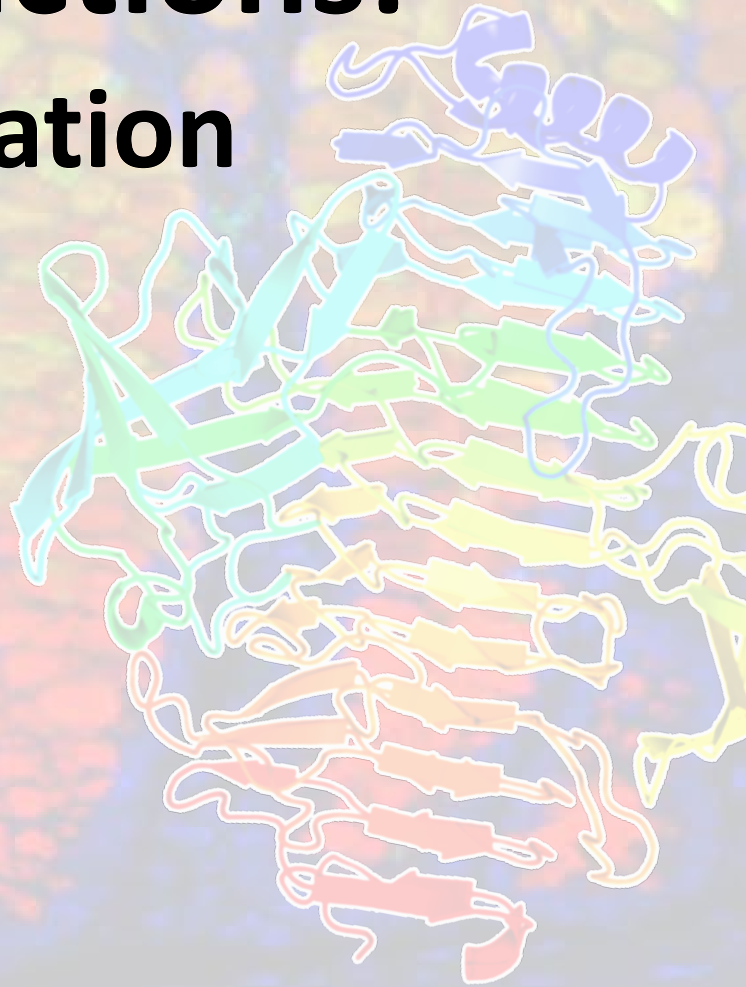
Future of the Microbiome
2024 Winter Summit
November 19-21, 2024

