented at the Society of Dairy Technology (SDT) Spring Conference held in UCC in April 2017.

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