An evolutionary link between bifidobacterial probiotics and milk

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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

CHR. HANSEN ← back to main site

Risk to losing probiotics to other foods?

Are there more overt links between dairy and probiotics?

Health & Nutrition →
Animal Health & Nutrition →
Consumers' Guide →
Documentation →
Contact →

well-documented probiotic BB-12®.

Shelf life is an issue

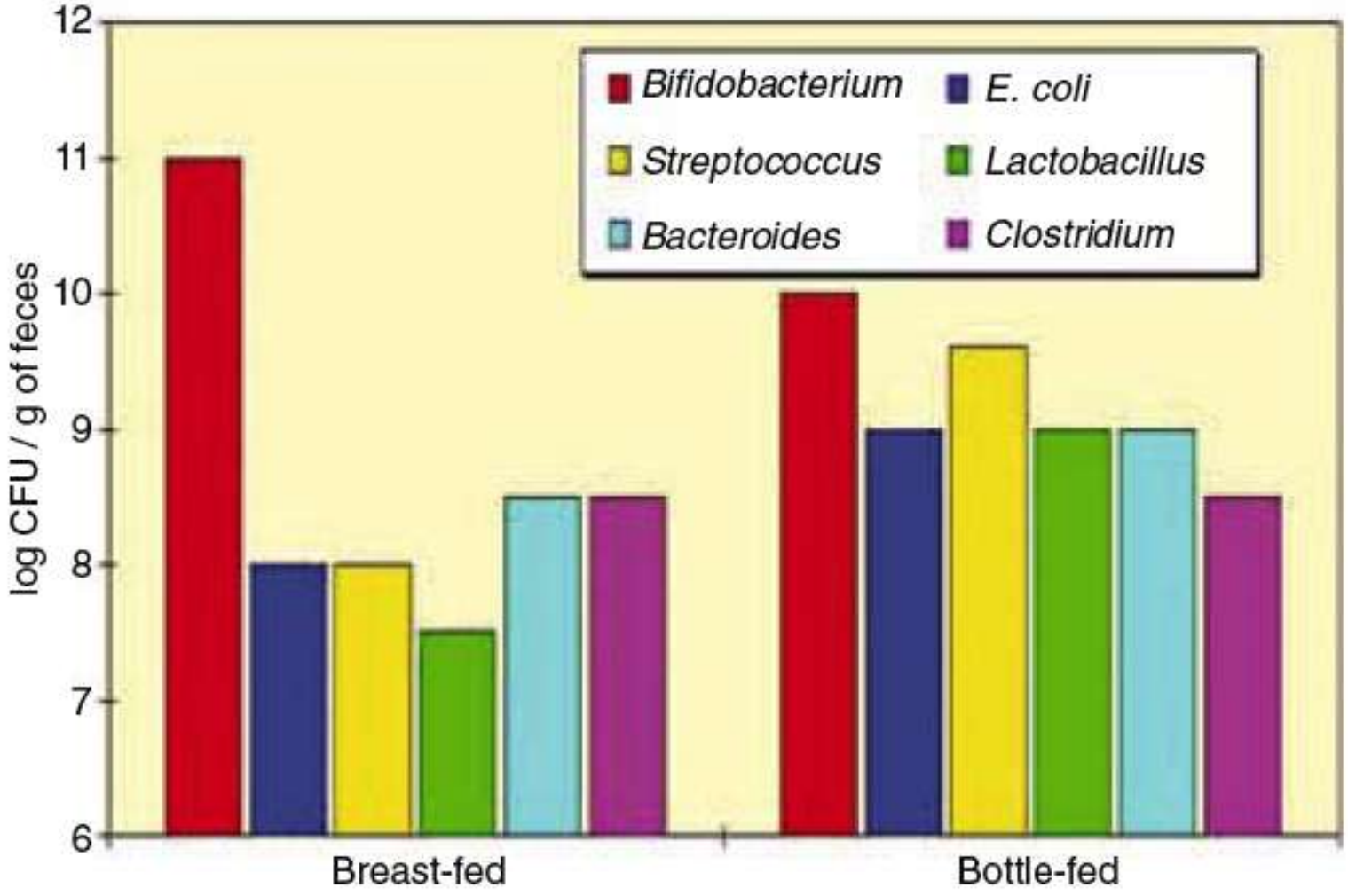
Ensuring the dose of probiotics remains optimal throughout the product's shelf life was key to m successful, so extensive and thorough trials at C Hansens's facilities in the UK have been done.

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

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)

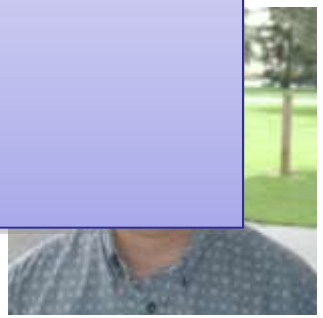
10-15% sialylated

α2-3/6

Lactosamine
0-25 units
(DP)

Lactose

Sialic acid

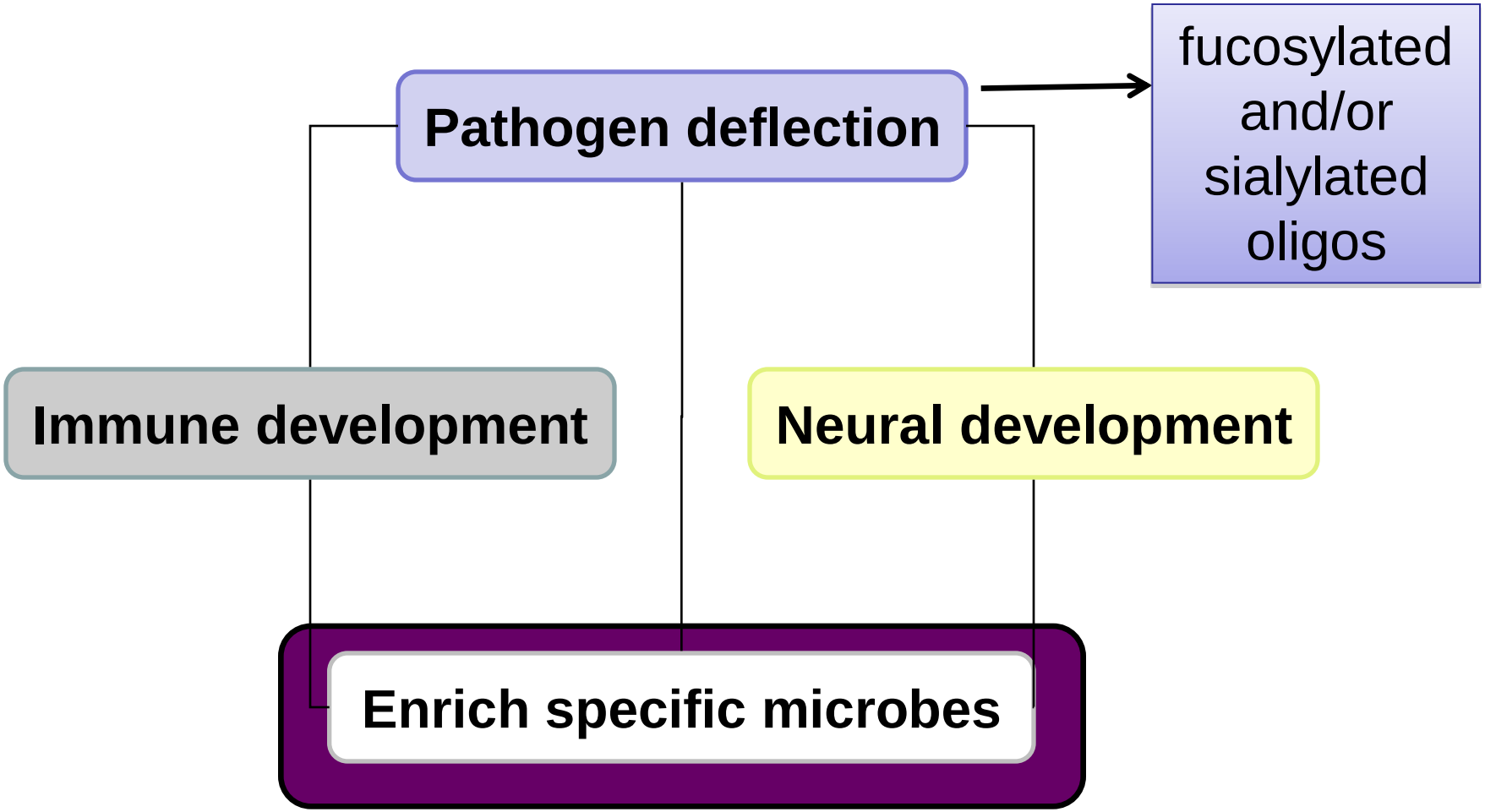


Carlito Lebrilla
UCD Chemistry

Niñonuevo, et al. 2006

Why Make Oligosaccharides?

(if they are not consumed by the infant)



Reviewed in Bode, L., *J. Nutr.* 2006

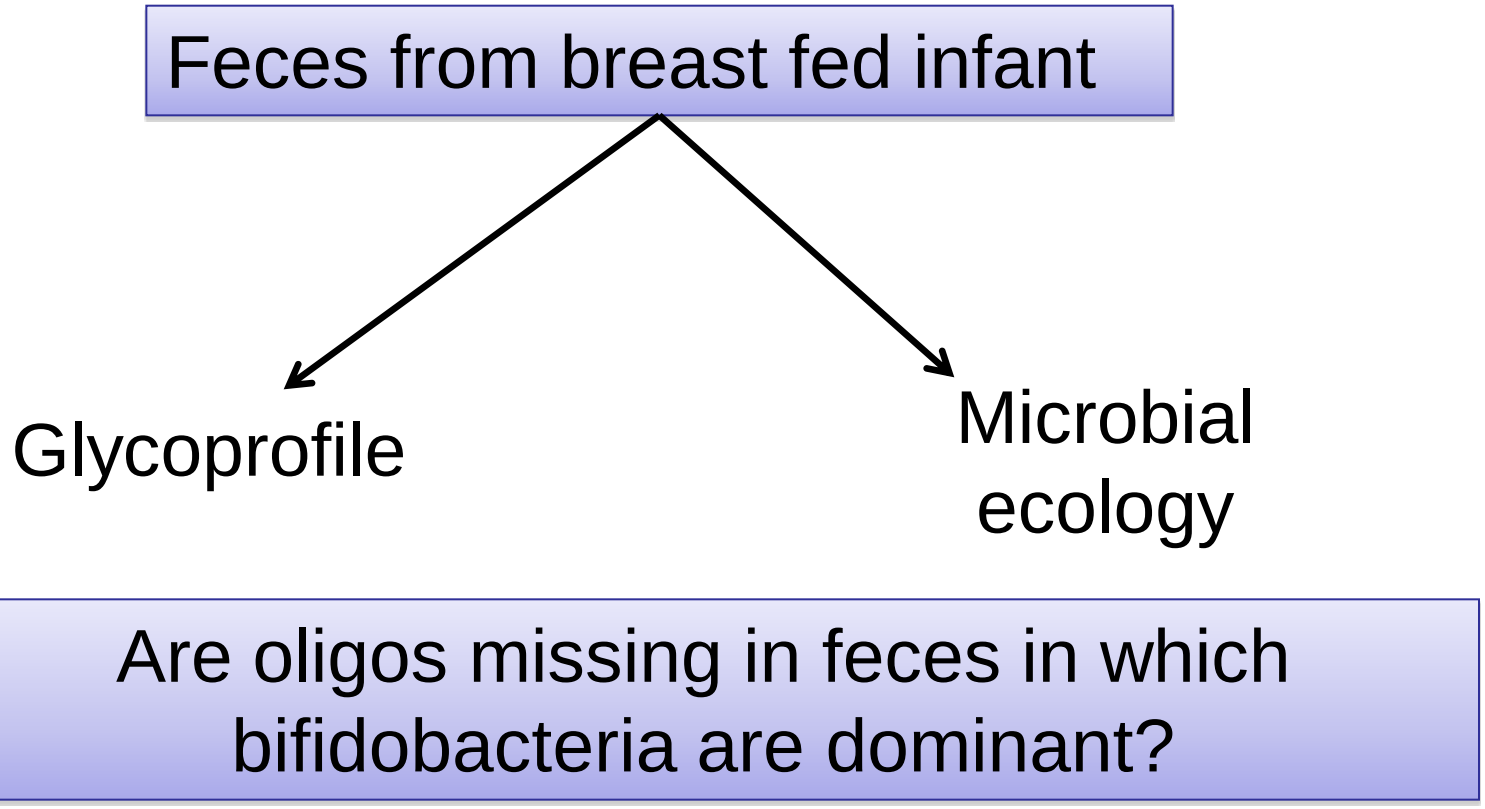
Are there differences in growth on HMOs of bifidobacteria vs. other gut bacteria?

