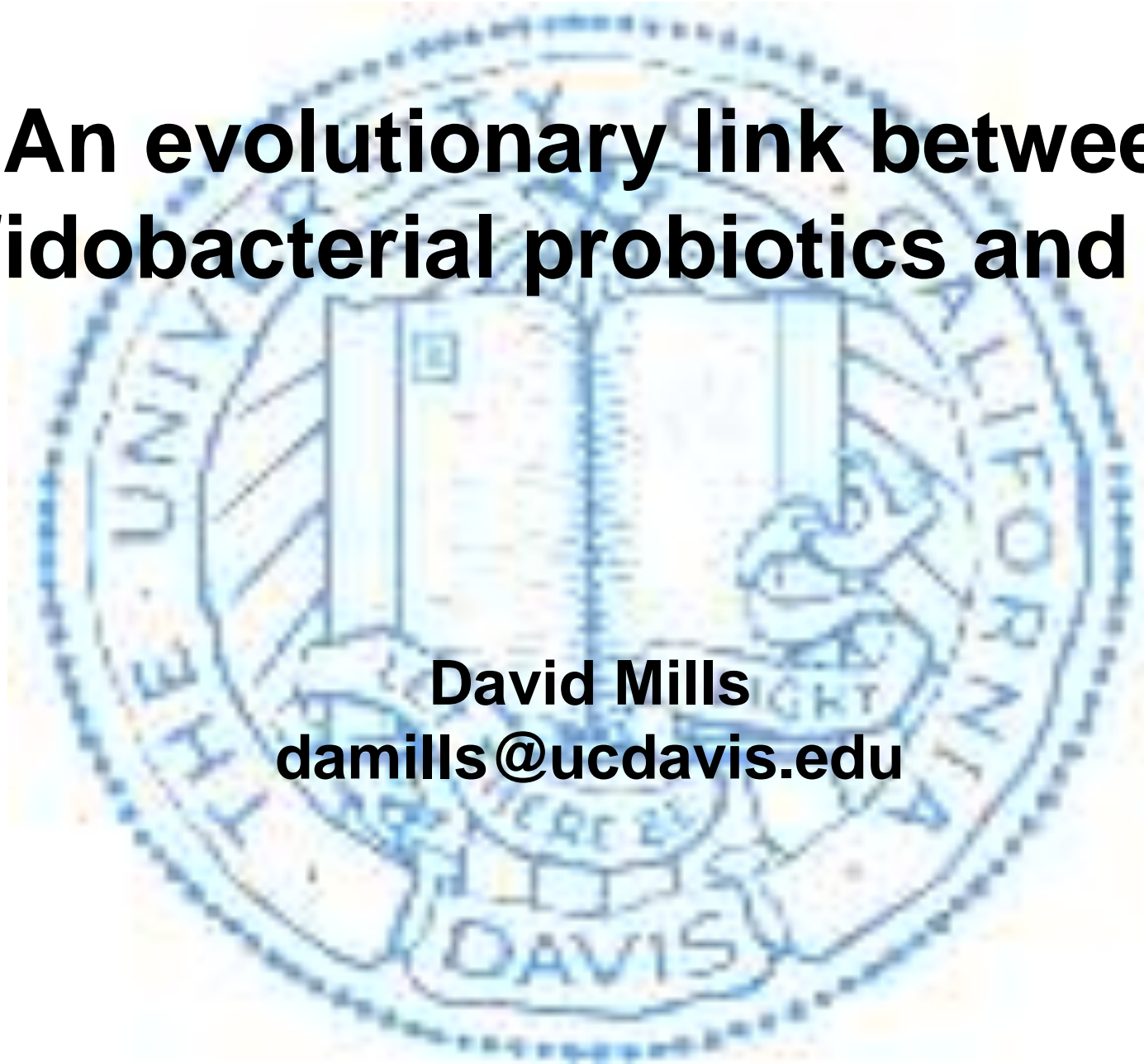


An evolutionary link between bifidobacterial probiotics and milk

David Mills
damills@ucdavis.edu



Probiotics are historically linked to dairy products, however...

- Many controlled studies have documented effects of probiotics **not delivered in dairy ingredients**
- Probiotic supplements are available in other forms, including capsules, chewable tablets, freeze-dried powders, wafers and beverages



Risk to losing probiotics to other foods?

Food & Beverage

Vitamin Fortified
Probiotic Fruit Juice
Contains Organic Fruit Juices Only

British
Batteries

Are there more overt links between dairy and probiotics?

Bio-Kefir

Orange juice

Health & Nutrition



Animal Health & Nutrition



Consumers' Guide



Documentation



Contact



well-documented probiotic BB-12[®].

Shelf life is an issue

Ensuring the dose of probiotics remains optimal throughout the product's shelf life was key to a successful, so extensive and thorough trials at CH Hansens's facilities in the UK have been done.



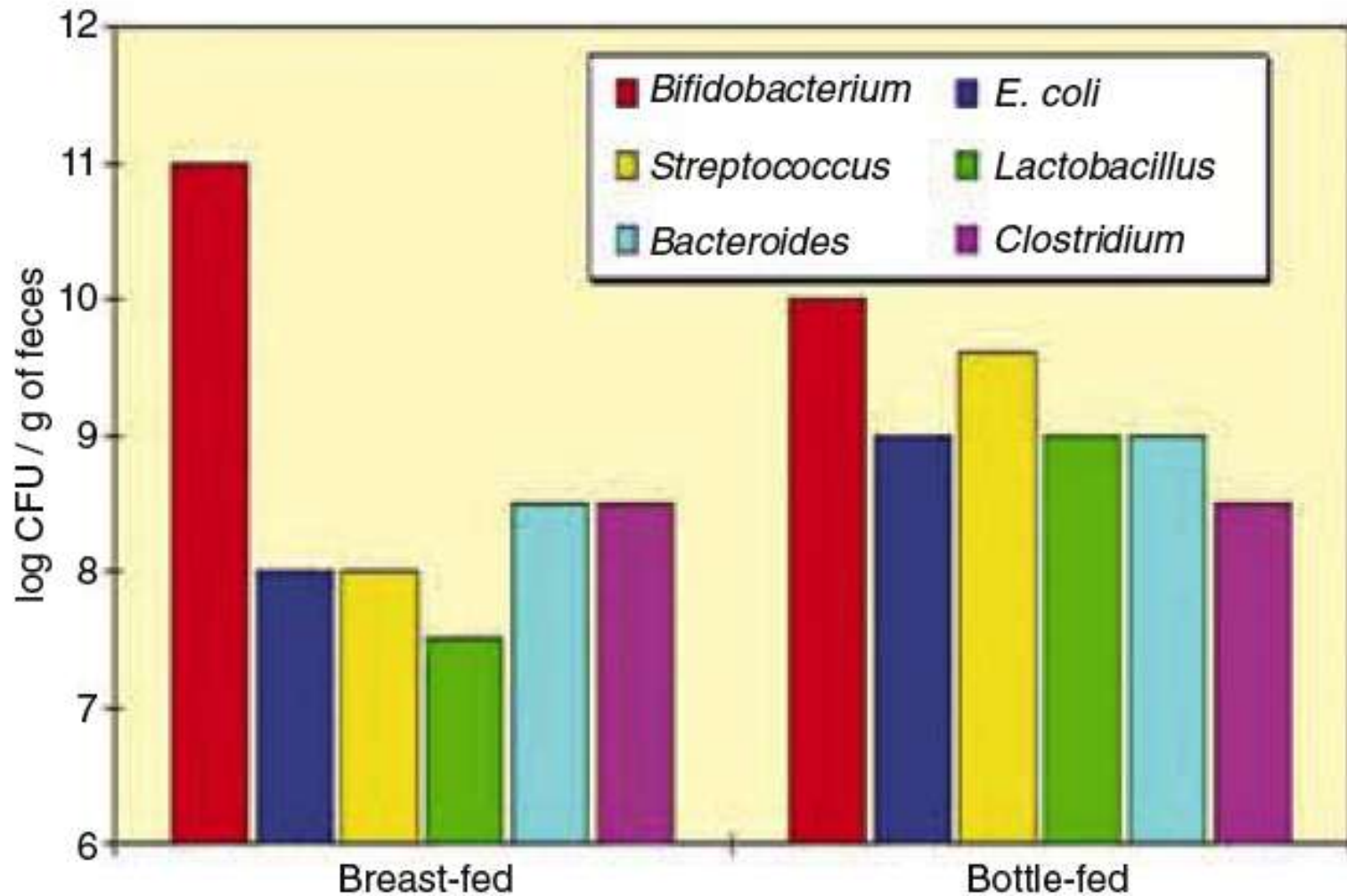
Where do you look for connections
between milk and beneficial
bacteria (probiotics)?

Let evolution be your guide

Human milk and baby guts!



Breast milk enriches bifidobacterial populations

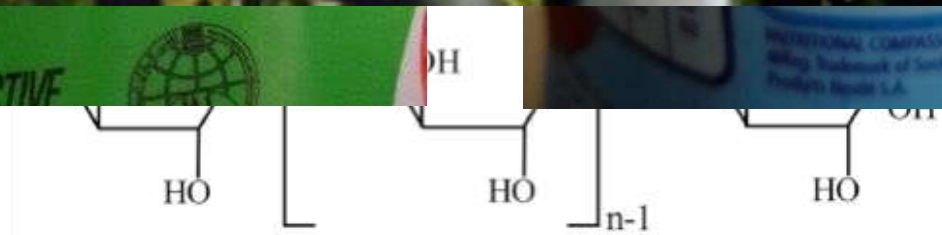


Biavati and Mattarelli 2006

Something in milk must
be acting as a prebiotic
to enrich bifidobacteria

fructooligosaccharides

Inulin



Human Milk Constituents

- Lactose ~70 g/L
- Fats ~40 g/L (glycolipids)
- Oligosaccharides ~5-15 g/L
- Proteins ~8 g/L (glycoproteins)

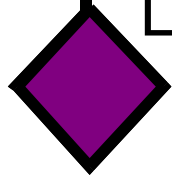
Human milk oligosaccharides

Nearly 200 compositions in pooled breast milk

80% fucosylated and many
large oligos (DP > 4)

10-15% sialylated

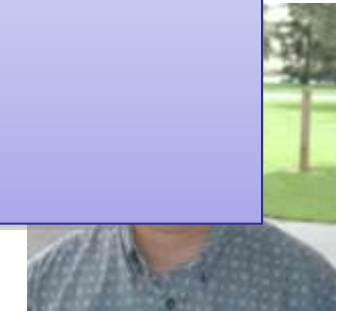
α 2-3/6



Sialic acid

Lactosamine
0-25 units
(DP)

