Preference for Milk and Host-Glycans in *Bifidobacterium infantis*

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Breast milk dictates the composition of the infant microbiota in the first years of life
Carbon Sources in the Breast-Fed Infant Colon

- Human Milk Oligosaccharides
- Mucin glycans
- Intestinal Glycoconjugates

Infant bifidobacteria
Carbon Sources in the Breast-Fed Infant Colon

Human milk oligosaccharides (HMO)

- Lacto-N-biose (type 1 chain)
- N-Acetyl lactosamine (type 2)
- Lacto-N-tetraose (LNT)
- Lacto-N-neotetraose (LNnT)

Epithelial glycoconjugates

Blood group antigens

Essentials of Glycobiology 2nd edition
What features are required for complex oligosaccharide consumption?

1. Transport mechanisms (ABC importers)

2. Glycolytic enzymes

- Fucosidase
- Hexosaminidase
- Sialidase
- Galactosidase

ATP → ADP → ATPase → Family 1 Solute Binding Protein (F1SBP) → Permease → Cytoplasm
**Bifidobacterium infantis**

- Commonly found in the infant colon
- Archetypical HMO consuming bacteria
- Specialization for carbohydrate metabolism

*B. infantis* ATCC 15697 HMO Cluster I

<table>
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<tr>
<th>Sequenced genome</th>
<th>Pfam01547 hits</th>
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<td><em>Bifidobacterium adolescentis</em> ATCC 15703</td>
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<td><em>Bifidobacterium animalis lactis</em> AD011</td>
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<td><em>Bifidobacterium animalis</em> subsp. lactis Bl-04</td>
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<td><em>Bifidobacterium bifidum</em> NCIMB 41171 (Draft)</td>
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<td><em>Bifidobacterium breve</em> DSM 20213 (Draft)</td>
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<td><em>Bifidobacterium longum</em> DJO10A</td>
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<td><em>Bifidobacterium longum infantis</em> ATCC 15697</td>
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<td><em>Bifidobacterium pseudocatenulatum</em> DSM 20438 (Draft)</td>
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• F1SBPs might represent a critical adaptation that might indicate the range of complex oligosaccharides *B. infantis* is able to import and consume

• SBP oligosaccharide affinity
  • Mammalian Glycan Array

• Induction by different prebiotics
  (HMO, inulin, FOS, GOS)
Analysis by qPCR and proteomics
Blon2347 (and Blon2344)

Type 2 (Galb1-4GlcNAc) binding proteins
HMO and epithelial glycoconjugates

Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAc
Tri lactosamine
RFU: 27537

Galb1-4GlcNAcb1-3Galb1-4GlcNAc
Di-lactosamine
RFU: 22369

Galb1-4GlcNAcb1-3Galb1-4Glc
Lacto-N-neotetraose
RFU: 18606

GalNAca1-3(Fuca1-2)Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAc
Human colonic mucin glycan
RFU: 14667

Galb1-4(Fuca1-3)GlcNAcb1-4Galb1-4(Fuca1-3)GlcNAc
Difucosyl Di LacNac
RFU: 7559

Galb1-4GlcNAcb1-3(GlcNAcb1-6)Galb1-4GlcNAc
Human colonic mucin glycan
RFU: 10898

Galb1-4GlcNAcb1-3(GlcNAcb1-6)Galb1-4GlcNAc
Branched trilactosamine
RFU: 17959
Concordance in proteomic with expression data

Sole carbon source in *B. infantis* culture

HMO Cluster I F1SBPs
**Blon2350-Blon2351-Blon2354**

Core subunit in mucin glycans

- **Galb1-3GalNAcb**
- **Galacto-N-biose**
- RFU: 1206

### B. bifidum, B. longum:
- One SBP binding LNB/GNB

### B. infantis:
- 2 SBPs binding LNB
- 5 SBPs binding GNB
**Type 1 chain F1SBPs**

**Blon0883**

- Galb1-3GlcNAc
  - Lacto-N-biose RFU:29986

- Galb1-3GalNAcb
  - Galacto-N-biose RFU:27955

- Fuca1-2Galb1-3GlcNAc
  - Type I blood group H-antigen RFU:14946

- Galb1-3(Fuca1-4)GlcNAc
  - Lewis Le\(^a\) antigen RFU:23132

**Blon2177**

- Galb1-3GlcNAcb1-3Galb1-4Glc
  - Lacto-N-tetraose RFU:17807

- Galb1-3GlcNAcb1-3Galb1-4GlcNAc
  - Di-LNB RFU:11944

- Iso-Lacto-N-octaose RFU:10883

- Galb1-3GlcNAc
  - Lacto-N-biose RFU:8160

- Galb1-3GalNAcb
  - Galacto-N-biose RFU:24879

• SBP unique to *B. infantis*

• Homologues in other members of the microbiota

• Found in conserved LNB/GNB cluster in several bifidobacteria

**BL1638 (B. longum NCC2705):**

GNB = LNB

**Blon2177 (B. infantis):**

GNB >>> LNB
• HMO-binding SBPs are turned on regardless their affinities for type 1 or type 2 chains
Type 1 chain F1SBPs

- Expected HMO binding protein. Catabolic repression? More active import of mucin glycans?
B. infantis growing on HMO
• **Blon_2061**: Candidate for fructan import in *B. infantis*
• A plant-derived oligosaccharide induces expression of SBPs that bind host glycans
Inulin has a long history of consumption by humans
- Prebiotic fermentable by bacteria in distal colon
- Induction of host glycan SBPs could be interpreted as a (vestigial) signal that prepares the bacteria to the encounter of host-derived glycans
SBPs Gene Expression

FOS and GOS induce SBPs involved in their own import.
• *B. infantis* devotes half of its F1SBPs to the import of host-derived glycans.

• Genes involved in HMO import were identified. Several are specific for *B. infantis*.

• F1SBPs affinities match the HMO structures consumed by *B. infantis*. This bacterium has developed strategies for garnering different isomers of HMO.

• HMO is able to induce the gene expression of several SBPs which bind host glycans.

• Inulin, but not FOS or GOS, also increased the expression of proteins that import host glycans.

• Some SBPs binding host glycans are potential candidates for interactions with the host.

• **Understanding of nutritional preferences of the intestinal microbiota will help in the selection of specific probiotics and specific prebiotic formulations.**
• Consortium for Functional Glycomics, Core H

• Mills Lab (Dave Sela, Riccardo Locascio)

• University of California Discovery Grant Program
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