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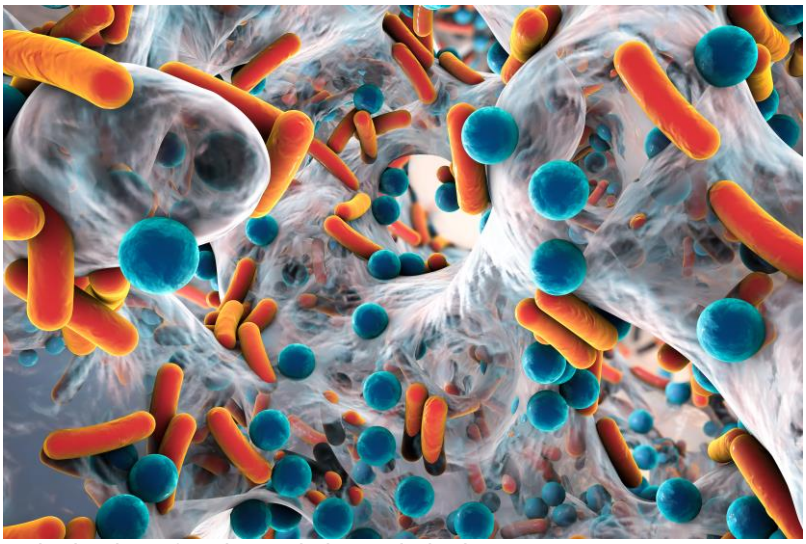
Buttermilk: potential to protect from infection



Colonization of pathogenic bacteria in the gut

Key external stakeholders:

Infant formula and Food for Health (functional food) manufacturers

Practical implications for stakeholders:

Buttermilk may act as a source of anti-infective compounds that reduces the threat of *E. coli* O157:H7 infection in humans. There is potential to include buttermilk fractions as functional ingredients in consumer products, such as in functional beverages, aiming to provide daily protection from infection. This work highlights an alternative use for buttermilk, increasing the worth of this under-valued dairy stream and, in turn, increasing the value of milk in the dairy industry as a whole.

Main results:

- A defatted Milk Fat Globule Membrane (MFGM) fraction reduced *E. coli* association with human intestinal cells
- Defatted MFGM does not target human cell receptors but instead a direct MFGM-bacterial interaction is likely responsible for the ant-infective activity.
- Temporal changes in bovine buttermilk glycosylation occur over lactation
- Temporal changes in the glycosylation of buttermilk proteins may have an important impact on the commercial isolation of such glycosylated ingredients.

Opportunity / Benefit:

Bovine Milk Fat Globule Membrane was demonstrated to prevent *E. coli* infection and hence the inclusion of this bioactive fraction in functional foods may benefit the general population, as well as immune-compromised individuals, including infants and the elderly.

Collaborating Institutions:

Teagasc, National University of Ireland Galway.

Teagasc project team: Dr. Rita Hickey (PI), Dr. Jonathan Lane, Sarah Ross
External collaborators: Prof. Lokesh Joshi and Dr. Michelle Kilcoyne (NUIG)

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1. Project background:

Infectious diseases are responsible for more than 17 million deaths worldwide each year, most of which are associated with bacterial infections. Moreover, the alarming increase in antibiotic-resistant bacteria makes the search for novel means of fighting infections imperative. For that reason, instead of searching for factors that kill bacteria, research should focus on compounds that can prevent the onset of infection. It is well documented that adhesion of enteric bacteria is required for colonization and subsequent development of disease. Therefore, one attractive possibility is the use of agents which interfere with bacterial adhesion to the host. Some of the most efficient anti-adhesion agents identified to date are present in foodstuffs - as best exemplified by human milk glycans which protect newborns against infections. Such glycans can display structural homology to host cell receptors or pathogenic lectins, thus functioning as receptor decoys. A novel approach to prevent *E. coli* infection may be found in bovine milk. A component of bovine milk, termed milk fat globule membrane (MFGM) which is a membrane that surrounds and stabilises milk fat droplets, could be key. MFGM can be sourced from buttermilk, a dairy fraction which is produced in high quantities in the dairy industry. The MFGM is composed of fats and proteins, many of which contain bound sugars. We generated a milk fraction, rich in these MFGM sugars, from bovine buttermilk and characterised its components.