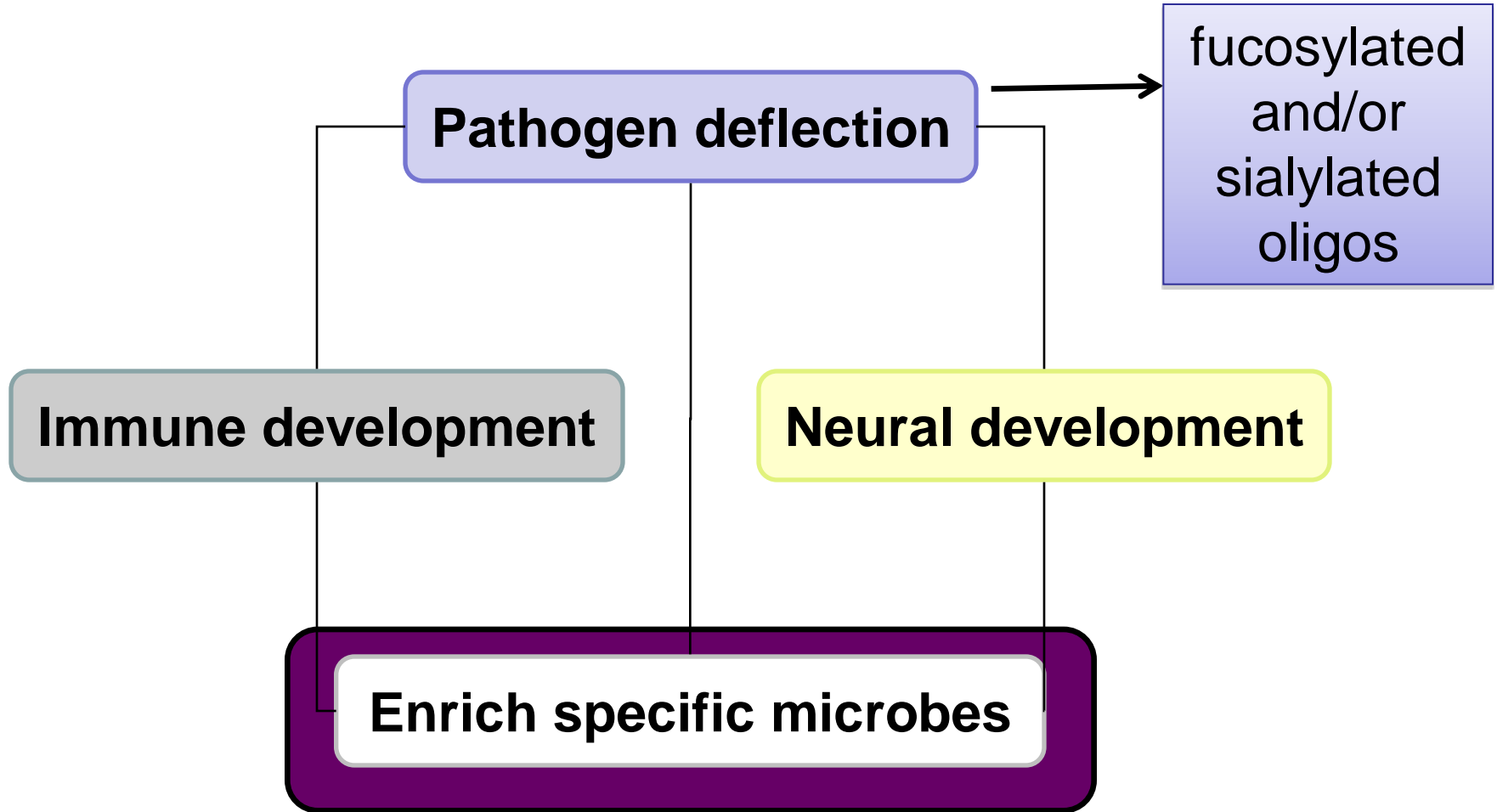
Lactose



Carlito Lebrilla
UCD Chemistry

Why Make Oligosaccharides?

(if they are not consumed by the infant)



Are there differences in growth
on HMOs of bifidobacteria vs.
other gut bacteria?

What bacteria grow with HMOs as a sole carbon source?

Select gut microbes	Clostridial sp.	No
	Lactobacillus sp.	Weakly
	Eubacterium sp.	No
	Streptococcus sp.	No
	Escherichia coli	No
	Enterococcus sp.	No
	Bacteriodes sp.	Yes
	Bifidobacterial sp.	Yes

Are oligos consumed by
bifidobacteria *in situ*?

Feces from breast fed infant

```
graph TD; A[Feces from breast fed infant] --> B[Glycoprofile]; A --> C[Microbial ecology]
```

Glycoprofile

Microbial
ecology

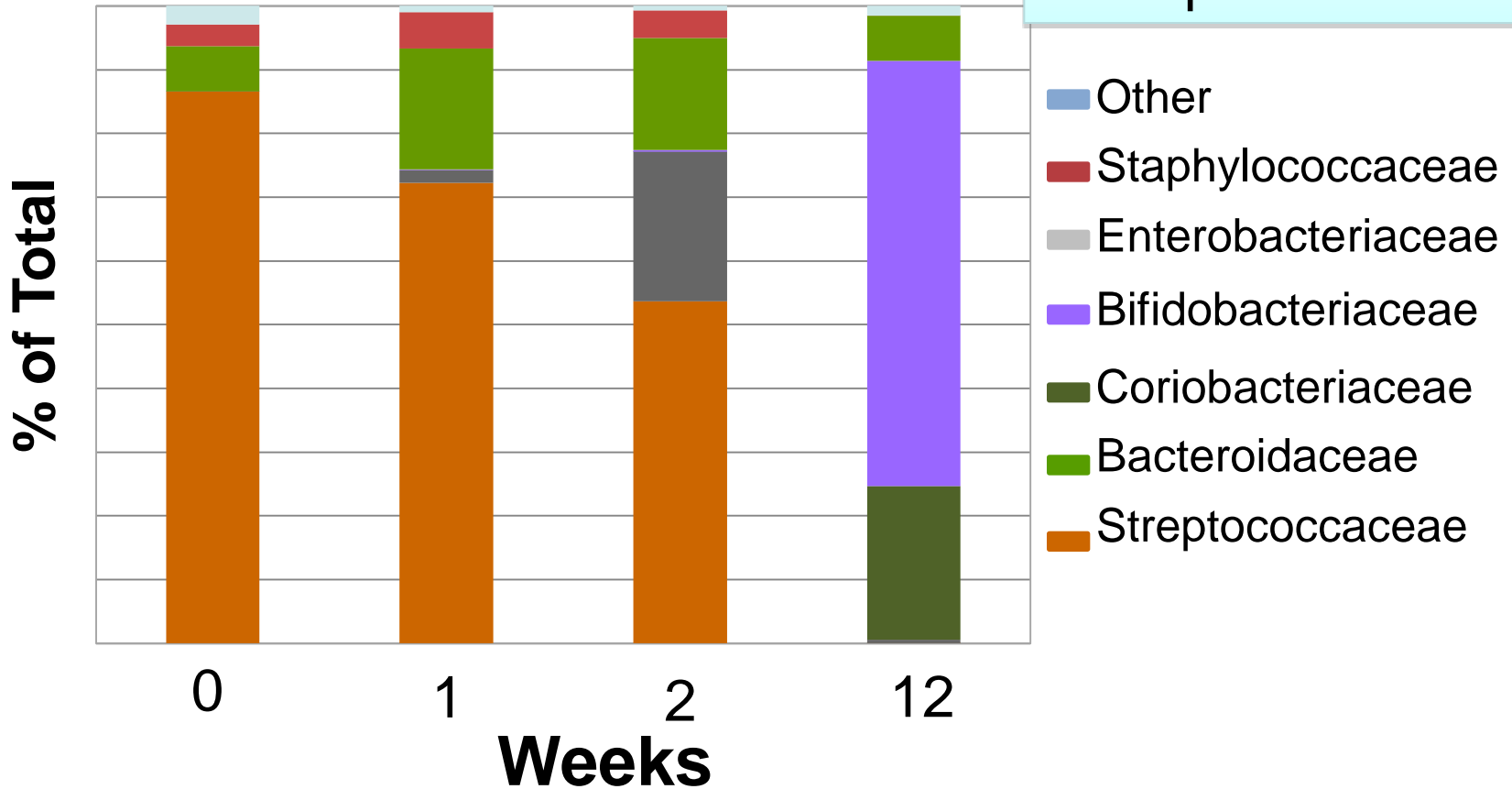
Are oligos missing in feces in which
bifidobacteria are dominant?

