Enzymatic Modification of Bovine Milk Oligosaccharides and their Functional Properties

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Project goal: This multi-disciplinary collaborative project aims at developing new strategies that will allow the dairy industry to profit from oligosaccharides recovery. Our approach involves using whey permeate oligosaccharides as "backbones" for the production of improved functional compounds that would add great /health value to dairy products.

Background: Human milk is the ideal food in terms of nutrition for newborns but not every infant can access it. The main components of human milk are lactose, lipids and human milk oligosaccharides (HMO), which are sugars present in high concentration in mother's milk and have been associated with important functions related with newborn development and health. HMO acts as prebiotics on a few select bacteria because of their unique structural complexity.

Despite the important role of HMO, these compounds are not well represented in infant formula since it only contains plant derived oligosaccharides (OS). Plant derived OS are used as an additive in a wide range of food products, but despite having a prebiotic effect on the intestinal microbiota, they lack many of the other beneficial effects provided by HMO. This difference is mainly caused by the simplicity of their chemical structure. Increasing the structural complexity of the oligosaccharides present in infant formula, paired with the appropriate bacteria, could potentially lead to improved health in babies. In the search of sources of HMO-mimics, bovine milk oligosaccharides (BMO) were discovered a few years ago.

Cheese whey permeate is a by-product of whey protein production and currently has no commercial value. Because it is considered an environmental pollutant and cannot be readily discarded in the wastewater, the dairy industry currently must manage the costs of disposing of it as if it was hazardous waste. Our group was the first to extract, identify, and characterize a multitude of functional compounds (oligosaccharides) in cheese whey permeate, albeit in low concentration. Considering that over 50 million pound of cheese whey is produced daily just in the State of California (mostly deriving from whey protein extraction which is now a very successful and growing market) the available quantities of whey permeate available for further extraction of bioactive oligosaccharides make it an attractive source.

Results: We successfully utilized whey streams as starting material for the development of an enzymatic approach to produce HMO-mimetics. Combining large-scale isolation of whey OS by membrane filtration, with pilot scale sialylation and fucosylation lead to the production of oligosaccharides much more similar to HMO and in enough quantities to test their expected improved biological activities.

Sialylation of whey OS was completed by enzymatic addition of sialic acid by PmST1_M144D, an α2-3-sialyltransferase mutant from *Pasteurella multocida*. Fucosylation of whey OS was also successfully achieved by enzymatic addition of fucose by Hp1-3FT, an α1-3-fucosyltransferase from *Helicobacter pylori*, attaining eight fucosylated structures. The addition of sialic acid and fucose and the disappearance of the "donor" whey OS was monitored by nanoLC QToF MS. After further purification, all

the fractions containing the new sialylated and fucosylated OS were tested for their improved ability to prevent the in vitro uptake of two common pathogens Methicillin-resistant Staphylococcus aureus (MRSA) and Enterohaemorrhagic Escherichia coli (EHEC) by Caco-2 cells. Interestingly, both the fucosylated and sialylated OS decreased the uptake of MRSA but only sialylated BMO decreased the uptake of EHEC by Caco-2cells.

Further scale-up of this approach will enable obtaining enough quantities to run further functional studies and eventually incorporate the new HMO-mimics into functional dairy foods.