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

Ward et al. AEM 2006, Marcobal JAFC 2010

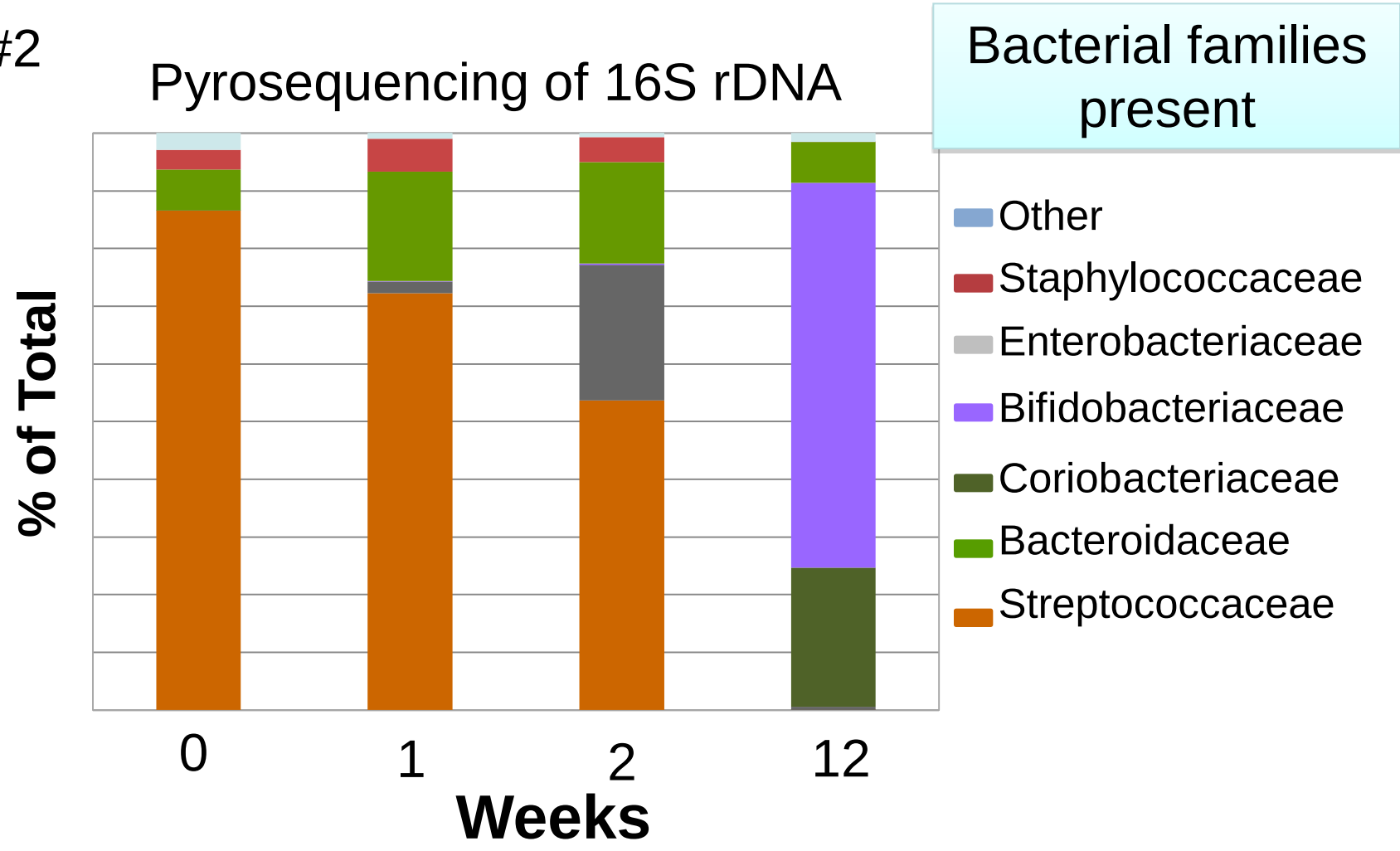
Are oligos consumed by bifidobacteria *in situ*?



Are oligos missing in feces in which bifidobacteria are dominant?

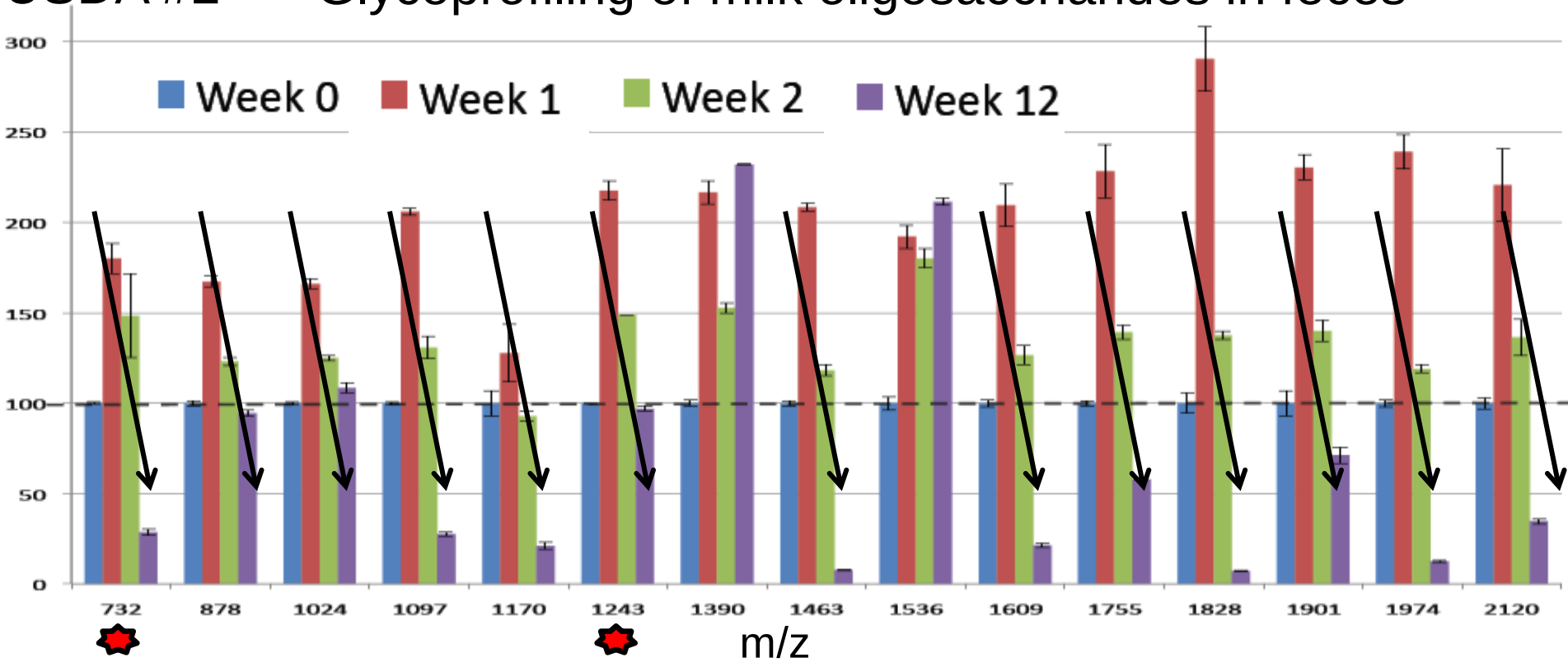
Infant microbial succession over breastfeeding

USDA #2



Infant microbial succession over breastfeeding

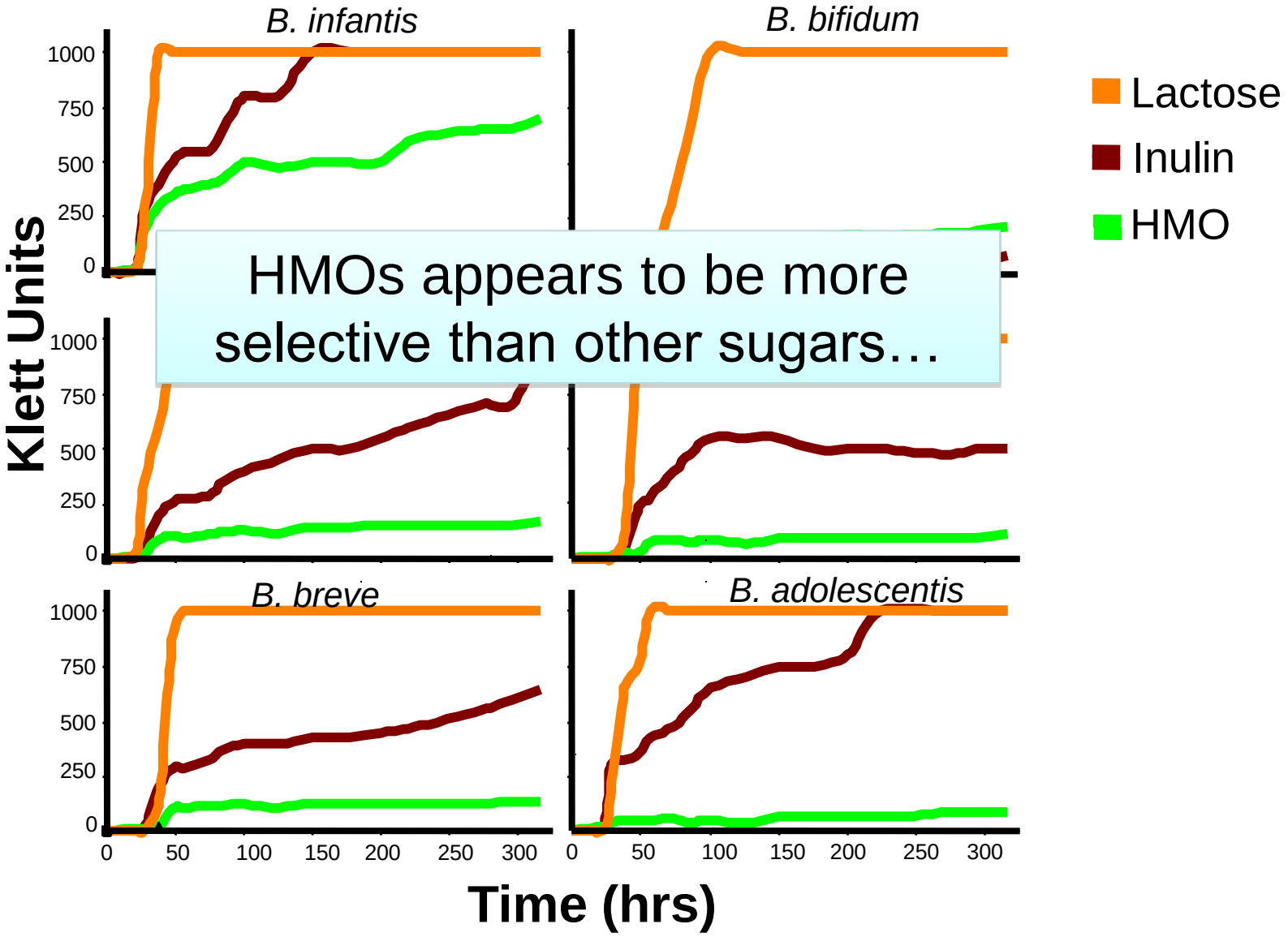
USDA #2 Glycoprofiling of milk oligosaccharides in feces



Different oligosaccharide compositions in feces as determined by Mass Spec

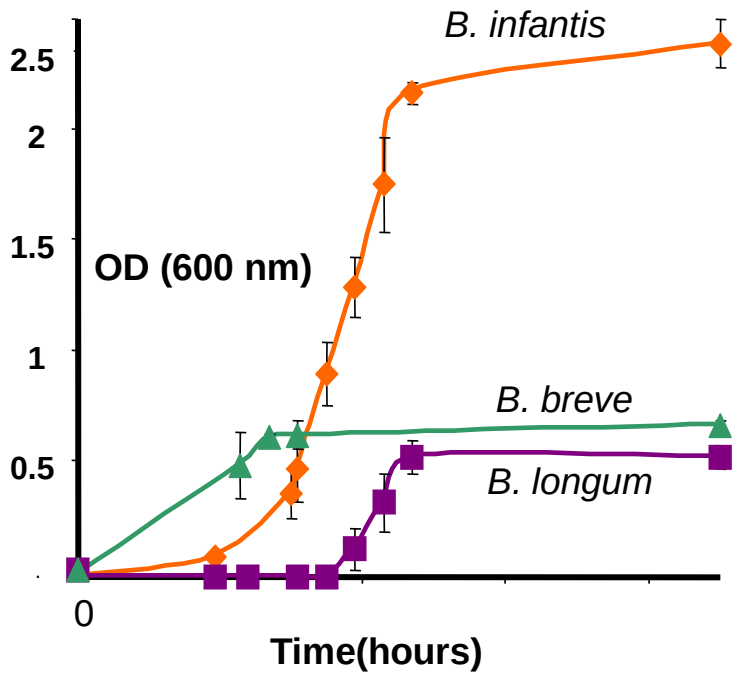
Do bifidobacterial species isolated from different GIT environments grow differently on HMO (i.e. adult vs. infant-borne)?