Microbiota- mucin interactions:
key enzymes in gut colonization



Ana Luis

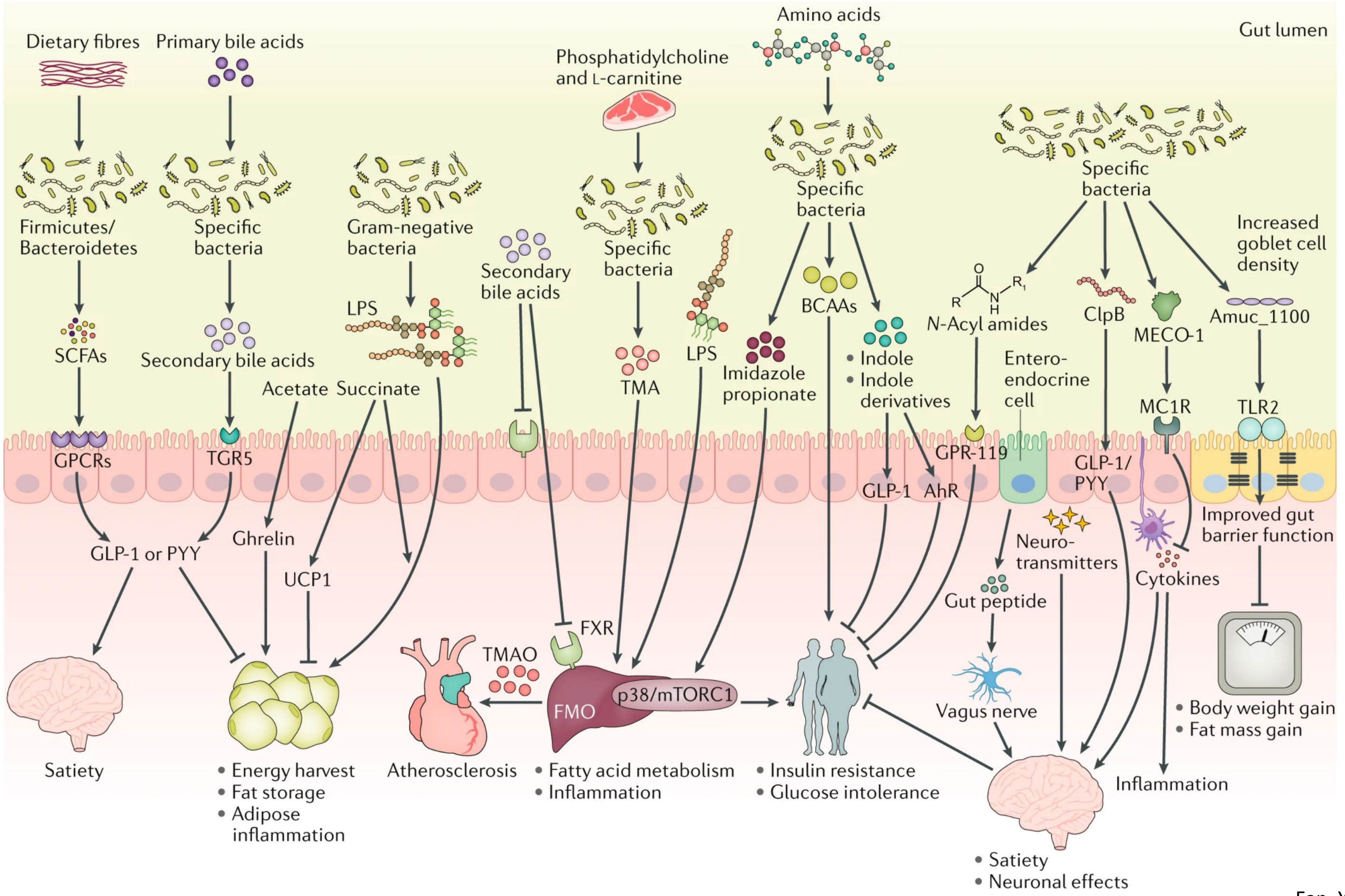
University of Gothenburg, Sweden

19/11/2024

MUCIN BIOLOGY GROUPS
UNIVERSITY OF GOTHENBURG