Infant microbial succession over breastfeeding

USDA #2

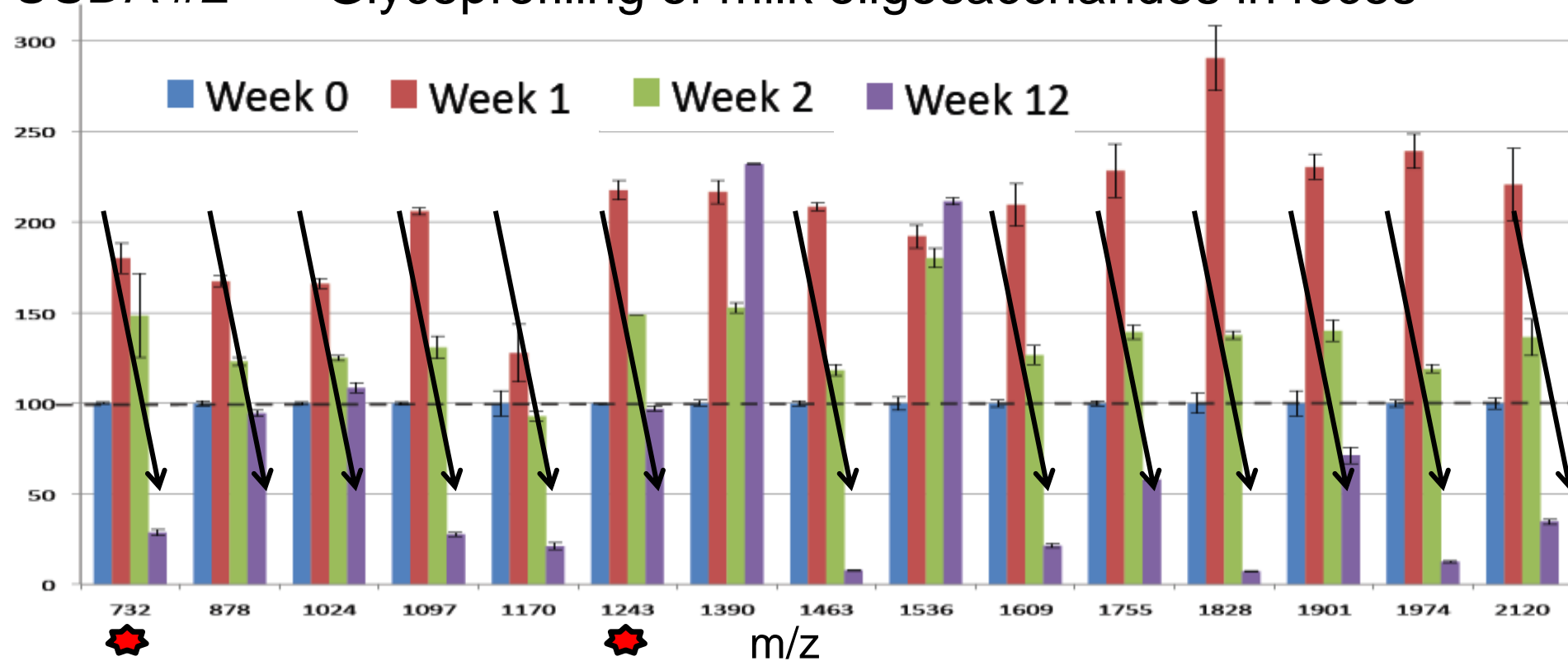
Pyrosequencing of 16S rDNA

Bacterial families present



Infant microbial succession over breastfeeding

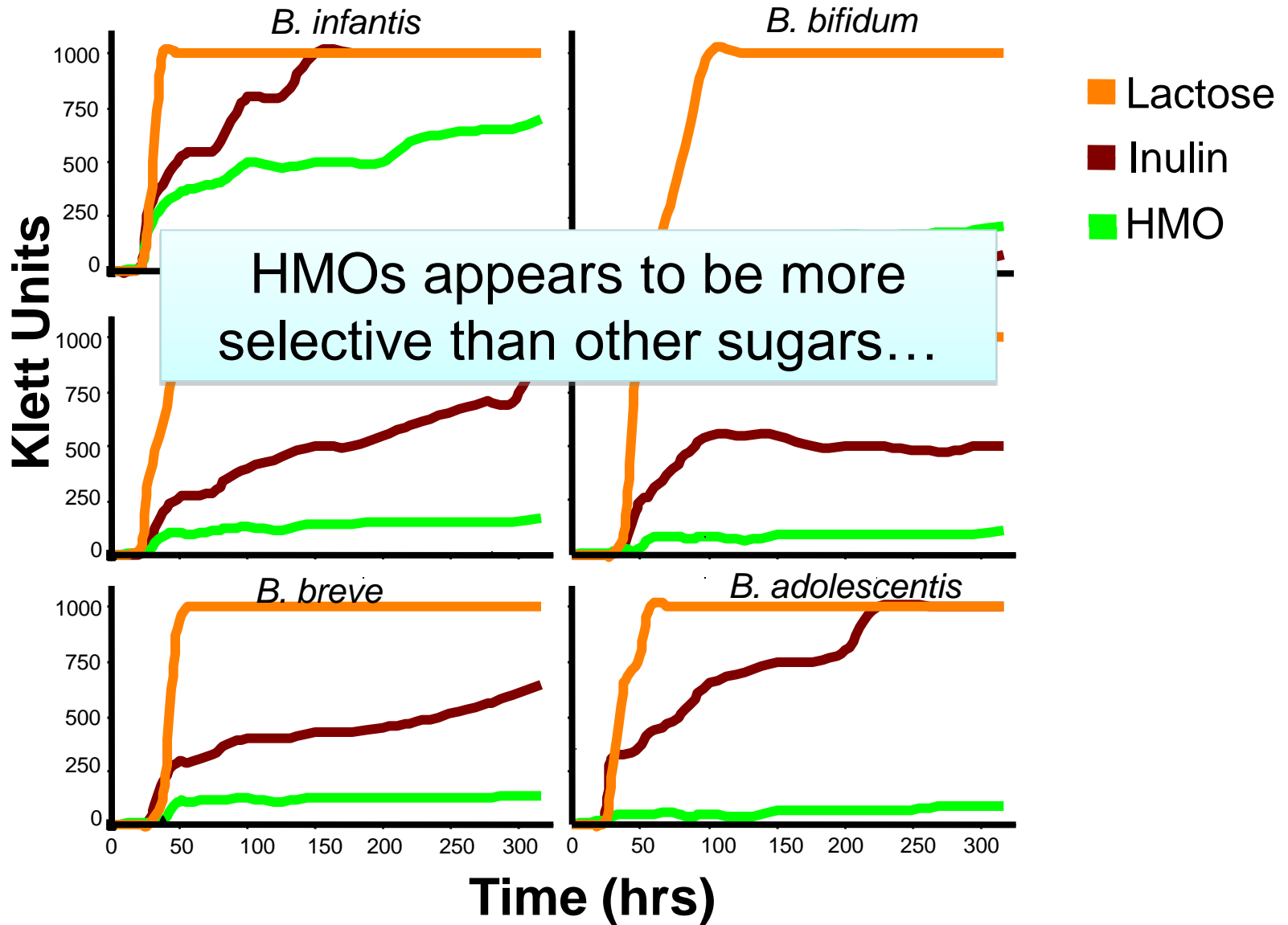
USDA #2 Glycoprofiling of milk oligosaccharides in feces



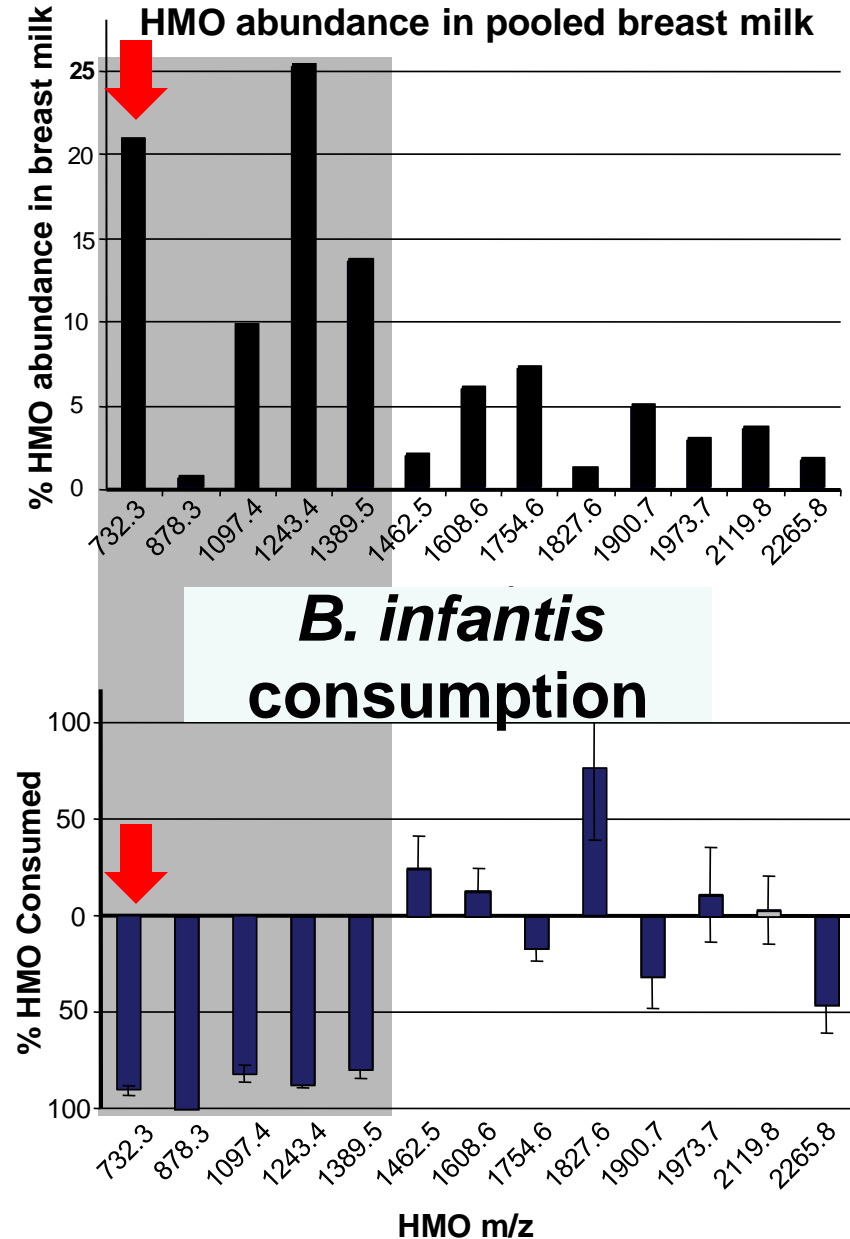
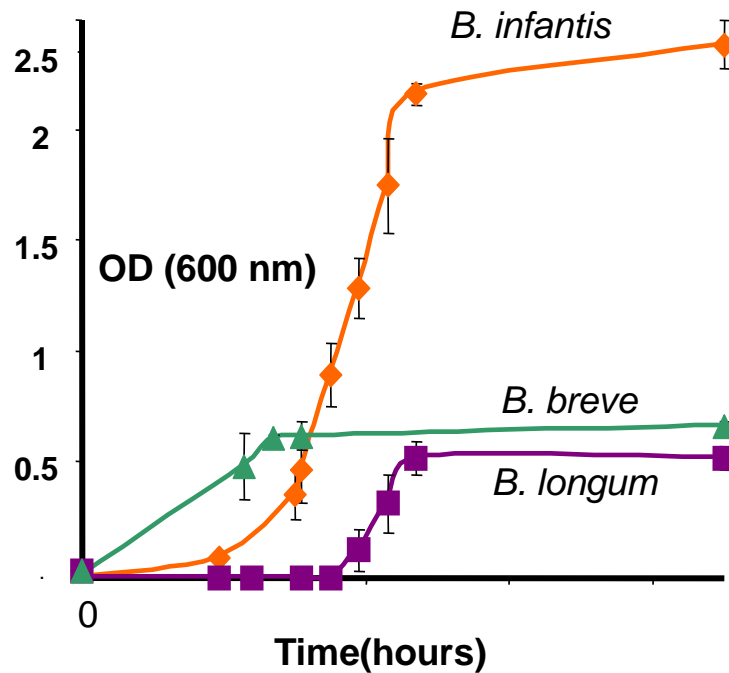
Different oligosaccharide compositions in feces as determined by Mass Spec

Do bifidobacterial species isolated from different GIT environments grow differently on HMO (i.e. adult vs. infant-borne)?

Bifidobacteria & HMOs



Bifidobacterial HMO Glycoprofiling

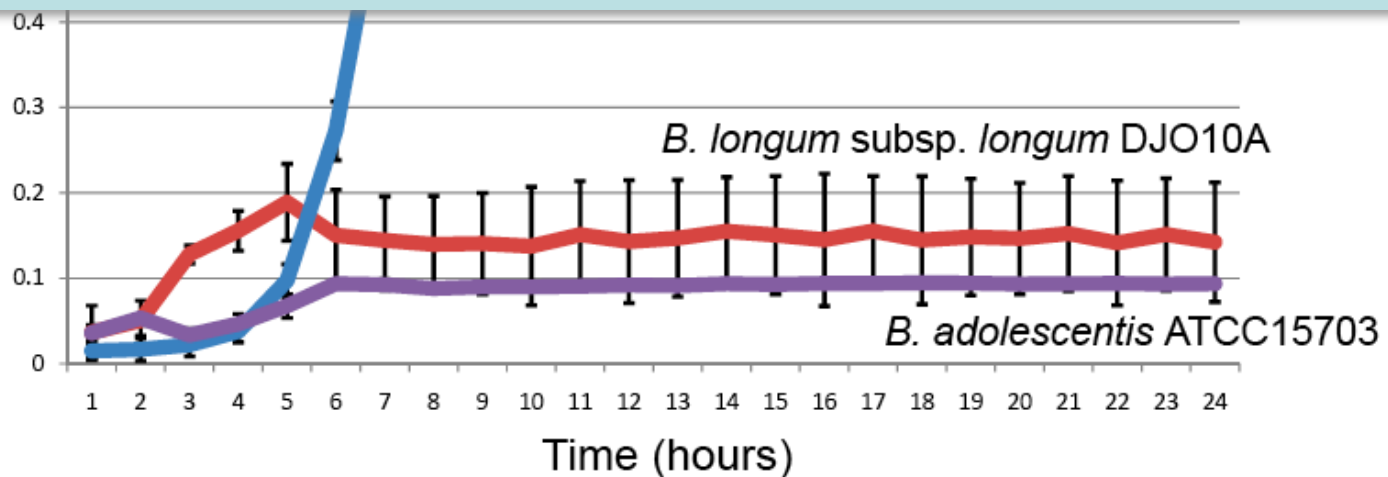


Several small MW oligosaccharides

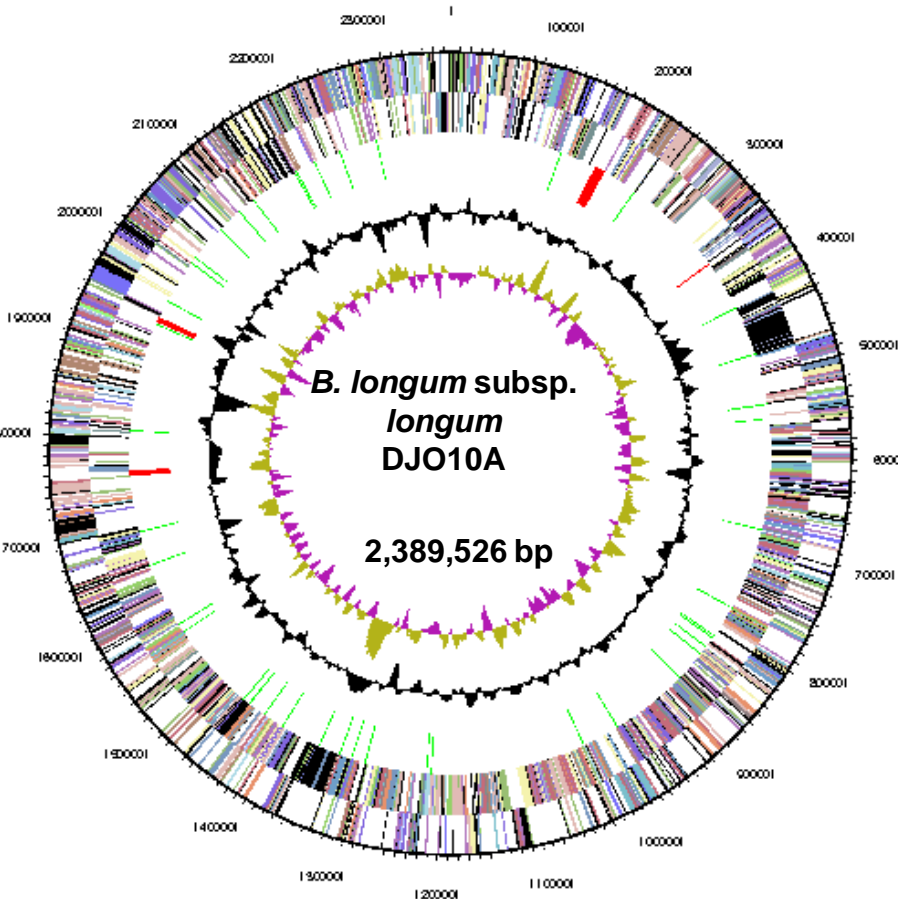
Single HMO composition consumed by other bifidobacteria