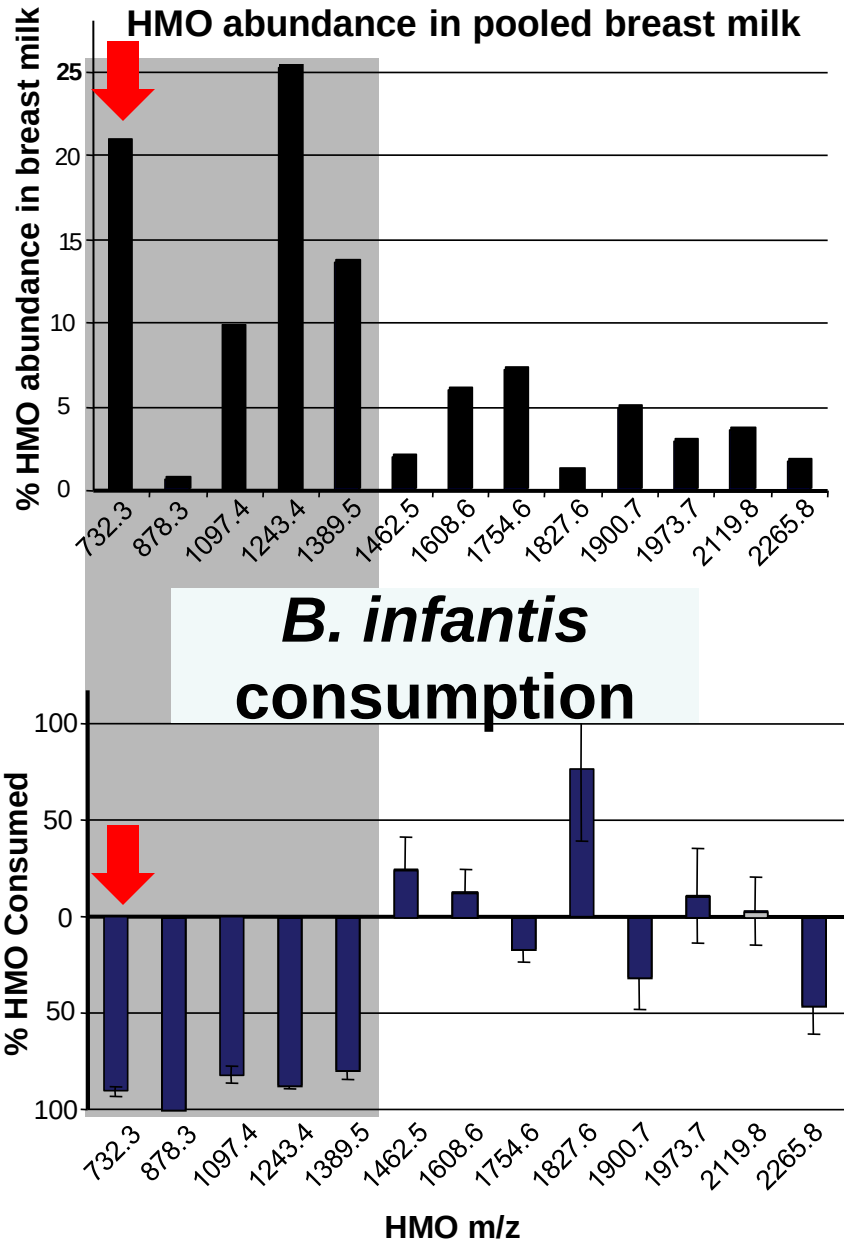
Bifidobacteria & HMOs



Ward et al., Mol. Nutr. Food Res. 2007

Bifidobacterial HMO Glycoprofiling



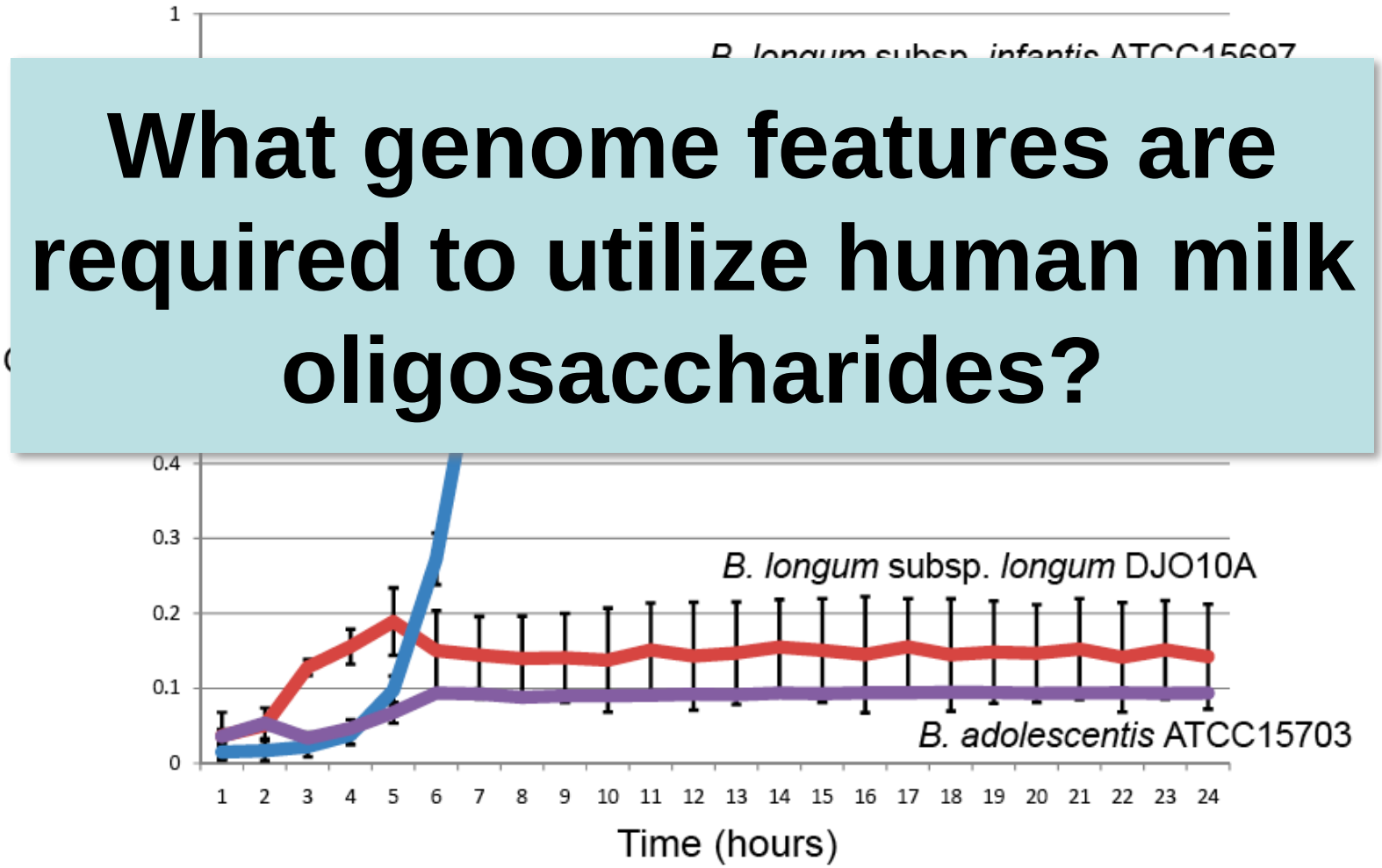


Several small MW oligosaccharides

Single HMO composition consumed by other bifidobacteria

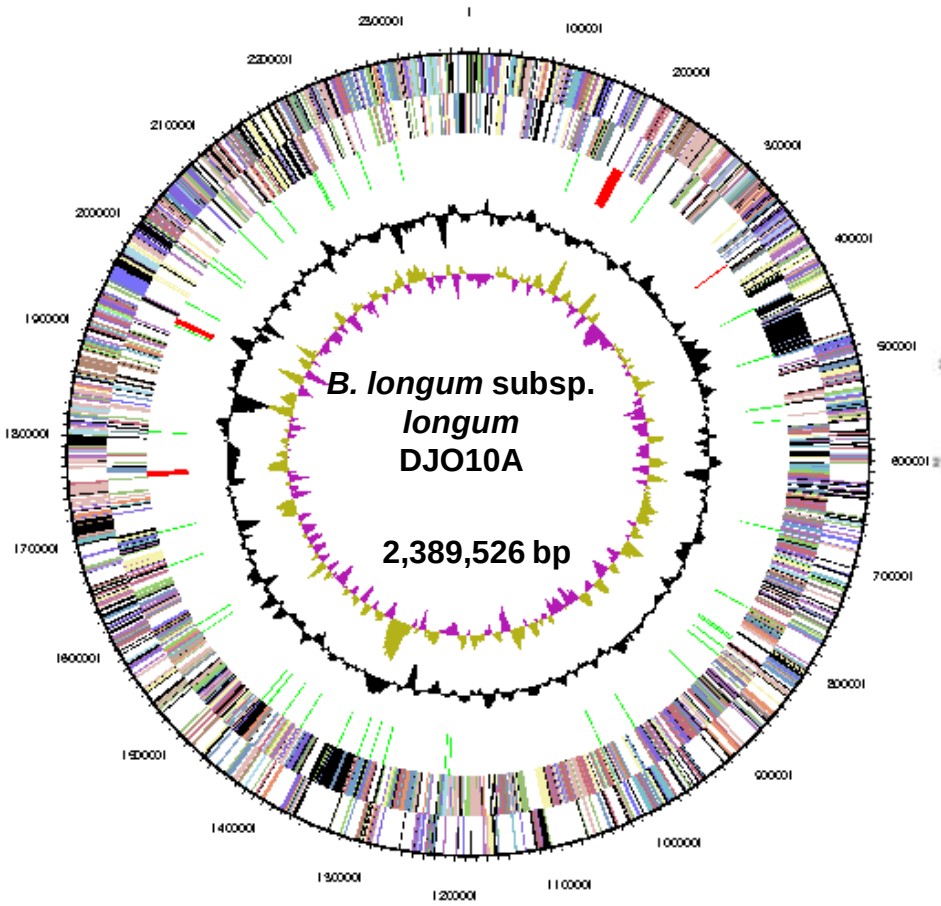
Locascio et al JAFC 2007

HMO utilization by Bifidobacteria



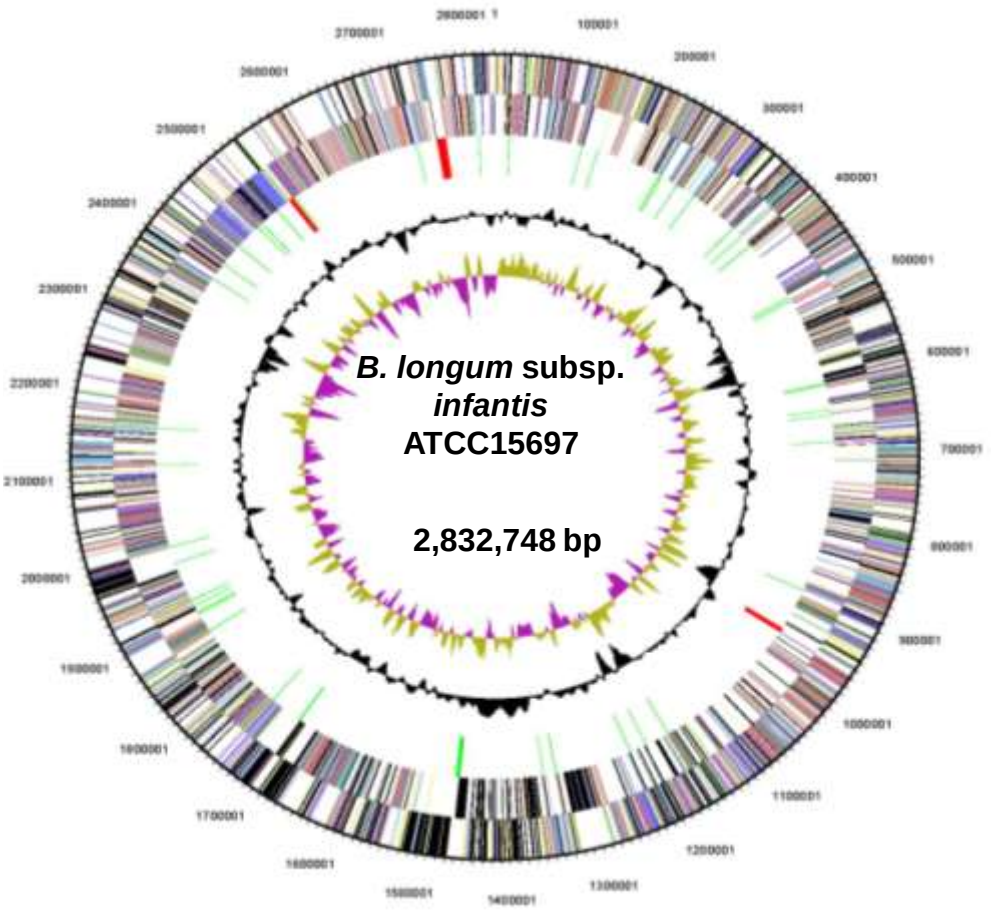
What genome features are required to utilize human milk oligosaccharides?