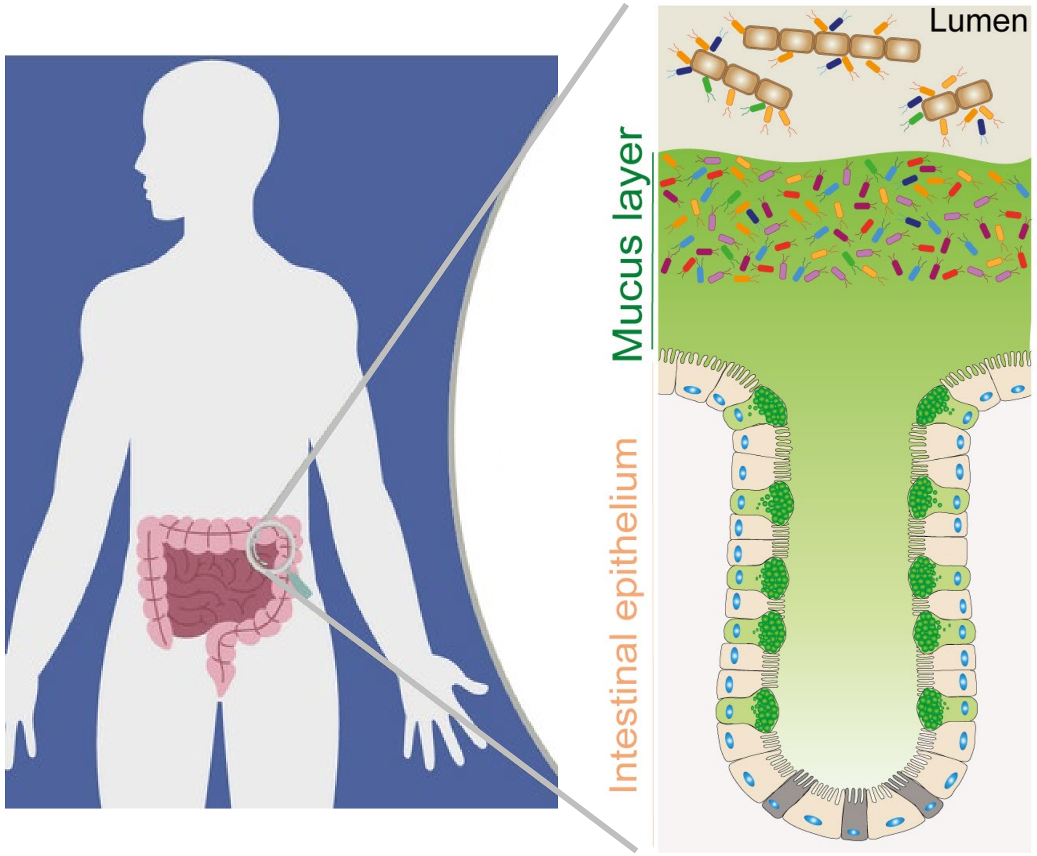
SciLifeLab

Gut microbiota – impact in human heath and disease

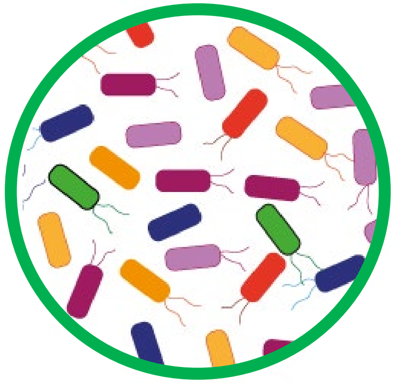


Fan, Y., *et al* (2021)Nat Rev Microbiol

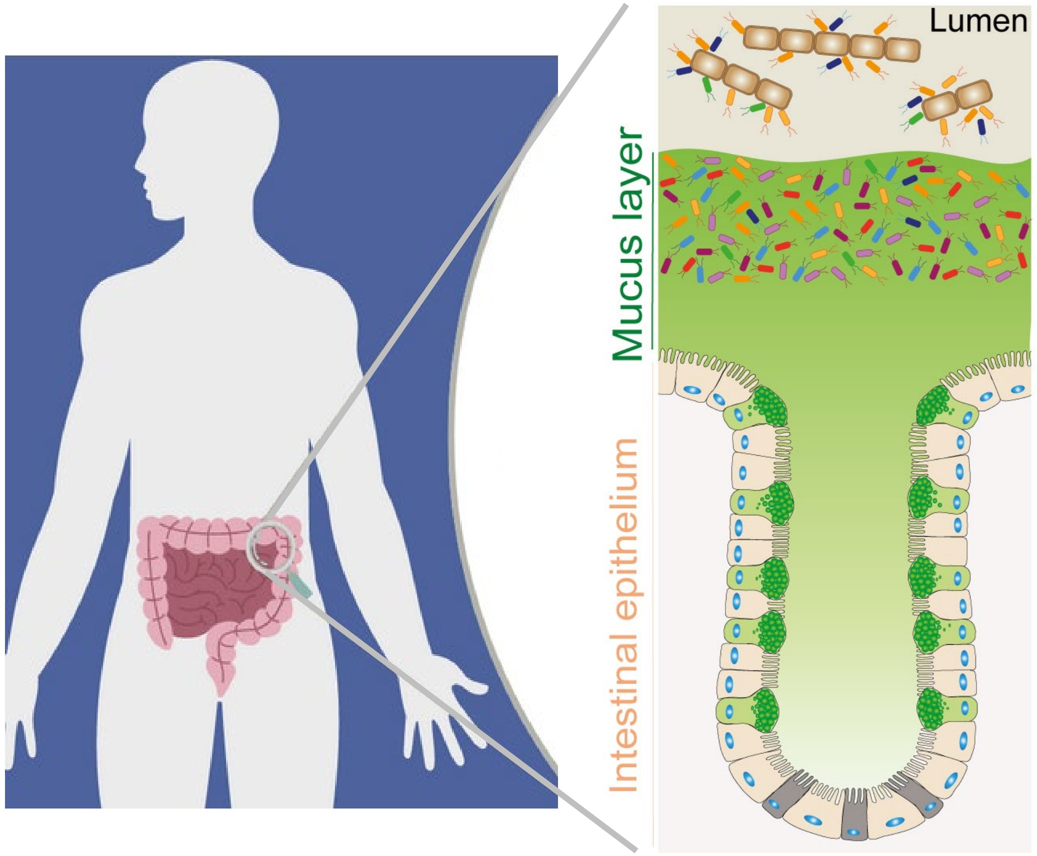
