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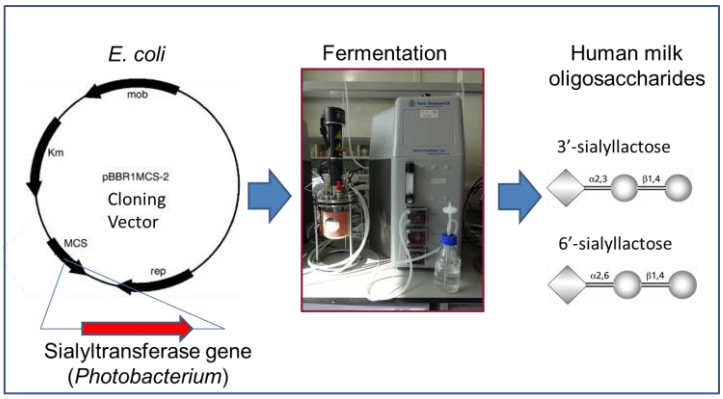
Enzymatic generation of sialylated lactose from waste whey using marine-derived sialyltransferases



Key external stakeholders:

Infant formula companies, the Irish and international dairy processing industry, Irish dairy exporter organisations, the marine sector and functional and medical food manufacturers

Practical implications for stakeholders:

Many biological functions have been attributed to sialylated human milk oligosaccharides (HMOs) which account for about 20% of all HMOs. These oligosaccharides can act as binding sites for specific pathogens and toxins, are thought to play a role in brain development and can regulate the immune response. However, the large amounts of HMOs which are required for clinical intervention are unavailable. Although many of these same MOs are present in bovine milk their levels are very low. This research therefore focuses on alternative sources and methods of producing two major sialylated HMOs, 3' and 6'sialyllactose. Marine species present a valuable source of robust genes which could open the way to sequence key genes of native Irish species for novel sialyltransferases. The high purity and low cost of HMOs generated in this manner should make their use possible in new fields such as the food or pharmaceutical industries.

Main results:

- Knockout strains of *Escherichia coli* were constructed using λ red recombination with the aim of generated a strain that was incapable of degrading the produced HMOs
- Sialyltransferase genes from marine bacteria (*Photobacterium)* were selected and cloned into this *E coli* strain aswell as the genes for the production of sialic acid
- This final *E. coli* strain is capable of sialic acid synthesis from simple carbon sources and transfer of this sialic acid to lactose to produce 3'- and 6'-sialyllactose simultaneously
- Optimised fermentation conditions were established whereby whey can act as the source of lactose for oligosaccharide production

Opportunity / Benefit:

Breast-feeding is not always possible, and therefore there is a consumer need for the availability of an infant formula, which more closely mimics that of human breast milk. Supplementing infant formula with synthetic HMO's has been considered as a way to improve infant nutrition. On a commercial level, infant nutrition companies are interested in adding HMO to their formula, however, a key obstacle is that the large quantities of purified HMO's needed for this are currently unavailable. The methods described here for the production of HMO, can be up-scaled to produce high yields of the sialylated oligosaccharides.

Collaborating Institutions:

Teagasc, National University of Ireland Galway.

Teagasc project team: Dr. Rita Hickey (Teagasc PI) and Dr. Aoife Thompson
External collaborators: Prof. Lokesh Joshi (Project PI) and Dr. Michael Cairns (NUIG)

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1. Project background:

Glycomacropeptide (GMP) is a C-terminal part (residues 106–169) of kappa-casein which is released in whey during cheese making by the action of chymosin. In recent years, interest in GMP has increased, as the peptide exhibits biological and nutritional properties which have been linked to a number of health benefits. For instance, the peptide is known to inhibit viral or bacterial adhesion to cells, promote proliferation of beneficial bacteria, neutralize enterotoxin, inhibit gastrointestinal secretions and exert immune regulation and plays a role in the nutritional management of phenylketonuria and has also been shown to stimulate cholecystokinin release and reduce gastric secretion. Many of these bioactivities have been attributed to the O-linked glycosylation associated with GMP and particularly the sialic acid (N-acetylneuraminic acid) component.

However, few studies have examined the exact mechanisms by which GMP exerts its beneficial effects particularly at the genetic level. The ability of GMP to inhibit the adhesion of a variety of pathogenic *Escherichia coli* strains to HT-29 and Caco-2 intestinal cell lines and improve barrier function was examined. In addition, the transcriptional response of HT-29 cells to GMP was investigated to gain insight into how GMP contributes to immunomodulation in the gastrointestinal tract.

2. Questions addressed by the project:

- Can we identify novel bioactivities associated with GMP which have the potential to protect against infection and modulate immune function?
- Can we establish the mechanism by which GMP prevents *E. coli* adherence to human cells and determine if it is through either direct (bacterial binding) or indirect (cell-line binding) inhibition?
- Can we determine the ability of GMP to suppress pathogen induced tight junction (TJ) barrier function impairment?
- Can we determine how GMP influences immune function associated gene expression in the gastrointestinal tract?
- Can we find a way to add value to traditional foods such as cheese whey?