Comparative Bifidobacterium Genomics



Adult derived strain

BMC Genomics 2008

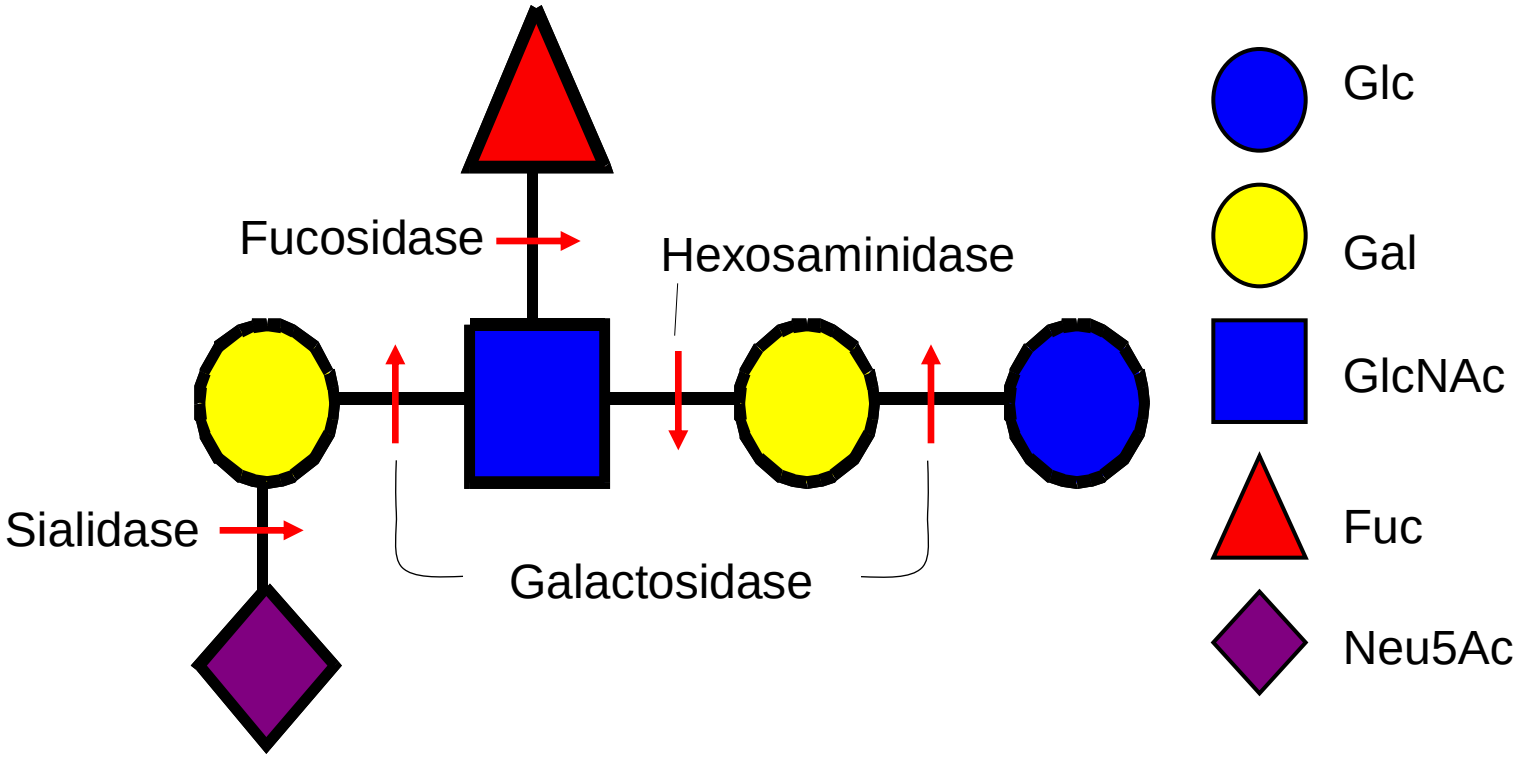


Infant derived strain

PNAS 2008

What's Needed to Deconstruct HMO?

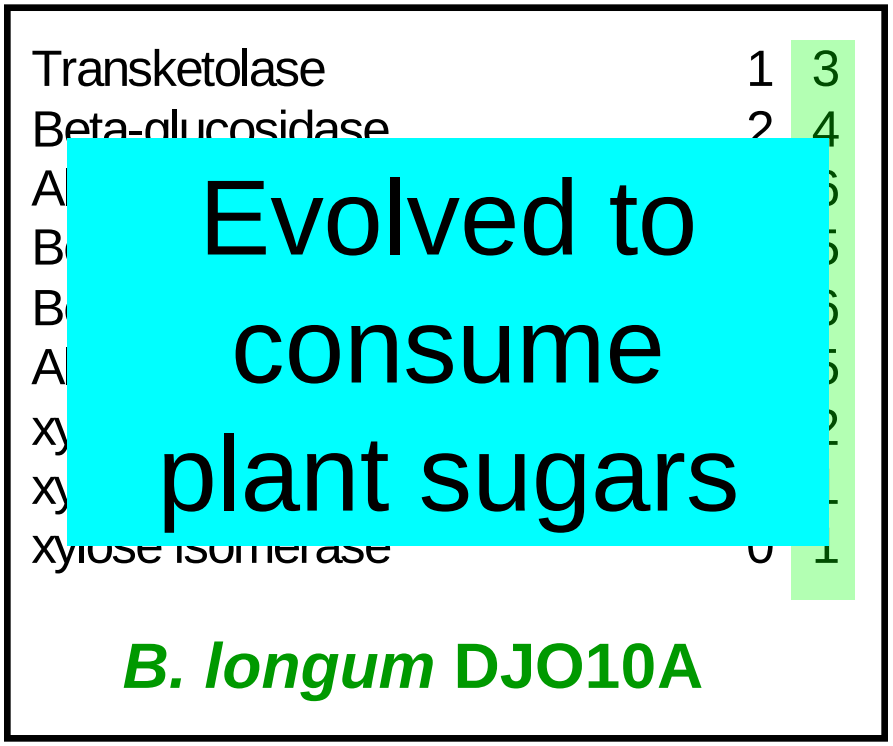
- Transport systems for oligo & monosaccharides
- Glycosyl hydrolases

