TANDEM MASS SPECTRAL ANALYSIS OF FREE OLIGOSACCHARIDES IN MILK OF VARIOUS MAMMALS

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U.S. Department of Commerce

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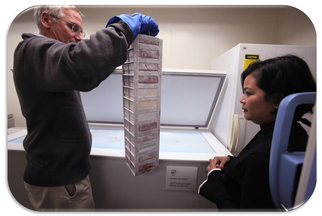
INTRODUCTION

Human milk is regarded as the gold standard for infant feeding.

Human milk oligosaccharides feed good bacteria in the gut where 70% of the immune system exists [1].

NIST SRMs



Smithsonian National Zoo Milk Repository

This study focuses on the identification and molecular fingerprinting of oligosaccharides derived from the milk of mammals using the Hydrophilic Interaction Liquid Chromatography and Mass Spectrometry for building NIST prebiotics/glycans MS library.

EXPERIMENTAL

Human milk SRM 1953
- ➢Removal of lipids & proteins

Sample reduction & clean up
- ➢SPE removes salts. 20% and 40% acetonitrile separates neutral and acidic oligosaccharides.

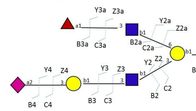
Analysis of HMOs & BMOs by HILIC-ESI-MS
- ➢Identification of neutral and acidic oligosaccharides.

Molecular structure analysis using spectral library and SIM Glycan

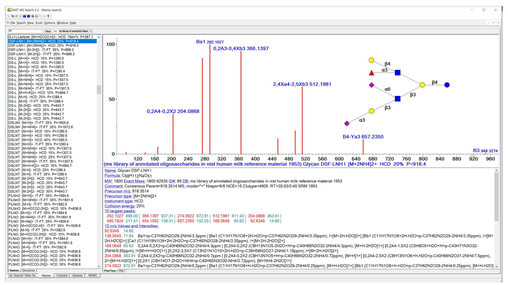
Building of NIST oligos MS Library

Nomenclature of oligosaccharide

- Glucose
- Galactose
- GlcNAc
- Fucose
- NeuAc



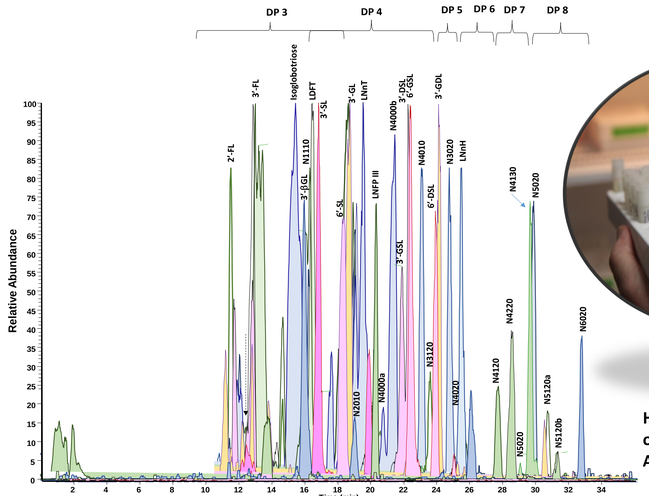
NIST Tandem MS Library



- ➢High resolution MS^2 values
- ➢negative and positive mode
- ➢Different collision energies
- ➢Different adducts

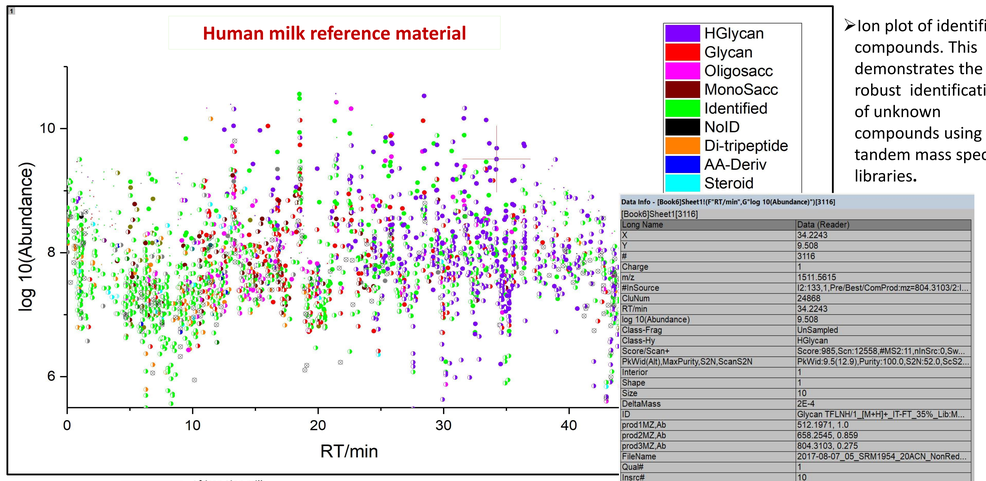
RESULTS

I. Separation based on size, net charge & type of oligosaccharides



HILIC MS profile of oligosaccharides present in African lion milk.

II. Data analysis using different analytical platforms



➢Ion plot of identified compounds. This demonstrates the robust identification of unknown compounds using tandem mass spectral libraries.