3. The experimental studies:

To further explore if bovine GMP can offer an approach to prevent *Escherichia coli* infection by inhibiting attachment of the pathogen to host cells, we employed a commercially available GMP ingredient (kindly donated by Agropur Food Ingredients) which was rich in sialic acid (8%). We investigated the ability of this ingredient to prevent the association of several enteropathogenic and enterohaemorrhagic *E. coli* strains with human colonic adenocarcinoma, HT-29 and Caco-2 cells using anti-infective assays.

To investigate the effect of GMP on *E. coli* translocation, transwell inserts containing Caco-2 cells were employed. To confirm the formation of a tight cell monolayer in the presence of GMP, TEER measurements were carried out on Caco-2 cells. Microarray analyses were employed to investigate the response of colonic epithelial cells (HT-29) to GMP at the genetic level. The microarray data was further validated by means of real time-PCR.

4. Main results:

- We demonstrated that the GMP ingredient reduced *E. coli* association with HT-29 and Caco-2 cells in a concentration dependent and strain specific manner.
- The results suggest that GMP does not target host cell receptors for *E. coli* and instead a direct GMP-bacterial interaction is likely responsible for the ant-infective activity.
- GMP is capable of maintaining the structural integrity of Caco-2 tight junctions. Furthermore, GMP delays the paracellular movement of *E. coli* through the tight junctions of Caco-2 monolayers.
- GMP majorly influenced the expression of immune-modulatory chemokines and cytokines in HT-29 cells including chemokine (C–X–C motif) ligand 1 (CXCL1), chemokine (C–X–C motif) ligand 2 (CXCL2), chemokine (C – C motif) ligand 20 (CCL20), chemokine (C – X – C motif) ligand 10 (CXCL10) and interleukin 17C (IL-17C). Other important regulatory genes differentially regulated by GMP include DUOX2, CAMP, IL-33, GJB6 and IL-20Rb.

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5. Opportunity/Benefit:

Our findings suggest that GMP 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 consumption of GMP may promote the maturation of the naive cytokine responses and contribute to increased immunological well-being in infants and adults, especially the immunocompromised. This knowledge should allow manufacturers of whey ingredients, infant formula and nutritional beverages to develop new products centred on scientifically proven functional attributes.

6. Dissemination:

Main publications:

- Feeney S., Ryan J.T., Kilcoyne M., Joshi L. and Hickey R.M. (2017) Glycomacropeptide Reduces Intestinal Epithelial Cell Barrier Dysfunction and Adhesion of Entero-Hemorrhagic and Entero-Pathogenic *Escherichia coli in vitro.* Foods. 6(11). pii: E93
- Feeney S., Lane J.A., O Callaghan J., Kilcoyne M., Joshi L. and Hickey R.M. (2018) Transcriptional response of HT-29 intestinal epithelial cells to bovine glycomacropeptide. *Under review -Food and Nutrition Research.*

Book Chapter:

- Feeney S., Joshi L. and Hickey R. M. (2018) Biological roles and production technologies associated with bovine glycomacropeptide" In *Novel Proteins for Food, Pharmaceuticals and Agriculture: Sources, Applications and Advances.* Wiley. Editor M. Hayes

Conference proceedings:

- Feeney S., Ryan J.T., Kilcoyne M., Joshi L. and Hickey R.M.: "Glycomacropeptide Reduces Intestinal Epithelial Cell Barrier Dysfunction and Infection of Enterohemorrhagic and Enteropathogenic *Escherichia Coli In-vitro*. Journal of Clinical Gastroenterology, 50, S224-S224, 2016 (Proceedings from the 8th Probiotics, Prebiotics & New Foods for Microbiota and Human Health meeting held in Rome, Italy on September 13-15, 2015)
- Feeney S., Ryan J. T., Kilcoyne M., Joshi L. and Hickey R. M. (2015) (Poster) 44th Food Research Conference, (December 14, TFRC, Moorepark, Fermoy, Co. Cork, IRELAND).
- Feeney S., Ryan J. T., Kilcoyne M., Joshi L. and Hickey R. M. (2015) Glycomacropeptide from bovine milk reduces intestinal *E. coli* colonisation and associated barrier dysfunction *in-vitro* (Poster) Walsh Fellow Seminar, (November 12**,** Royal Dublin Society, Dublin, IRELAND).
- Feeney S., Ryan J. T., Kilcoyne M., Joshi L. and Hickey R. M. (2014) Glycomacropeptide reduces infection of enterohemorrhagic and enteropathogenic *Escherichia coli in-vitro* (Poster) 43rd Annual Food Research Conference (9-11 December, University College Dublin, Dublin, IRELAND.

7.Compiled by: Dr. Rita Hickey

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