HMO utilization by Bifidobacteria

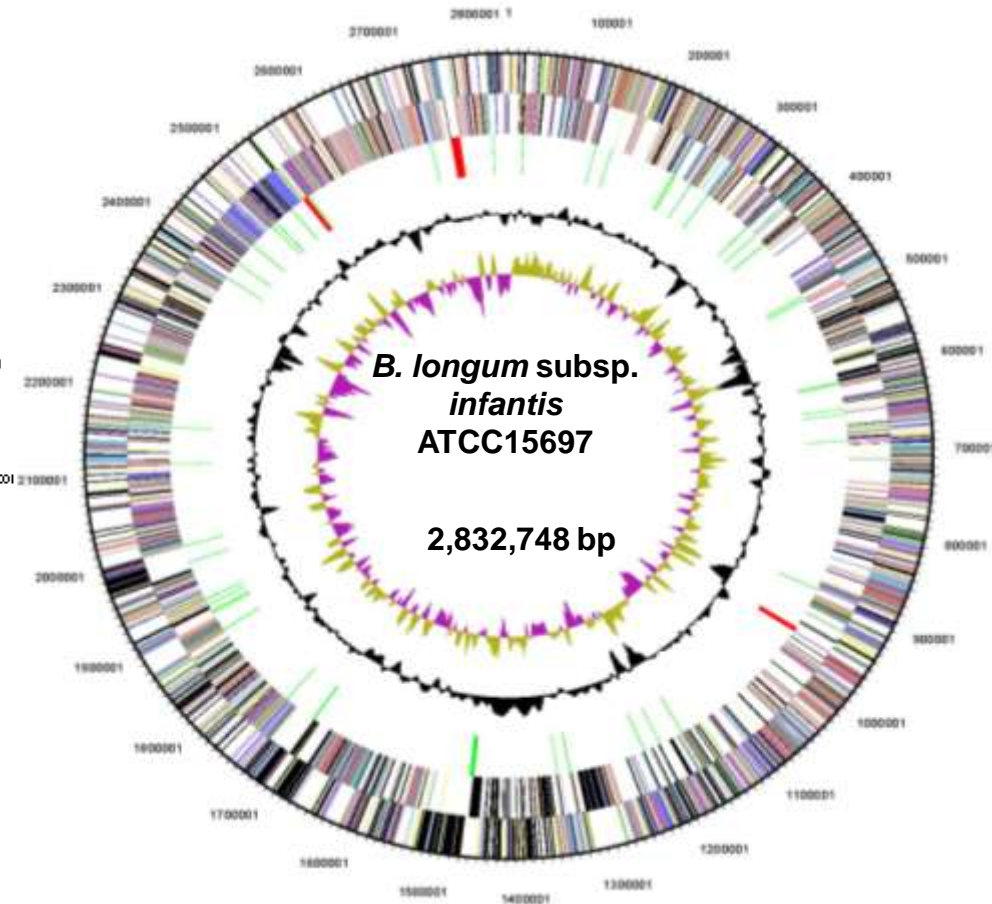
What genome features are required to utilize human milk oligosaccharides?



Comparative *Bifidobacterium* Genomics



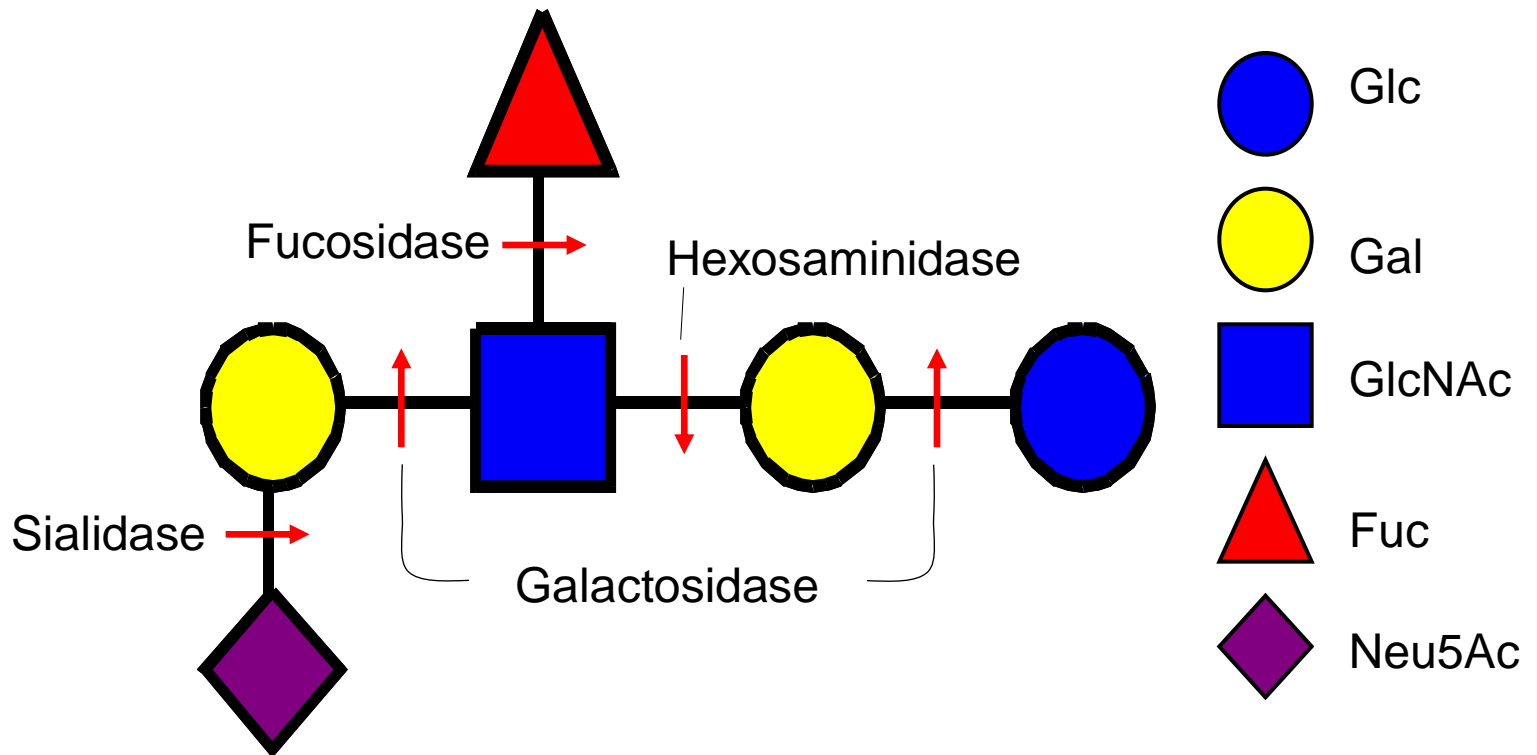
Adult derived strain
BMC Genomics 2008



Infant derived strain
PNAS 2008

What's Needed to Deconstruct HMO?

- Transport systems for oligo & monosaccharides
- Glycosyl hydrolases



Oligosaccharide Degradation Capacity

Plant-derived oligosaccharide catabolic genes

Transketolase	1	3
Beta-glucosidase	2	4
Alpha-glucosidase	3	5
Beta-galactosidase	4	6
Alpha-galactosidase	5	7
Xyloxyranase	6	8
Xyloxyranase	7	9
Xylose isomerase	8	10

Evolved to
consume
plant sugars

***B. longum* DJO10A**

Adult derived strain

Mammal-derived oligosaccharide catabolic genes

Beta-galactosidase	5	3
N-acetyl-beta-hexosaminidase	4	1
Alpha-galactosidase	3	0
Fucosidase	2	0
Sialidase	1	0
N-acetyl-beta-glucosaminidase	0	0
Sialidase	0	0
N-acetyl-beta-glucosaminidase	0	0
Enzyme	0	0