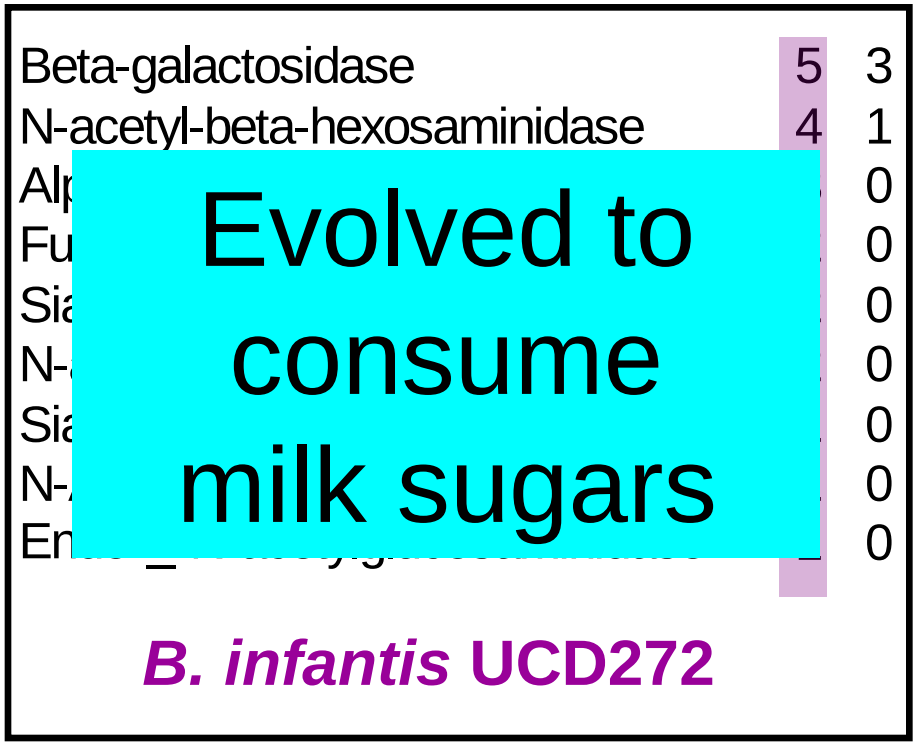


Oligosaccharide Degradation Capacity

Plant-derived oligosaccharide catabolic genes

Mammal-derived oligosaccharide catabolic genes



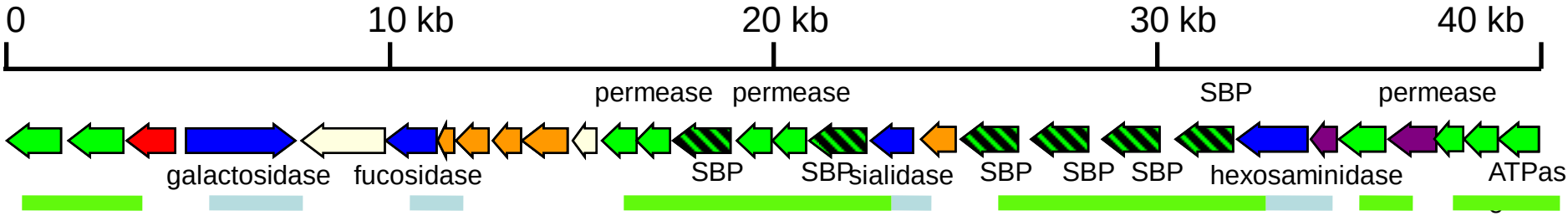


Adult derived strain

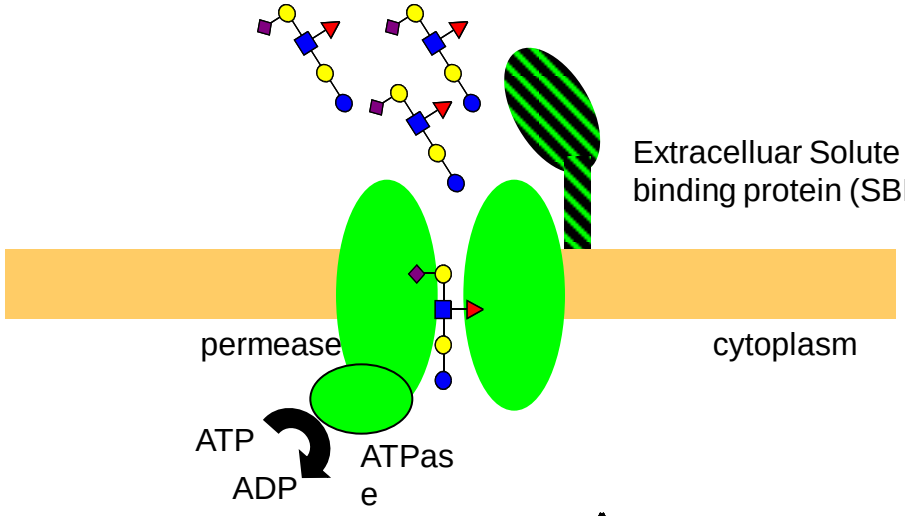
Infant derived strain

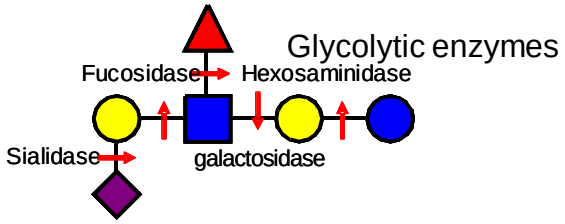
HMO cluster 1

All 4 glycosyl hydrolases **Array of oligosaccharide transporters**



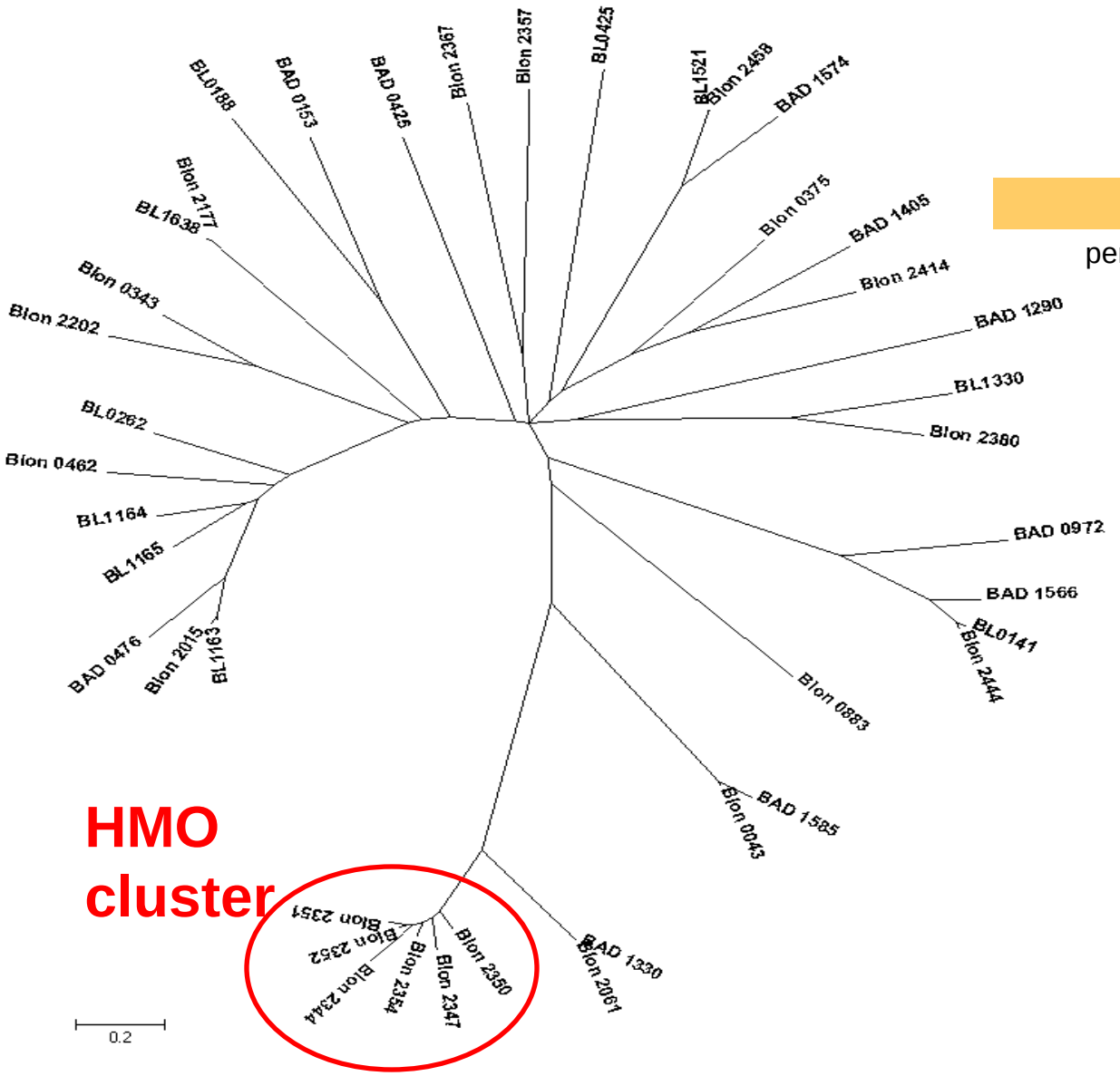
- HMOs are bound by SBP lipoproteins proximal to permeases
- ATP hydrolysis prompts transport of oligosaccharides across membrane
- Intracellular glycolytic enzymes deconstruct oligosaccharide



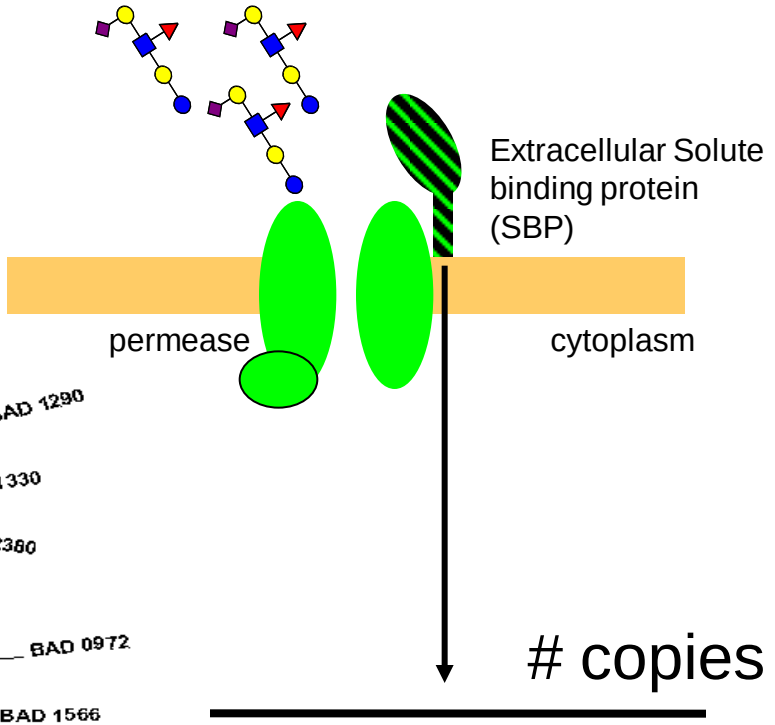


HMO cluster SBPs

Family 1 (oligosaccharide binding)



HMO cluster

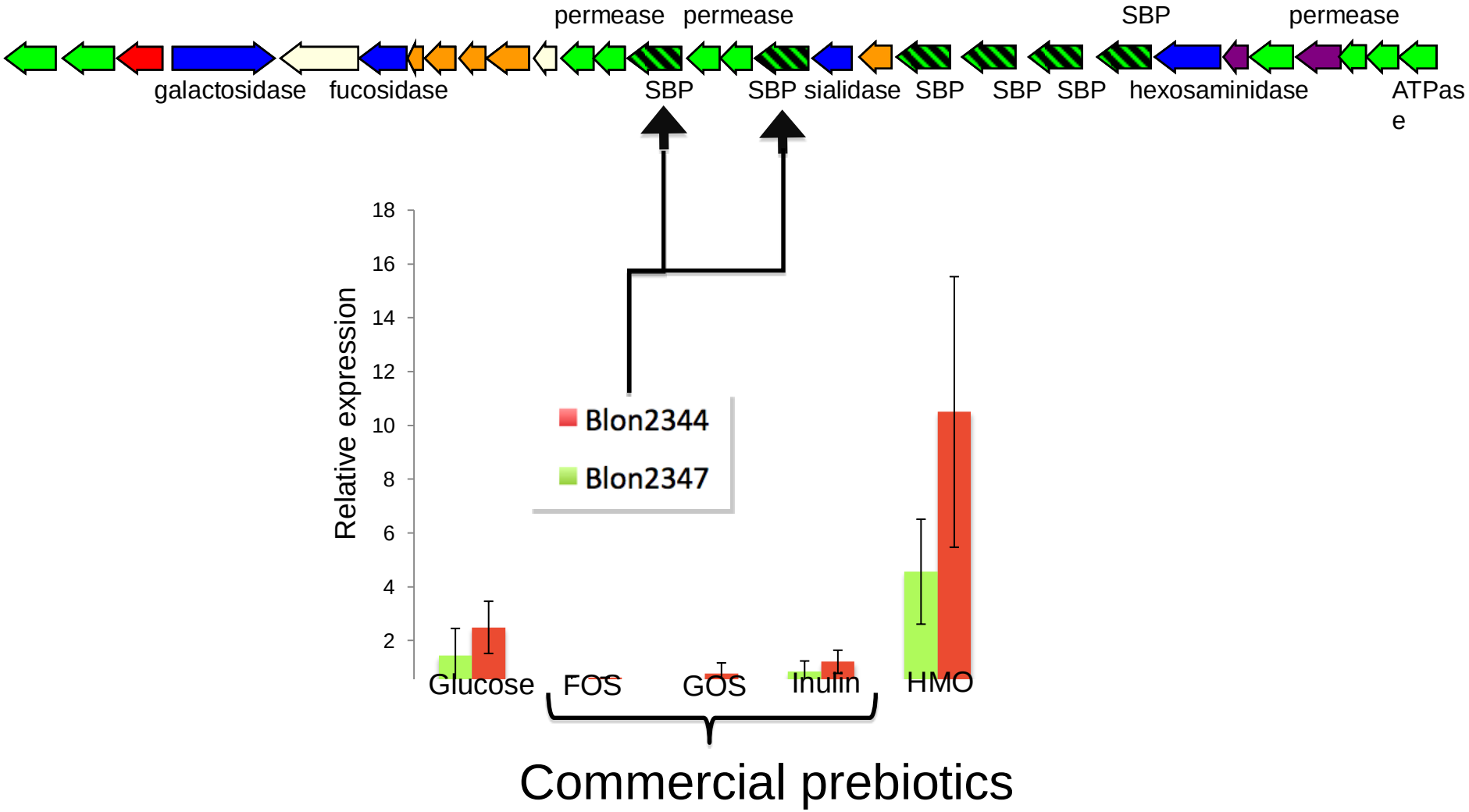


B. infantis 20

B. longum 10

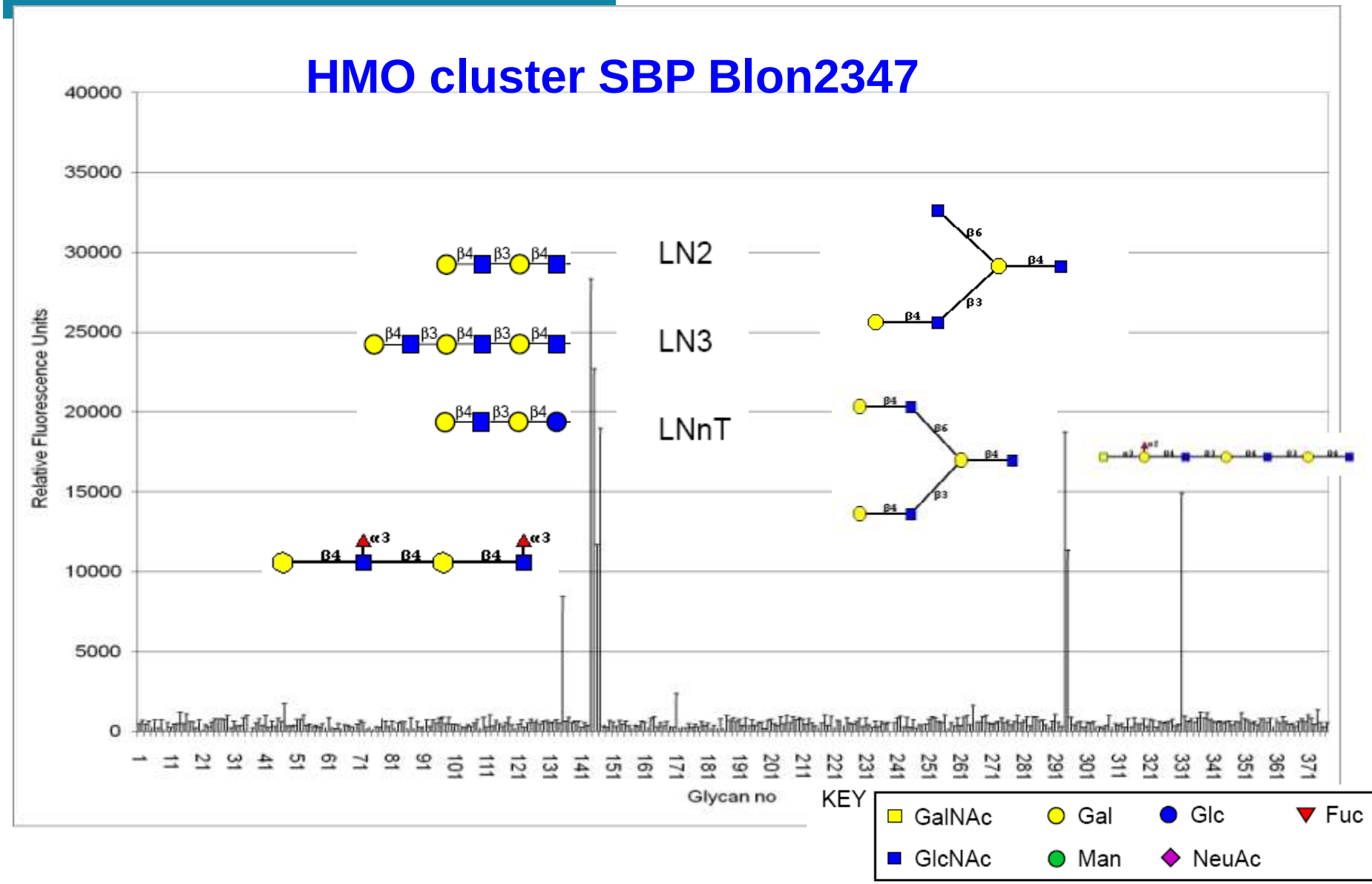
B. adolescentis 11

SPB expression in B. infantis



CFG nature functionalglycomicsgateway

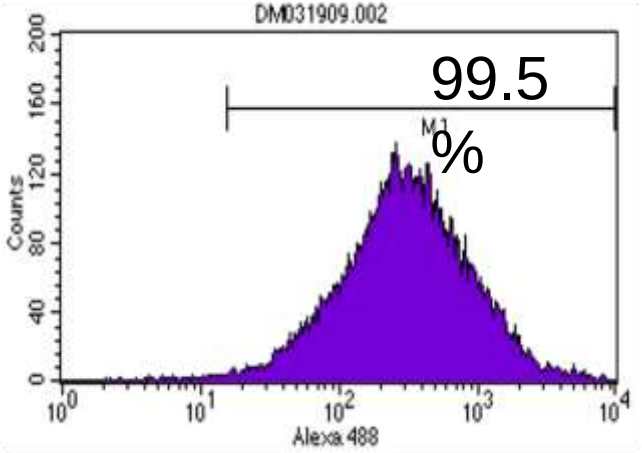
HMO cluster SBP Blon2347

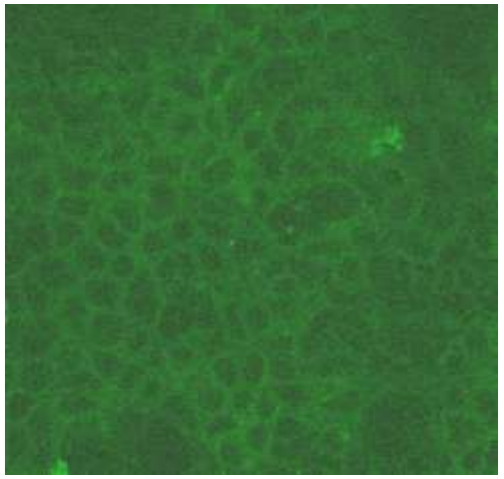


SBP Blon2347 binding to Caco2 cells

Caco-2 + UEA lectin
(Ulex europeus agglutinin)