Microbiota colonizes the colonic mucus layer



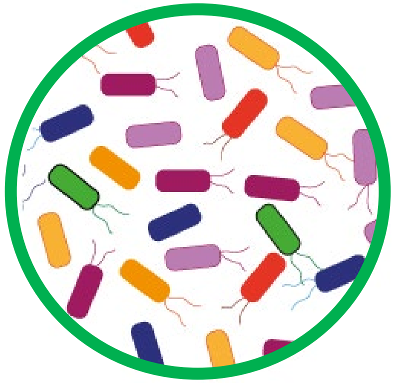
Microbiota



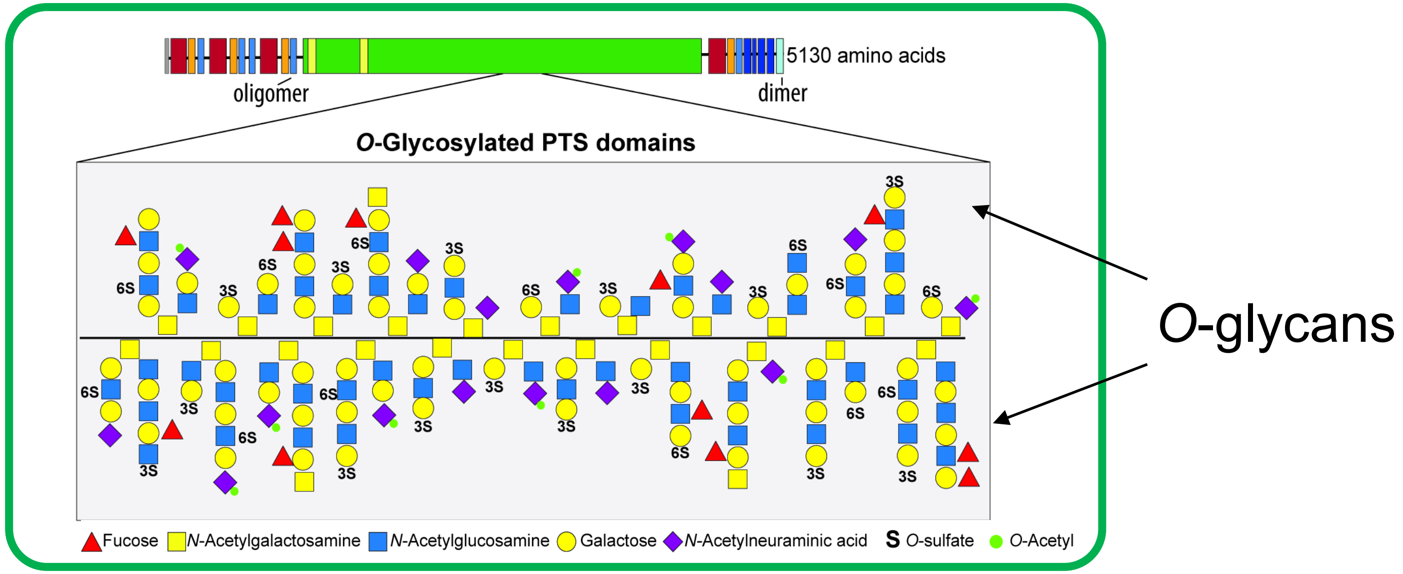
Microbiota colonizes the colonic mucus layer