2. Questions addressed by the project:

- Can we identify anti-infective fractions from MFGM material which have the potential to protect against infection?
- Can we find a way to add value to traditional foods such as buttermilk?

3. The experimental studies:

Since bovine milk fat globule membrane (MFGM) is a source of food-derived glycans that can offer an approach to prevent *Escherichia coli* O157:H7 infection by inhibiting attachment of the pathogen to host cells, we generated a defatted bovine MFGM fraction, rich in proteins and glycoproteins. We investigated the ability of this preparation to prevent the association of several enterohaemorrhagic *E. coli* O157:H7 strains with human colonic adenocarcinoma, HT-29 cells.

Bovine buttermilk is a commercially viable source of MFGM and is an under-valued by-product of butter making. We studied the changes in buttermilk glycosylation over the course of lactation to determine what the impact would be on commercial isolation of glycosylated ingredients. In this study, buttermilk was generated from three individual multiparous cows at 13 time points over the first three months of lactation. Buttermilk glycosylation was profiled using lectin microarrays and lectin blotting using technology developed at NUIG.

4. Main results:

- We demonstrated that our defatted MFGM fraction reduced bacterial association with HT-29 cells in a concentration dependent and strain specific manner.
- Results suggest that defatted MFGM does not target host cell receptors for E. coli O157:H7 and instead a direct defatted MFGM-bacterial interaction is likely responsible for the ant-infective activity.
- Lectin analysis demonstrated that temporal changes of bovine buttermilk glycosylation occurred during milk maturation which may vary between individual cows.

5. Opportunity/Benefit:

Our findings suggest that MFGM is effective at preventing infection *in vitro* and may present a new approach to mitigate the adverse health effects caused by *E. coli* infections in humans. The inclusion of this bioactive fraction in functional foods may be of great benefit to the general population, as well as immuno-compromised individuals, including infants and the elderly.
Buttermilk is a rich source of MFGM, however, if it is to be used as a source of anti-infective glycans, then commercial producers would need to be aware of the impact of seasonality on levels of glycans found on MFGM.

6. Dissemination:

Main publications:

- Ross S. A., Gerlach J. Q., Gill S. K., Lane J. A., Kilcoyne M., Hickey R. M., Joshi L. (2016). Temporal alterations in the bovine buttermilk glycome from parturition to milk maturation. *Food Chemistry.* 211:329-38.
- Ross S. A., Lane J. A., Kilcoyne M., Joshi L. and Hickey R. M**.** (2016) Defatted bovine milk fat globule membrane inhibits association of enterohaemorrhagic *Escherichia coli* O157:H7 with human HT-29 cells. *International Dairy Journal*. 59:36-43.

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Book Chapter:

- Ross S.A., Lane J. A., Kilcoyne M., Joshi L. and Hickey R. M. (2015) The milk fat globule membrane: A potential source of health-promoting glycans. In *Biotechnology of Bioactive Compounds: Sources and Applications*, First Edition. Edited by Vijai Kumar Gupta, Maria G. Tuohy, Mohtashim Lohani, and Anthonia O'Donovan.

Thesis:

- Ross, S. (2016). Targeting the glycome of the milk fat globule membrane for anti-infective properties. Submitted to the National University of Ireland, Galway for the Degree of Doctor of Philosophy.

Popular publications:

- Ross, S., Lane J.A. and Hickey R. M. (2016) Buttermilk: potential to protect from infection. *TResearch Spring edition* 11 (1) p10

Press:

- http://www.agriland.ie/farming-news/has-e-coli-finally-met-its-match-in-buttermilk/

Awards:

- Ross, S.A., Lane, J.A., Kilcoyne, M., Joshi, L. and Hickey, R. M. (2015) 'Anti-infective potential of defatted bovine milk fat globule membrane against *Escherichia coli* O157:H7.' (poster- joint winner of poster prize) Walsh Fellow Seminar, (November 12**,** Royal Dublin Society, Dublin, Ireland).

7.Compiled by: Dr. Rita Hickey

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