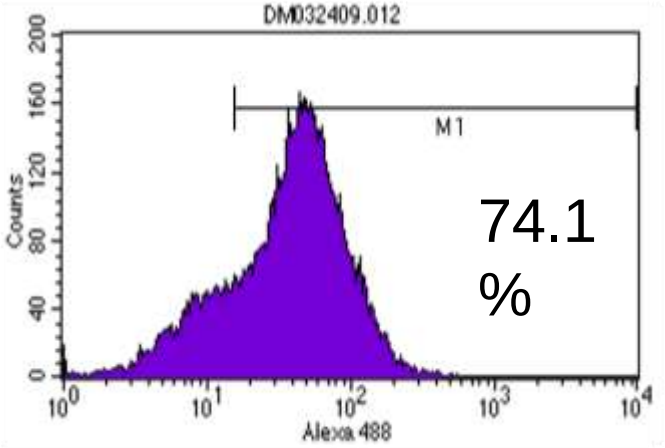
Major epitope:
Fuca1-2Galb1-4GlcNAc

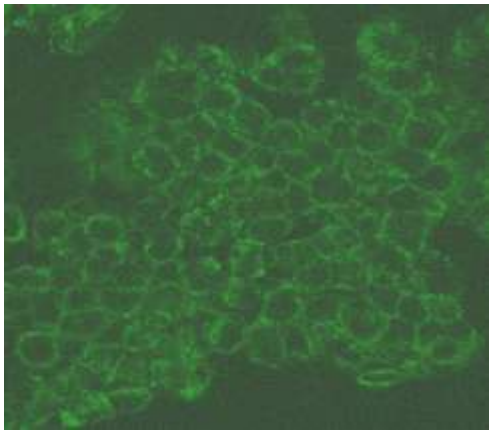




Caco-2 + Blon2347

GalNAca1-3**(Fuca1-2)Galb1-4GlcNAcb**1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAc

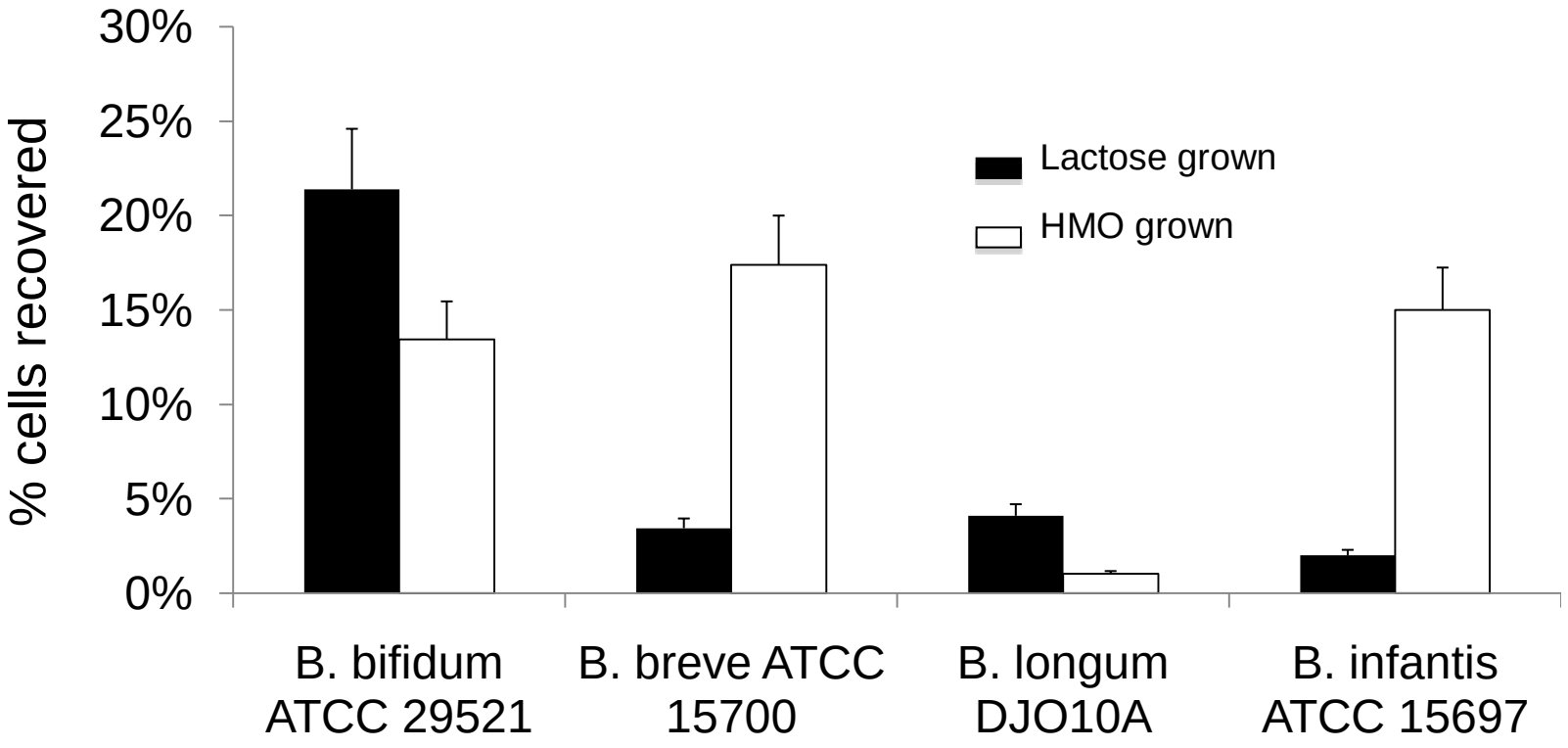




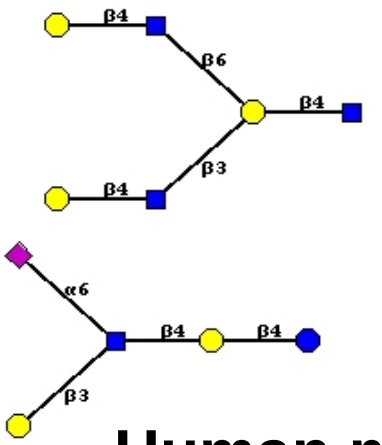
Daniel Garrido

Does consumption of HMO encourage colonization by Bifidobacteria?

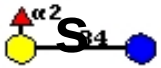
Growth on milk oligosaccharides helps bifidobacteria bind intestinal cells

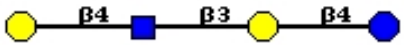


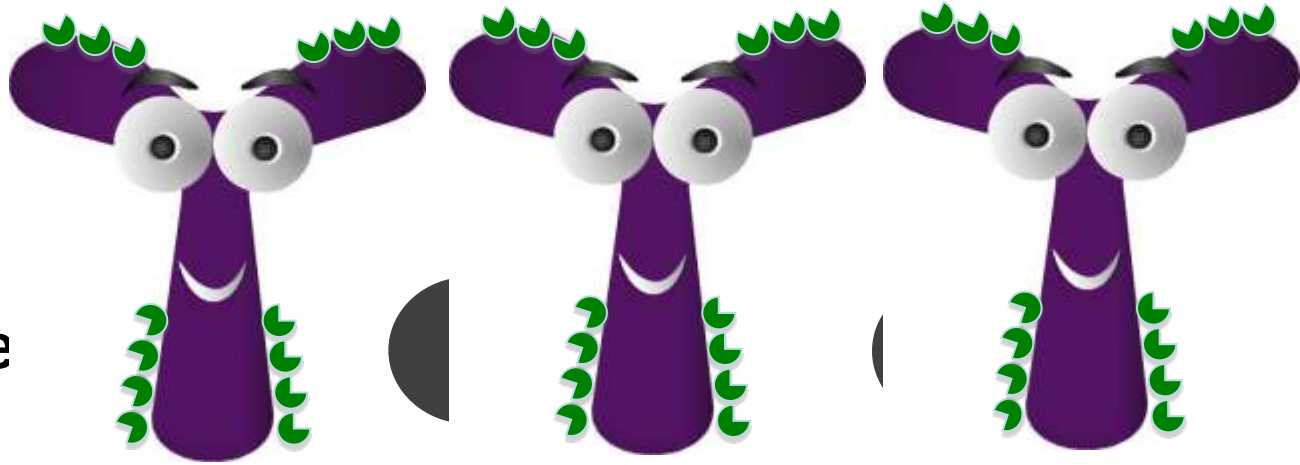
Model for *B. infantis* enrichment in the infant GIT