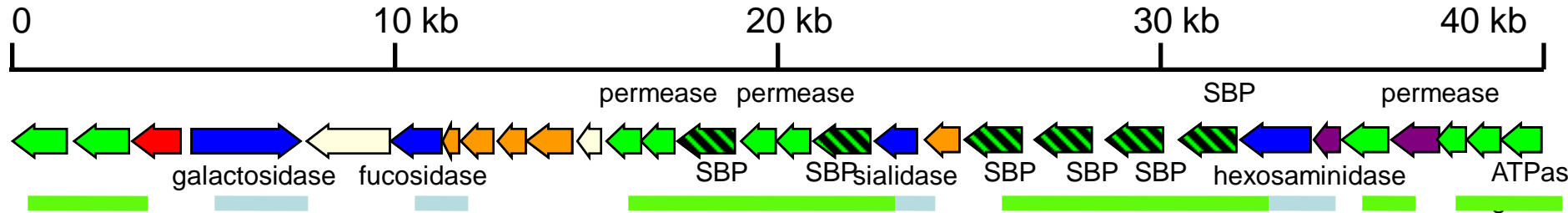
Evolved to
consume
milk sugars

***B. infantis* UCD272**

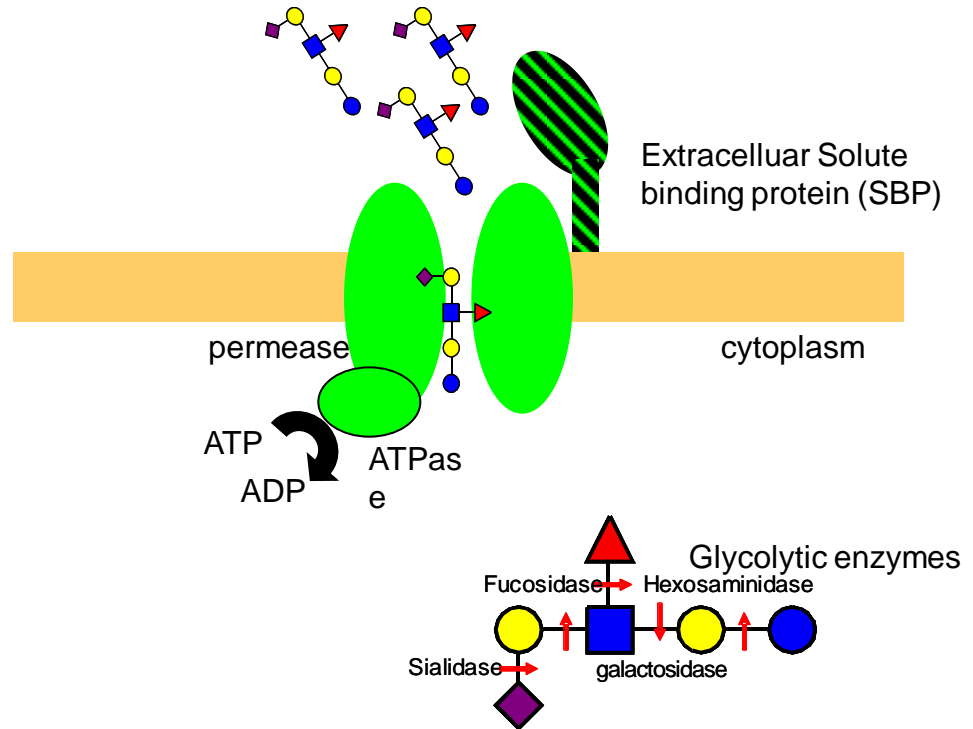
Infant derived strain

HMO cluster 1

All 4 glycosyl hydrolases Array of oligosaccharide transporters

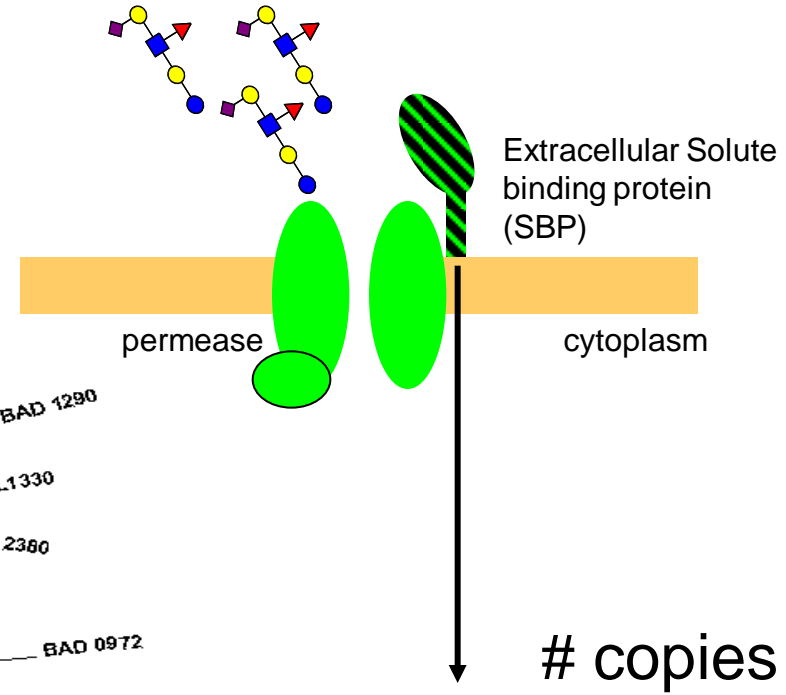
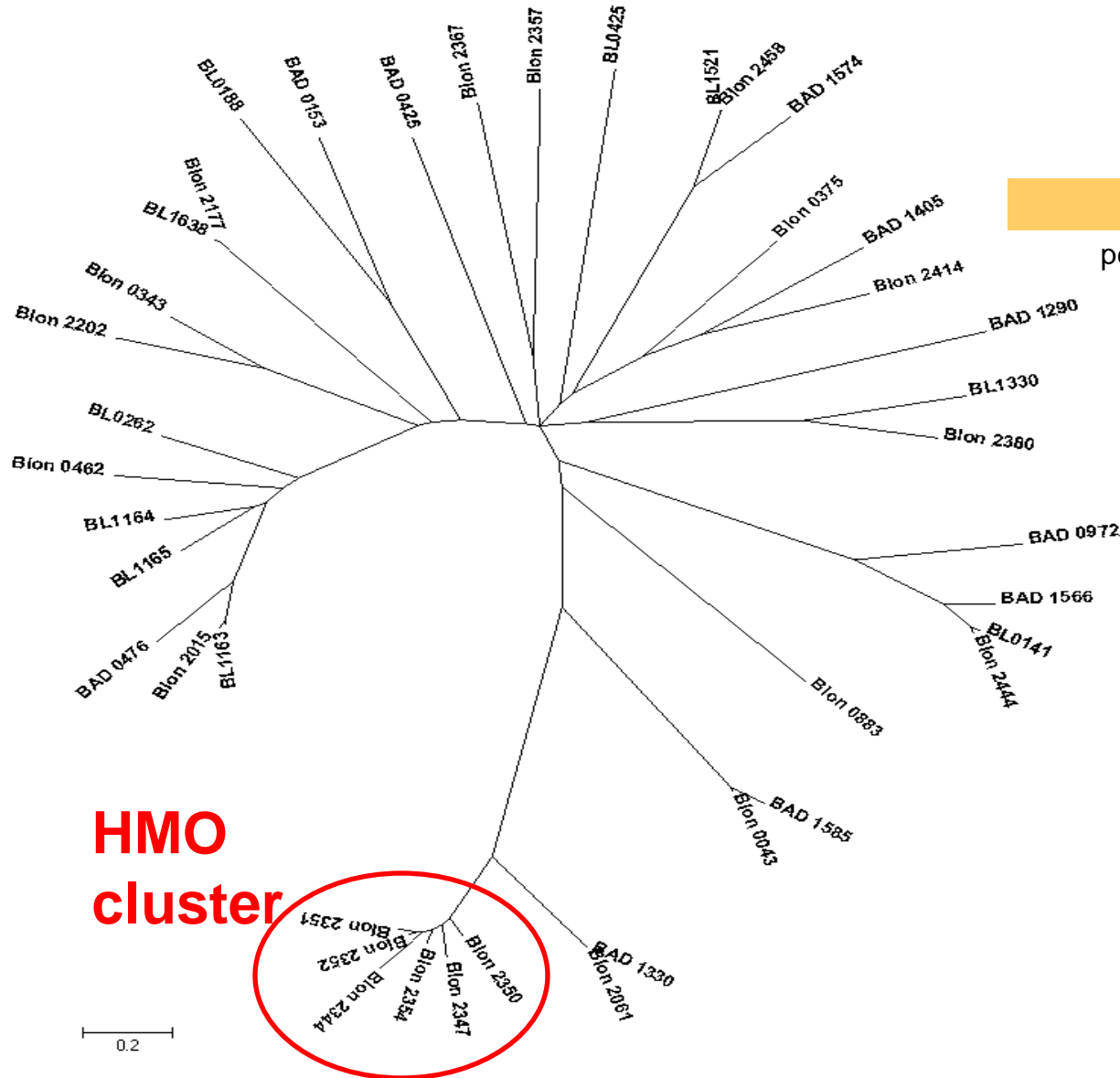


- HMOs are bound by SBP lipoproteins proximal to permeases
- ATP hydrolysis prompts transport of oligosaccharides across membrane
- Intracellular glycolytic enzymes deconstruct oligosaccharide



HMO cluster SBPs

Family 1 (oligosaccharide binding)

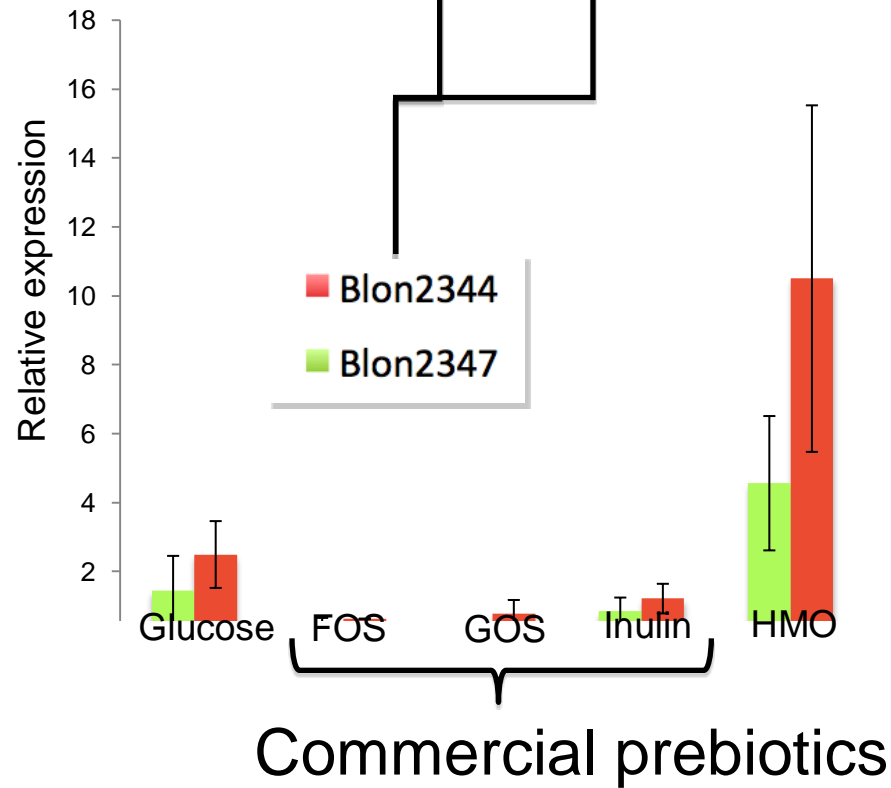
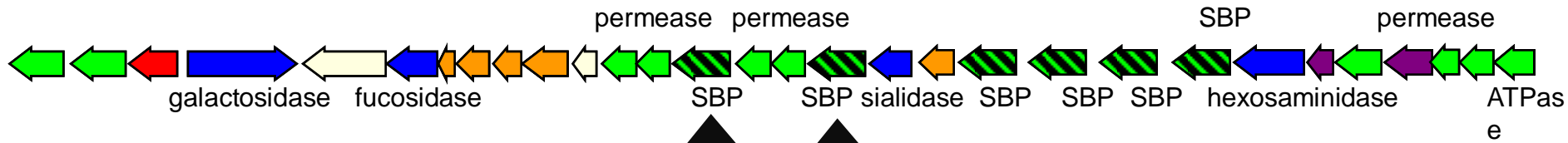