Data Info - [Book6]Sheet1!{F"RT/min",G"log 10(Abundance)"}[3116]

[Book6]Sheet1[3116]

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Y	9.508
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m/z	1511.5615
#InSource	[2:133,1,Pre/Best/ComProd:mz=804.3103/2:1...
CluNum	24868
RT/min	34.2243
log 10(Abundance)	9.508
Class-Frag	UnSampled
Class-Hy	HGlycan
Score/Scan+	Score:985,Scn:12558,#MS2:11,nInSrc:0,Sw...
PkWid(Alt),MaxPurity,S2N,ScanS2N	PkWid:9.5(12.9),Purity:100.0,S2N:52.0,ScS2...
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Shape	1
Size	10
DeltaMass	2E-4
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prod1MZ,Ab	512.1971, 1.0
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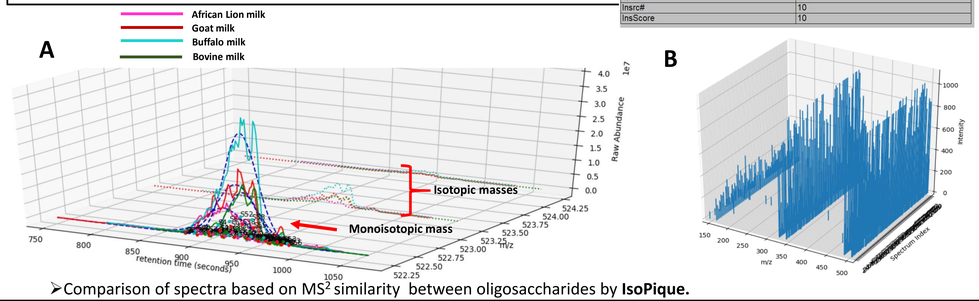
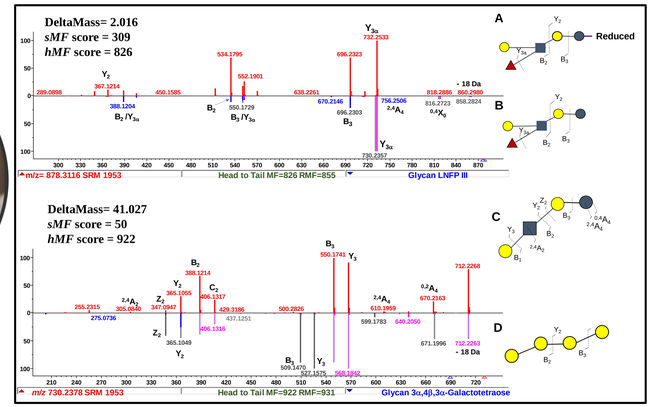


➢Comparison of spectra based on MS^2 similarity between oligosaccharides by **IsoPique.**

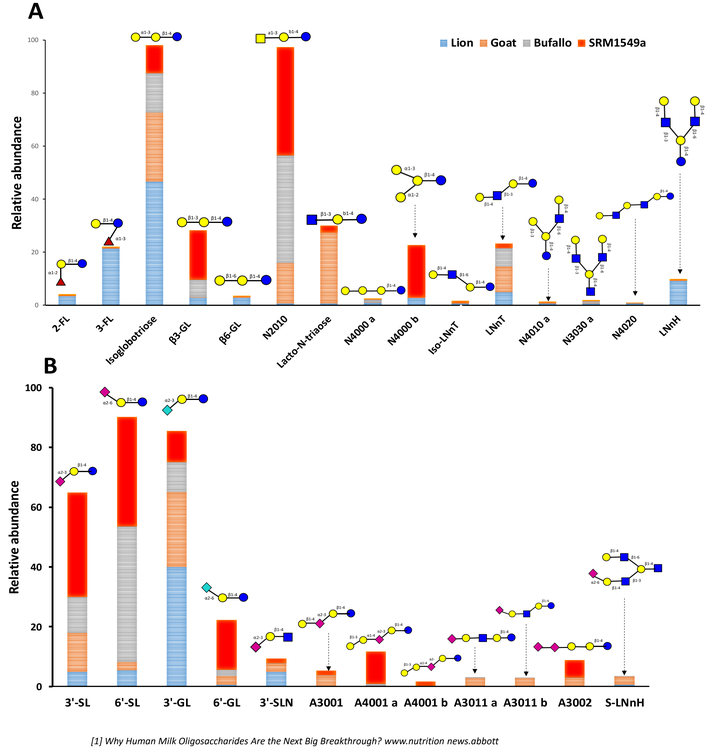
III. Hybrid MS Library Search facilitates the identification of unknown oligosaccharides



➢**Score or match factor** (MF) provides the degree of structure similarity.

➢**Error tolerance** of below 10 ppm.

IV. Quantitative analysis of oligosaccharides in milk of various mammals



CONCLUSIONS

- ➢In summary, 140 of human milk oligosaccharides and over 70 non human oligosaccharides have been identified in milk of various mammals.
- ➢The HILIC-ESI-MS method in combination with NIST MS search engine can now be used to accurately determine various fucosylated and sialylated oligosaccharides present in infant formula and other domestic animal milk.

[1] Why Human Milk Oligosaccharides Are the Next Big Breakthrough? www.nutrition news.abbott