Pilot scale isolation of bioactive glycans from dairy co-products: capturing the whey glycome

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Complex glycans in human milk have been demonstrated to exhibit a variety of health effects in developing infants, including prebiotic, immunomodulatory, and anti-pathogenic roles. We are isolating milk glycans from dairy co-products in a multifaceted strategy to reclaim economic value from currently underutilized streams and improve nutrition for infants worldwide. We have successfully developed several methods for pilot scale isolation of bovine milk oligosaccharides using fermentation, nanofiltration, and enzymatic treatment. These methods have yielded kg amounts of highly sialylated milk oligosaccharides, and represent a promising translational opportunity for the dairy industry to reclaim value in co-products. In order to capture an oligosaccharide pool that resembles even more those found in human milk, we have utilized a recombinant enzyme Endo BI-1 cloned from the commensal gut bacterium *Bifidobacterium longum* subsp. *infantis* whose primary function is to cleave N-linked glycans from glycoproteins in the infant gut. Use of this enzyme has thus far been successful in small scale, however in order to examine the multiple biological functionalities of the glycans released from bovine whey glycoproteins using Endo BI-1, the magnitude of glycan recovery must be increased. We will use this enzyme in conjunction with a variety of immobilization resins and membrane filtration to scale glycan release and optimize overall process dynamics. Examining total glycan release with cutting-edge mass spectrometry and chromatographic methods will inform development of novel functional ingredients. This immobilization and subsequent glycan release in large scale will enable recovery of sufficient quantities of N-glycans to further investigate their *in vitro* and *in vivo* mechanisms of bioactivity and evaluate potential synergistic effects with bovine milk oligosaccharides.