

A novel method for high-throughput analysis of bovine milk oligosaccharides

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Introduction

Oligosaccharides from mammalian milk are theorized to impart a variety of health benefits to consumers, as evidenced by studies demonstrating prebiotic and disease-preventing bioactivities. Beneficial oligosaccharides are highly concentrated in human milk, but are much less abundant in bovine milk and related commercial products. Previous studies have shown that both oligosaccharide abundance and complexity can vary between dairy bovine breeds. However, factors affecting such differences in bovine milk are not fully understood. This study has established a method for high-throughput oligosaccharide analysis using isobaric tagging and tandem mass spectrometry which will be used to profile milk oligosaccharides in a large set of bovine milk samples, allowing correlations between breed and oligosaccharide content to be identified.

Methods

Oligosaccharide standards were labeled with commercially-available carbonyl-reactive isobaric tags in a known ratio. An LC-MS/MS method was developed on an Agilent 6520 Accurate Mass quadrupole time-of-flight (Q-TOF) mass spectrometer which uses collision-induced dissociation (CID) energies optimized for both precursor m/z and charge state to fragment oligosaccharides. Specific reporter ions originating from the tags are then used as a basis for both relative oligosaccharide quantification and deconvolution of multiplexed samples. In near future oligosaccharides will be analyzed with the optimized method in 850 milk samples collected from two Danish dairy breeds. Further, genotyping has been performed on all cows using the bovine HD SNP-chip so that the genomes can be correlated with oligosaccharide abundances and genetic parameters (heritability and genetic correlations) of bovine milk oligosaccharides can be estimated.

Results

After collision energy optimization, relative oligosaccharide abundances taken from replicate injections of tagged standards showed excellent repeatability and sensitivity, with a coefficient of variation below 5% for each oligosaccharide standard. Oligosaccharide precursor ions of charge 1+ and 2+ demonstrated a predictable, linear trend in optimal collision energy (as evaluated by quantification accuracy, residual precursor abundance, and reporter ion signal), indicating that the method should be applicable to analysis of oligosaccharides for which standards are unavailable. A collision energy trendline for the largest bovine milk oligosaccharides, which ionize as 3+ ions, is under final optimization. The final method will have the capability to quantify the 19 most abundant bovine oligosaccharides, including fucosylated structures in trace abundance.

Relevance to the dairy industry

This work is important for the dairy industry because it will identify heritability and genetic correlations for oligosaccharides which can be used for optimizing breeding programs. In the absence of specialized software for automated oligosaccharide identification (similar to that already available for proteomics and genomics), novel tools need to be integrated into current investigative workflows to make such large studies possible.