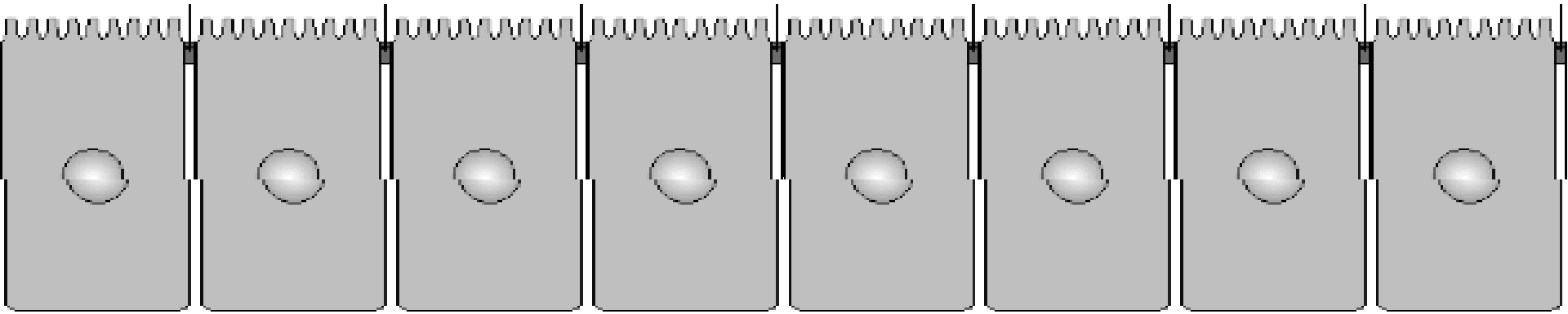


Human milk oligosaccharide





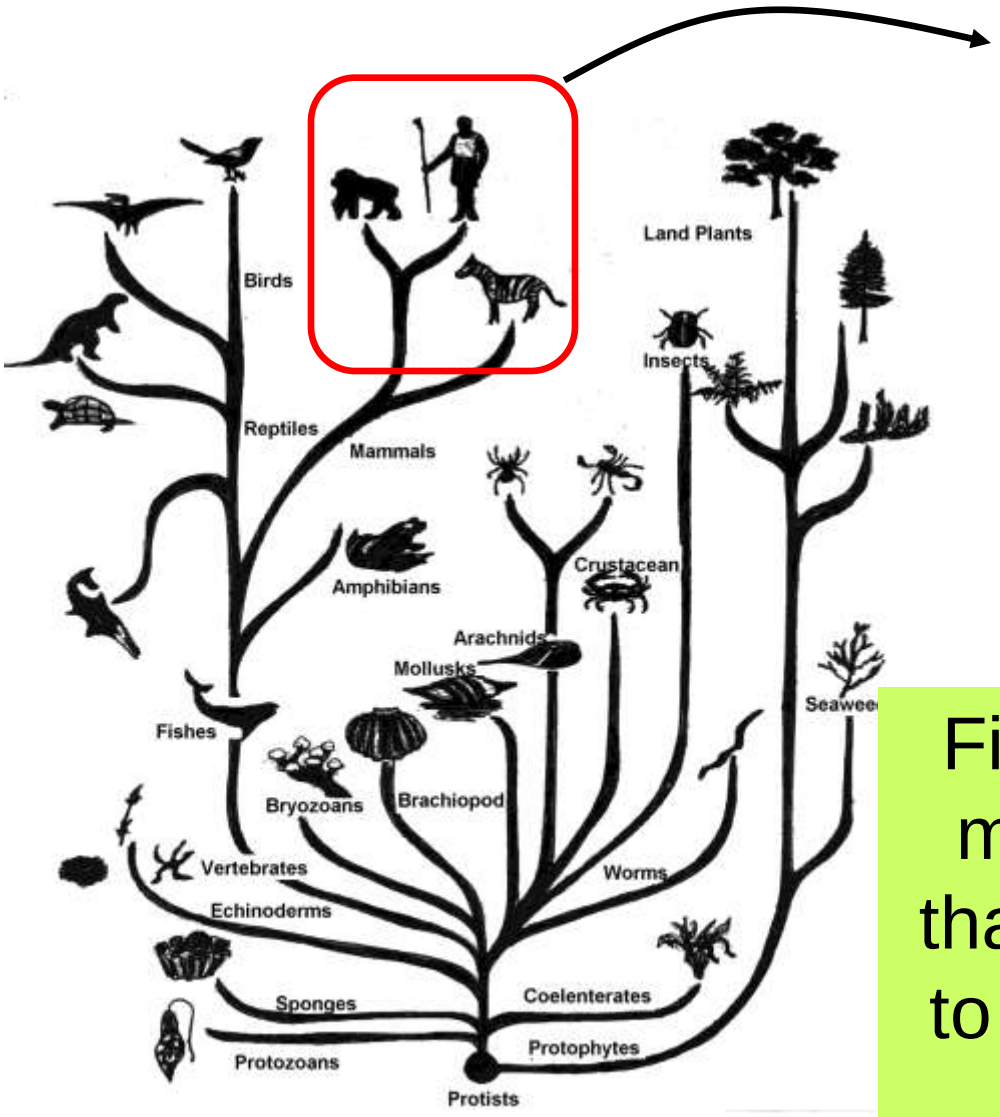


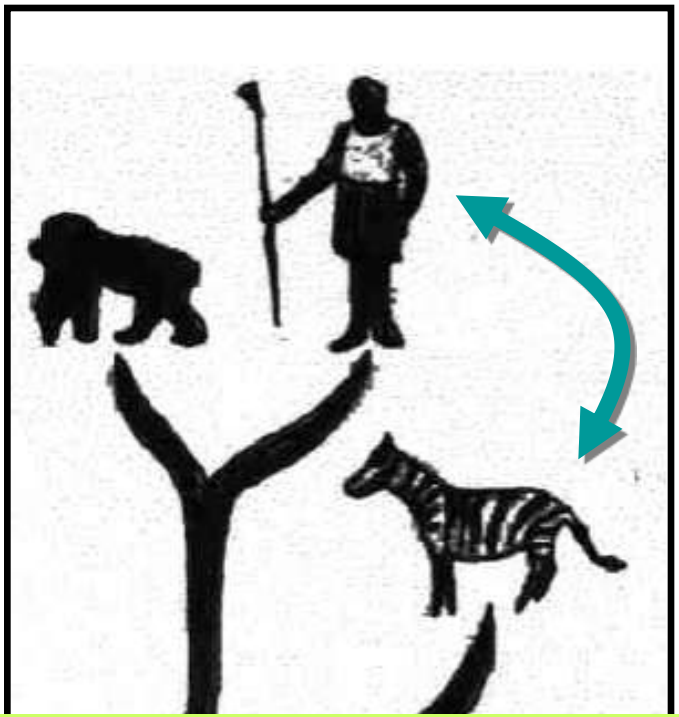


Dave…the human milk research is great…

…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

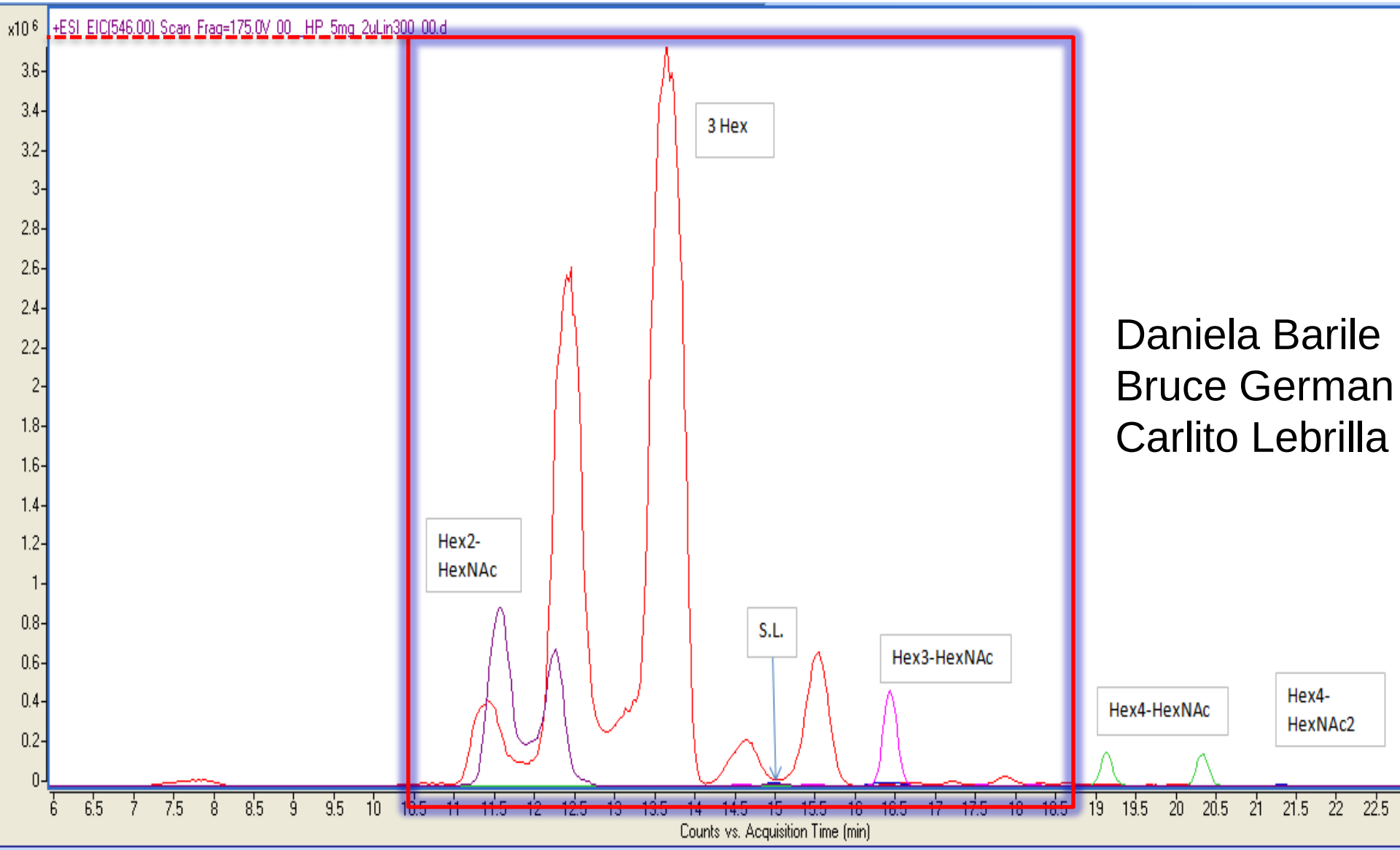
• **Bovine Milk**: Colostrum (0.7-1.2g/L); Mature Milk (trace amount)

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

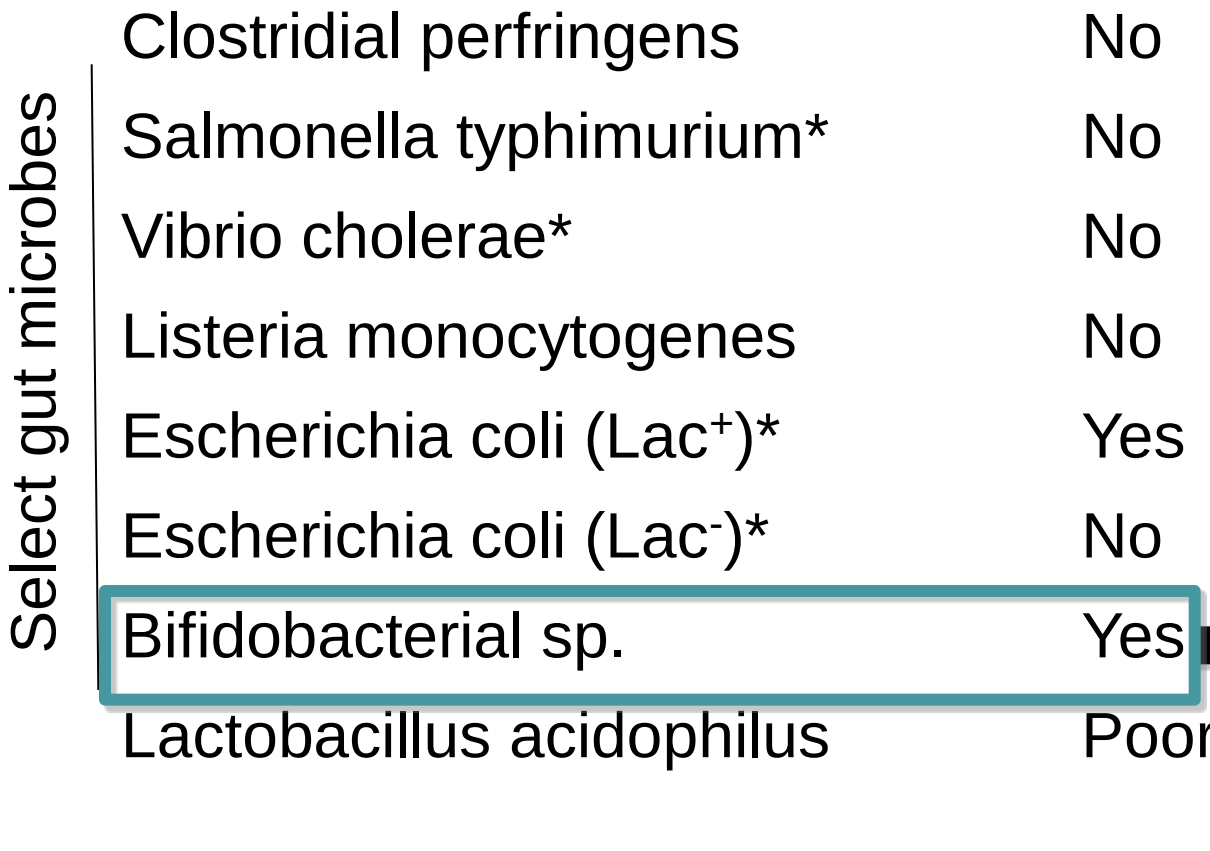
Tao et al., J. Dairy Science, 2008
Barile et.al. J. Dairy Science, 2010