B. infantis 20

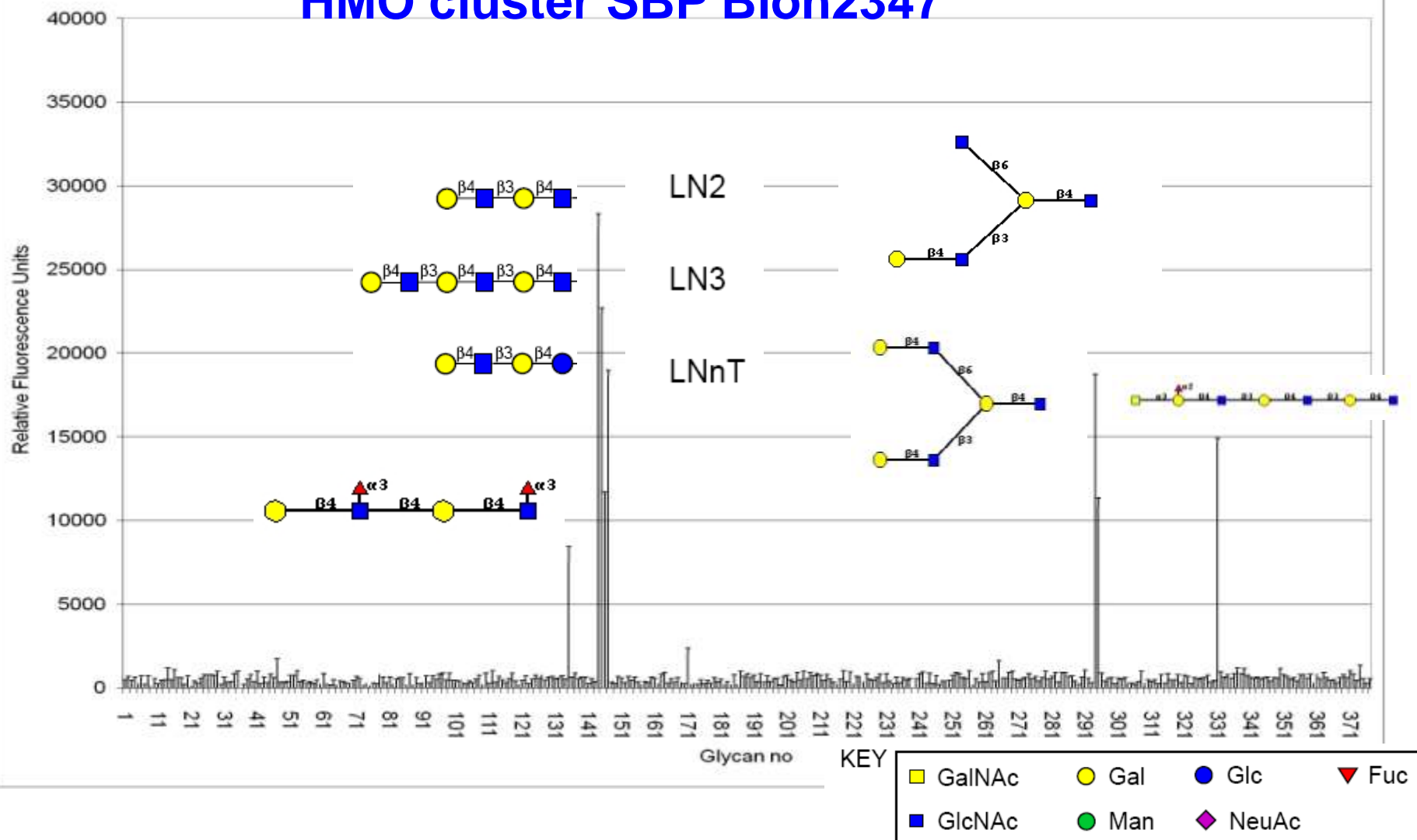
B. longum 10

B. adolescentis 11

SPB expression in *B. infantis*



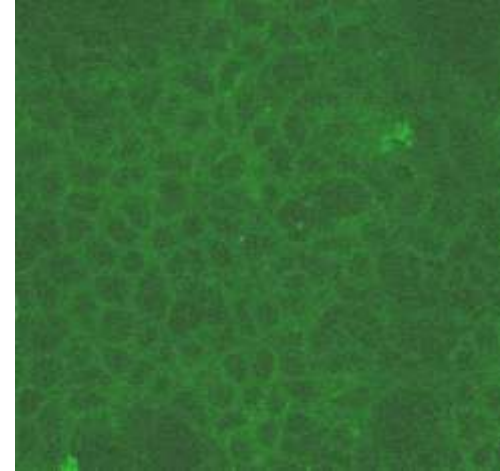
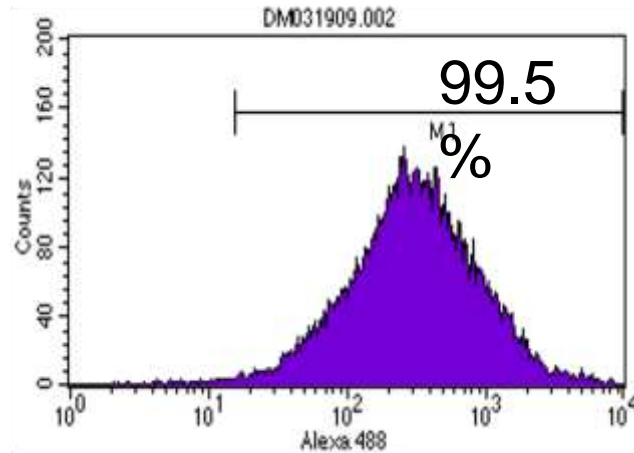
HMO cluster SBP Blon2347



SBP Blon2347 binding to Caco2 cells

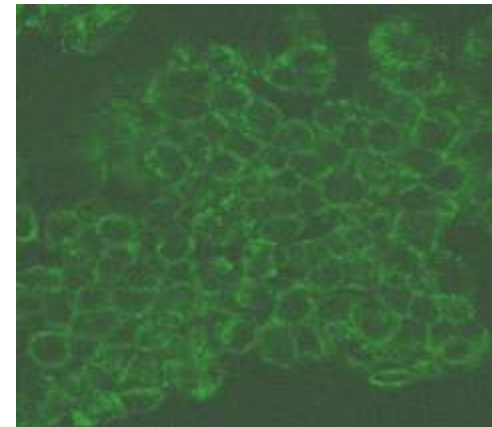
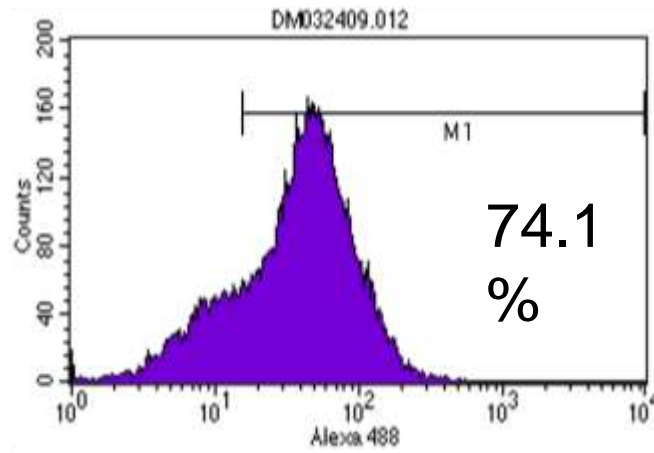
Caco-2 +
UEA lectin
(*Ulex europaeus*
agglutinin)

Major epitope:
Fuca1-2Galb1-4GlcNAc



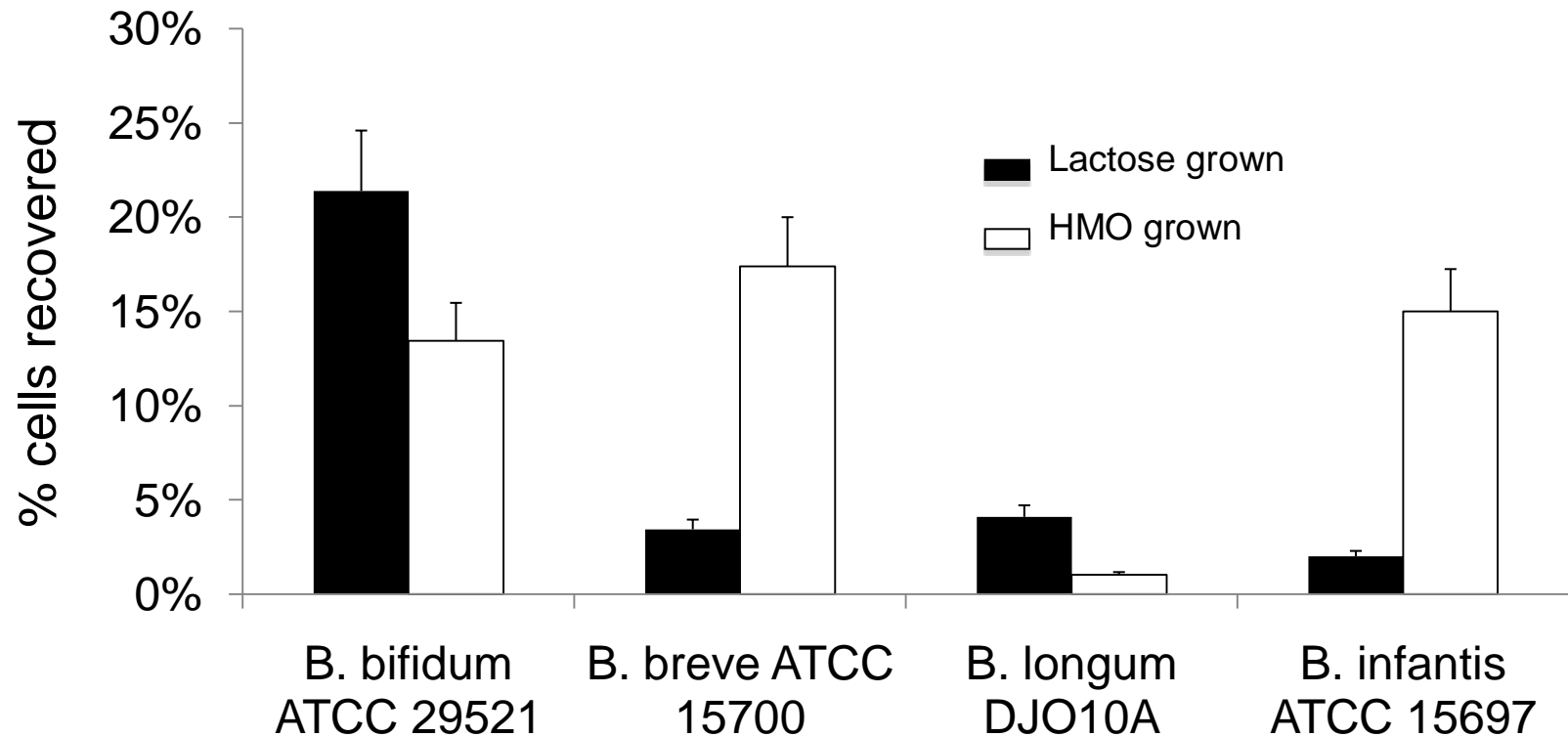
Caco-2 +
Blon2347

GalNAc1-3(Fuca1-2)Galb1-4GlcNAcb1-
3Galb1-4GlcNAcb1-
3Galb1-4GlcNAc

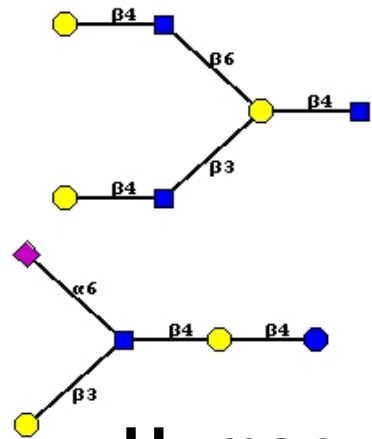


**Does consumption of HMO
encourage colonization by
Bifidobacteria?**

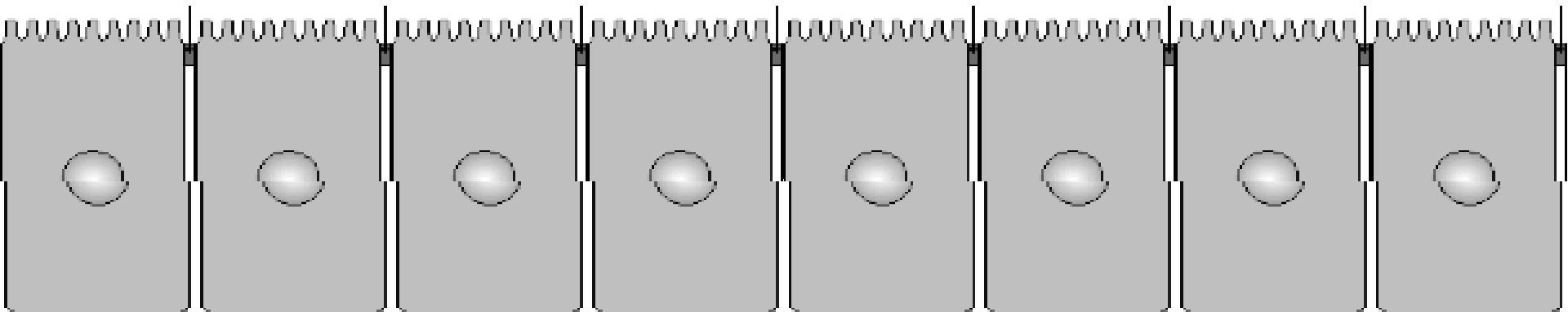
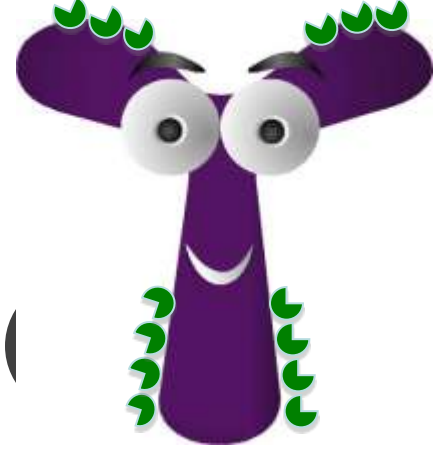
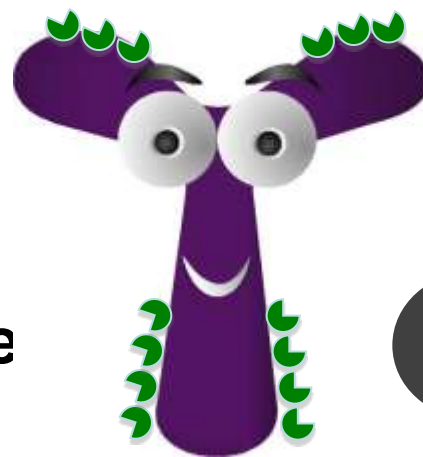
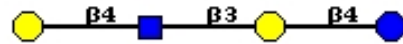
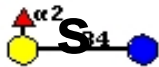
Growth on milk oligosaccharides helps bifidobacteria bind intestinal cells



Model for *B. infantis* enrichment in the infant GIT



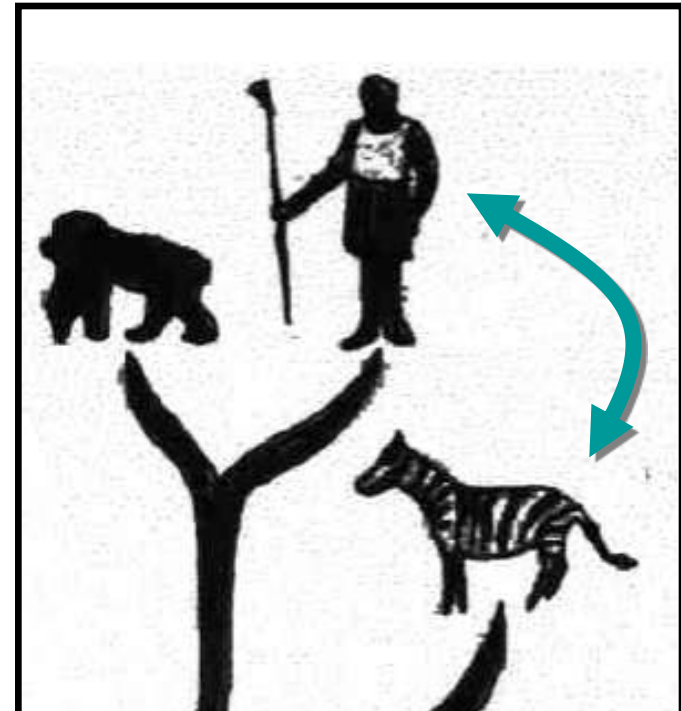
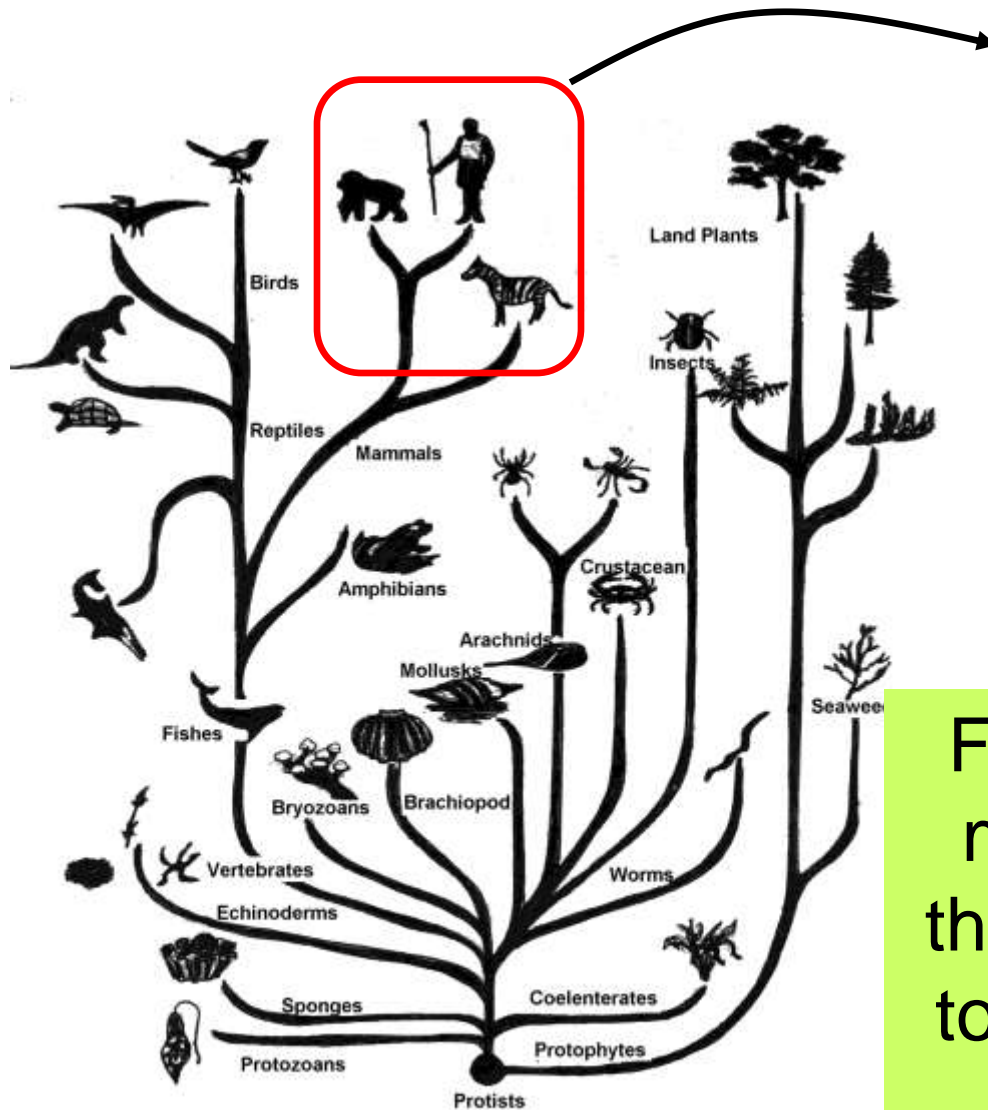
Human milk
oligosaccharide



Dave...the human milk research
is great...

...but human milk is not for sale at
my local grocery store

Value Proposition for the Dairy Industry



Find (or modify) bioactive molecules in bovine milk that serve similar functions to those present in human milk

Bovine Milk OS vs. Human Milk OS

- Compared to human milk, the concentration of BMO is 20 fold lower.

- **Human Milk**: Colostrum (20-23g/L) ; Mature Milk (12-14g/L):

majority are fucosylated (50-75%)

~200 species identified

- **Bovine Milk**: Colostrum (0.7-1.2g/L); Mature Milk (trace amount)

No fucosylated BMOs identified

Source. FSA.2002

Vet,R. J. Chromatogr. 212: 313-322

Urashima etc, Glycoconjugate Journal, 18,357,2001

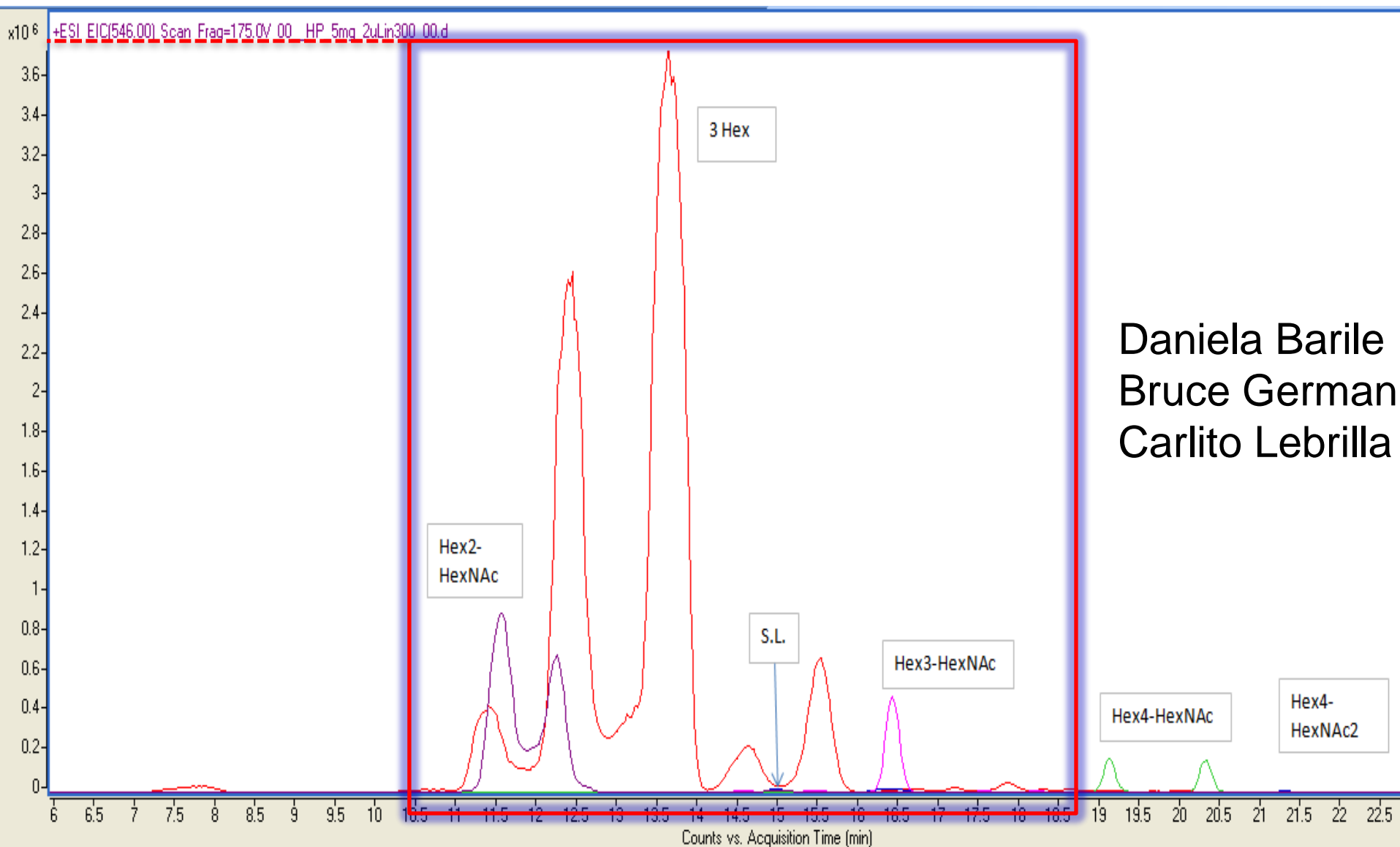
Boehm etc, Ch9 Oligosaccharides, CRC Press LLC, 2003