BMO profile - lactose reduced/removed



Daniela Barile
Bruce German
Carlito Lebrilla

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

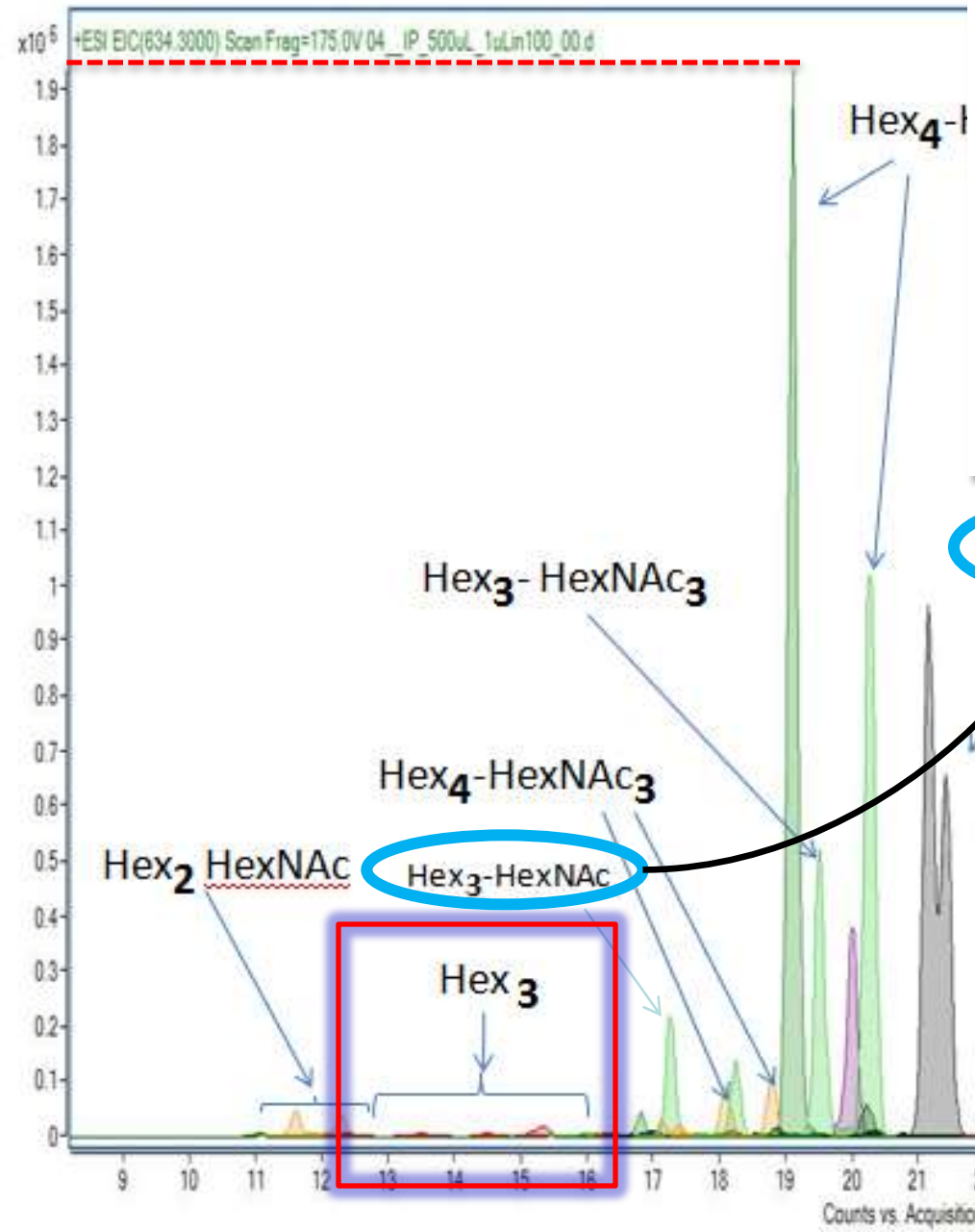


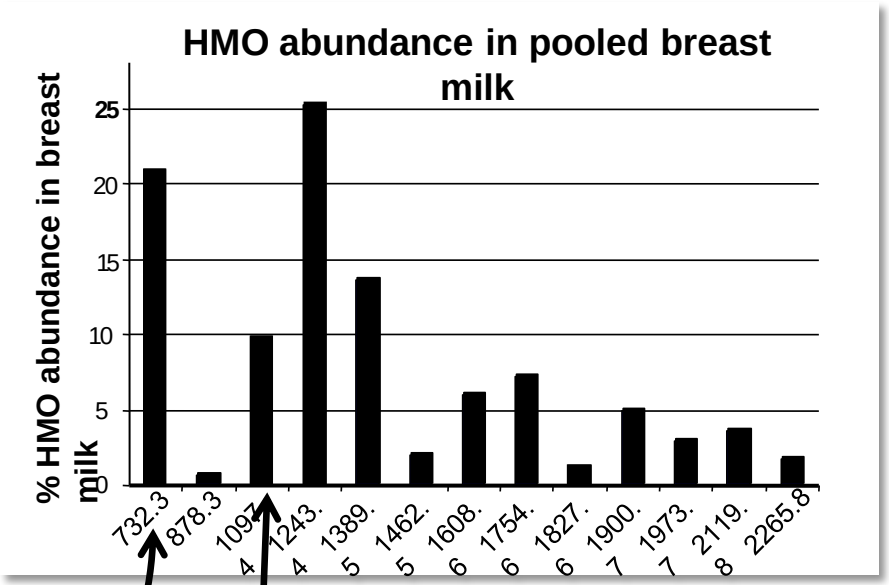
Not species/strain specific (unlike HMO)

* Tested for fermentation via MacConkey agar

Glenn Young

BMO profile - lactose and





Hex4-HexNAc2

Daniela Barile
Bruce German
Carlito Lebrilla

NEW BMOs!!!

Sialyl Lactose

BMOs have many oligos similar to HMOs

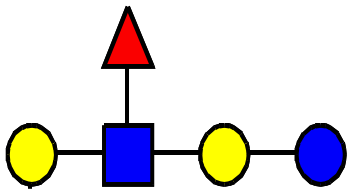
m/z $[M+Na]^+$	Hex	HexNAc	NeuAc (sialic acid)	Fuc	DP
527.133	3	0	0	0	2
568.154	2	1	0	0	2
656.156	2	0	1	0	2
730.179	3	1	0	0	3
771.197	2	2	0	0	3
892.196	4	1	0	0	4
933.213	3	2	0	0	4
1095.219	4	2	0	0	5
1136.234	3	3	0	0	5
1257.229	5	2	0	0	6
1298.241	4	3	0	0	6
1339.253	3	4	0	0	6
1419.225	6	2	0	0	7
1485.256	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

Existing HMO-like compositions

New discovery!

Large fucosylated oligosaccahrides



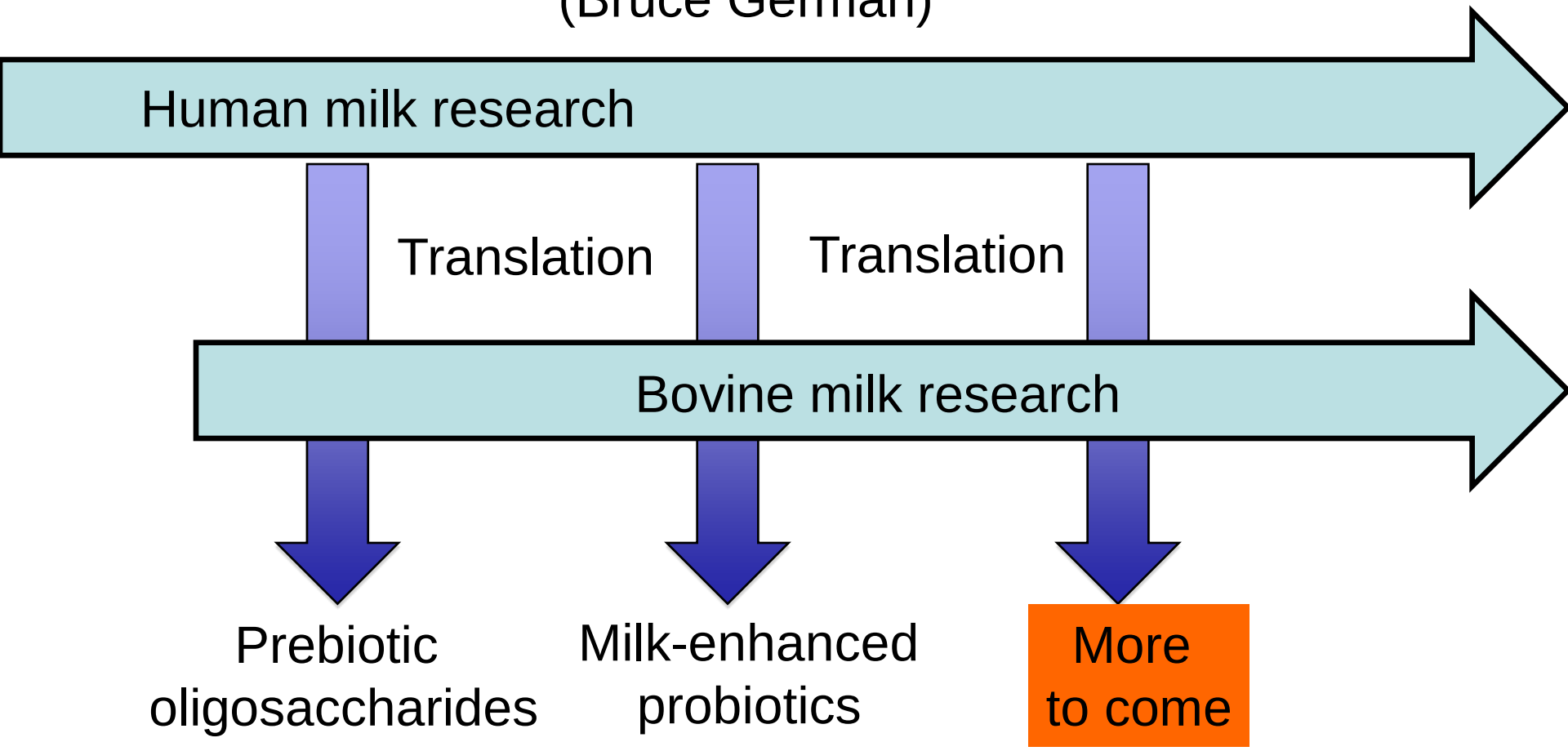
Daniela Barile
Bruce German
Carlito Lebrilla

BMO Summary

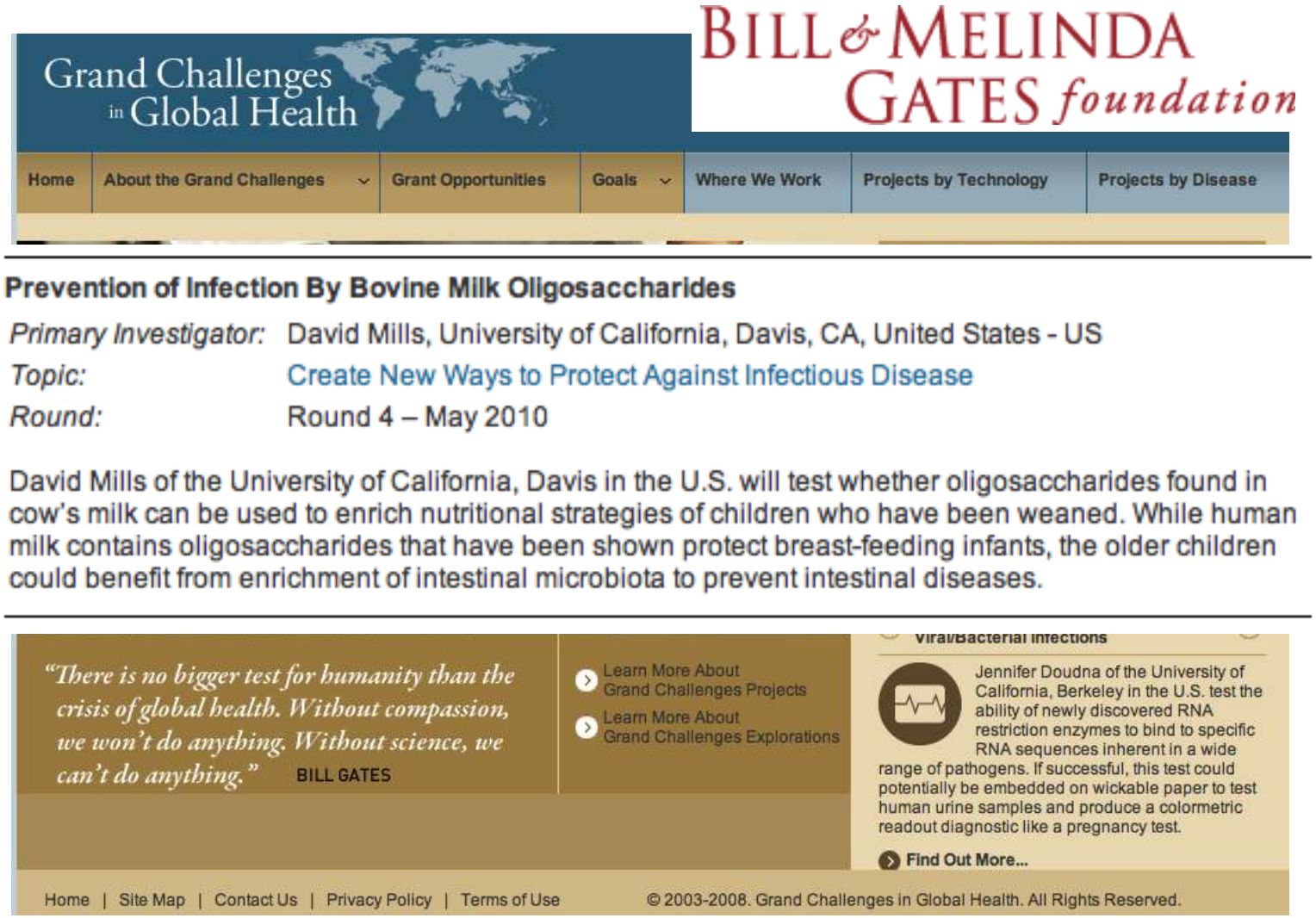
- Bovine milk oligosaccharides contain a major amount of triose sugars (sialyllactose and others)
- Triose-dominant BMOs enable growth of bifidobacteria non-specifically, and do not allow growth of most pathogens (tested)
- Further separation of BMO fractions to remove trioses has revealed oligosaccharides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

UC Davis Milk Bioactives Program

(Bruce German)



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

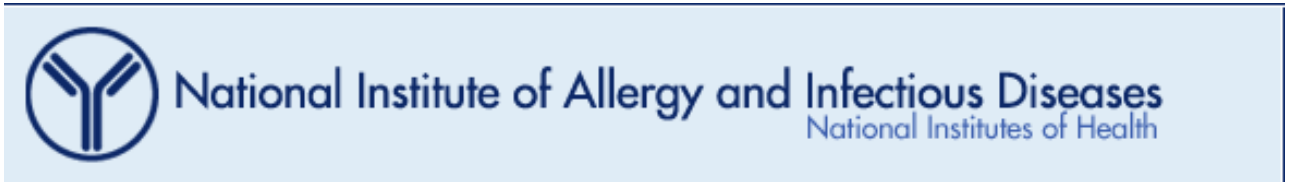


Summary

- 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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