Justine Butler,T.Colin, Jane Plant. Vegetarian & Vegan Foundation 2006

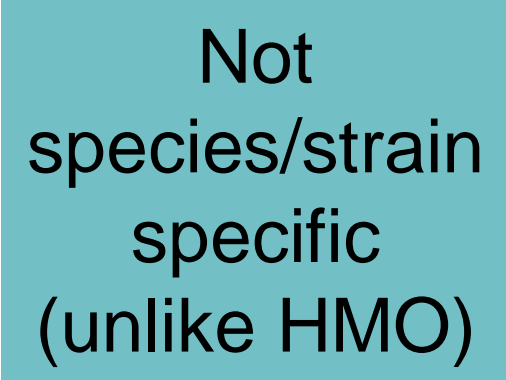
Tao et al., J. Dairy Science, 2008

Barile et.al. J. Dairy Science, 2010

BMO profile - lactose reduced/removed

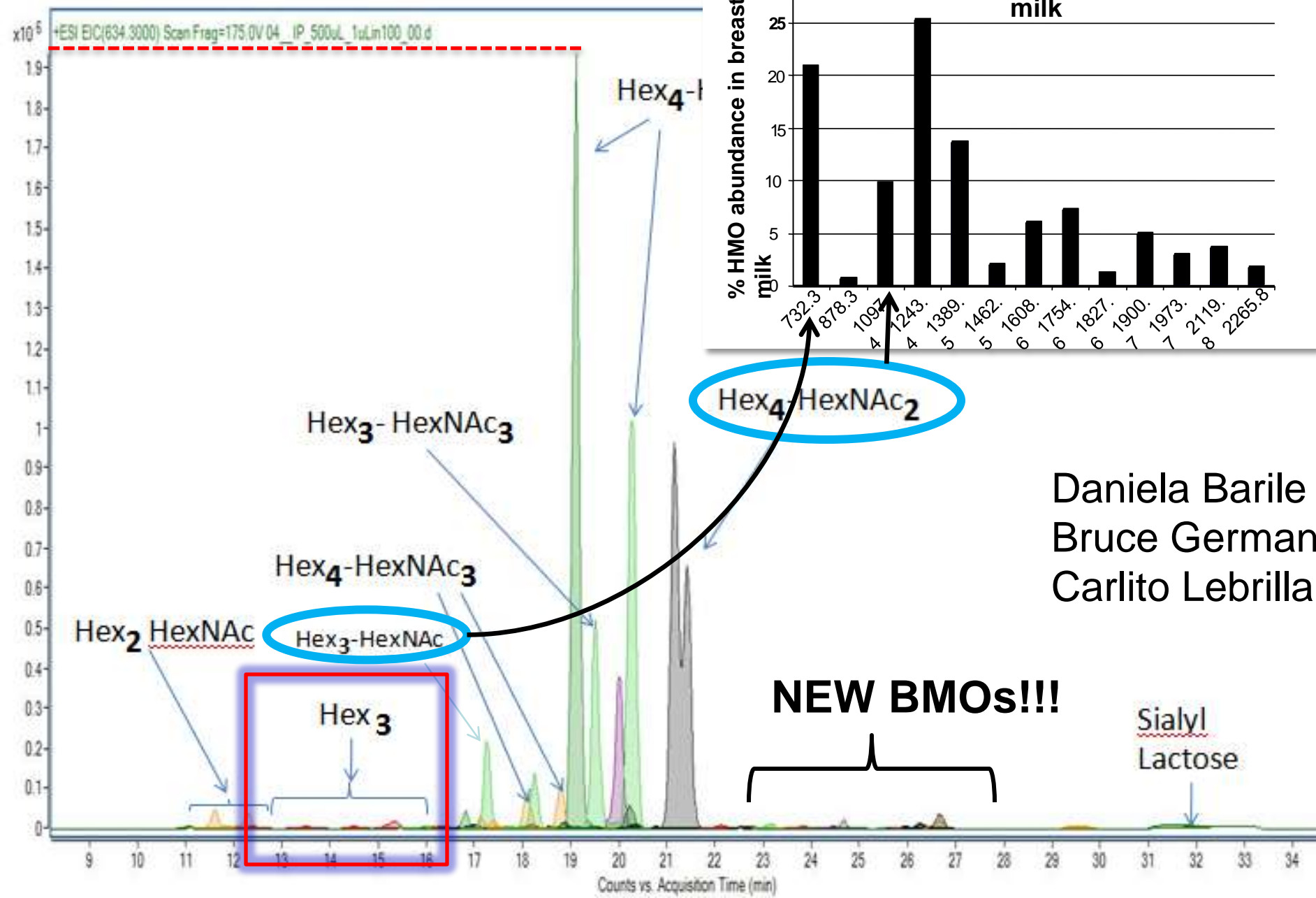


What bacteria grow/ferment (triose-dominant) BMOs as a sole carbon source?

Select gut microbes	Clostridial perfringens	No	
	Salmonella typhimurium*	No	
	Vibrio cholerae*	No	
	Listeria monocytogenes	No	
	Escherichia coli (Lac ⁺)*	Yes	
	Escherichia coli (Lac ⁻)*	No	
	Bifidobacterial sp.	Yes	
	Lactobacillus acidophilus	Poorly	

* Tested for fermentation via MacConkey agar

BMO profile - lactose and



Daniela Barile
Bruce German
Carlito Lebrilla

BMOs have many oligos similar to HMOs

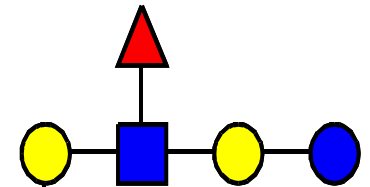
Existing HMO-like compositions

m/z [M+Na] ⁺	Hex	HexNAc	NeuAc (sialic acid)	Fuc	DP
527.133	3	0	0	0	2
568.154	2	1	0	0	2
656.156	2	0	1	0	2
730.179	3	1	0	0	3
771.197	2	2	0	0	3
892.196	4	1	0	0	4
933.213	3	2	0	0	4
1095.219	4	2	0	0	5
1136.234	3	3	0	0	5
1257.229	5	2	0	0	6
1298.241	4	3	0	0	6
1339.253	3	4	0	0	6
1419.225	6	2	0	0	7
1485.256	3	4	0	1	7
1499.184	4	4	0	0	7
1542.251	3	5	0	0	7
1645.240	4	4	0	1	7
1663.221	5	4	0	0	8
1688.24	3	5	0	1	8
1704.23	4	5	0	0	8
1745.228	3	6	0	0	8
1809.21	5	4	0	1	9
1850.227	4	5	0	1	9
1891.225	3	6	0	1	9

Sialyllactose

New discovery!

Large fucosylated oligosaccharides



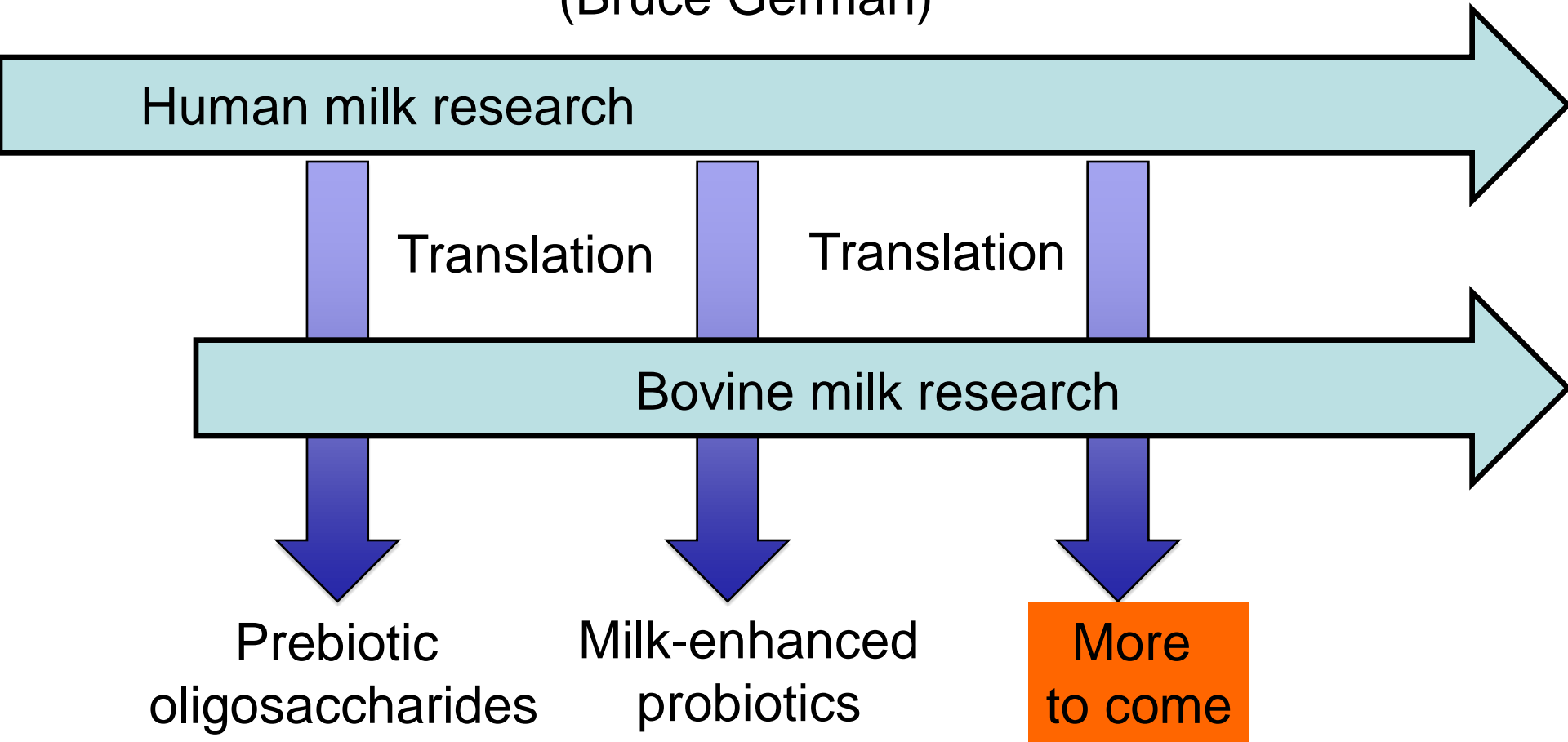
Daniela Barile
Bruce German
Carlito Lebrilla

BMO Summary

- Bovine milk oligosaccharides contain a major amount of triose sugars (sialyllactose and others)
- Triose-dominant BMOs enable growth of bifidobacteria non-specifically, and do not allow growth of most pathogens (tested)
- Further separation of BMO fractions to remove trioses has revealed oligosaccharides which resemble human milk oligos...*including previously unknown large fucosylated oligosaccharides*

UC Davis Milk Bioactives Program

(Bruce German)



We are not the only ones who think BMOs are potentially useful...



Prevention of Infection By Bovine Milk Oligosaccharides

Primary Investigator: David Mills, University of California, Davis, CA, United States - US

Topic: [Create New Ways to Protect Against Infectious Disease](#)

Round: Round 4 – May 2010

David Mills of the University of California, Davis in the U.S. will test whether oligosaccharides found in cow's milk can be used to enrich nutritional strategies of children who have been weaned. While human milk contains oligosaccharides that have been shown protect breast-feeding infants, the older children could benefit from enrichment of intestinal microbiota to prevent intestinal diseases.

"There is no bigger test for humanity than the crisis of global health. Without compassion, we won't do anything. Without science, we can't do anything."

BILL GATES

- Learn More About Grand Challenges Projects
- Learn More About Grand Challenges Explorations

Viral/Bacterial Infections



Jennifer Doudna of the University of California, Berkeley in the U.S. test the ability of newly discovered RNA restriction enzymes to bind to specific RNA sequences inherent in a wide range of pathogens. If successful, this test could potentially be embedded on wickable paper to test human urine samples and produce a colorimetric readout diagnostic like a pregnancy test.

[Find Out More...](#)

Summary

- Dairy industry is ideally situated to capture value in probiotics because of **evolutionary links to HMOs** through translation to cognate BMOs
- Next generation probiotics are being selected and tested on BMOs
- This process will result in inexorable links between dairy products containing dairy glycans and probiotics

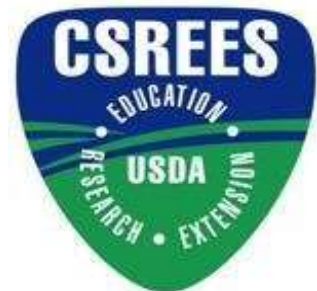
Acknowledgements



Joe O'Donnell



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