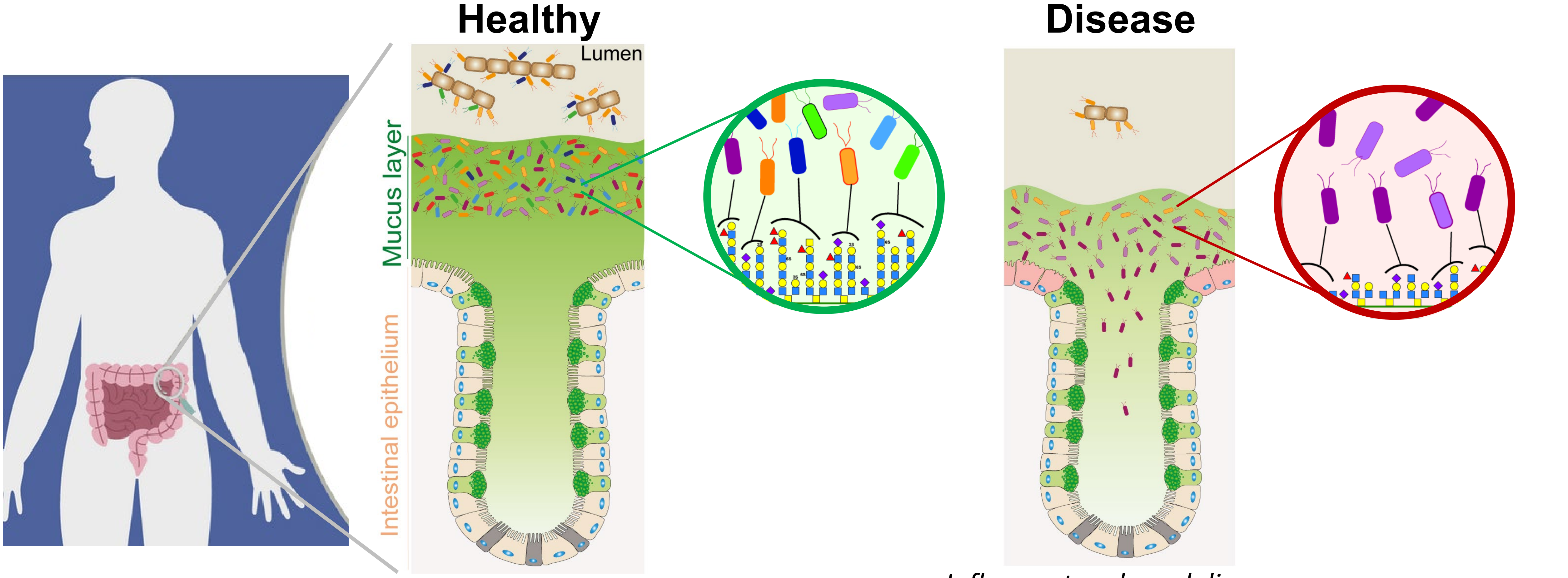
Microbiota



Mucin 2

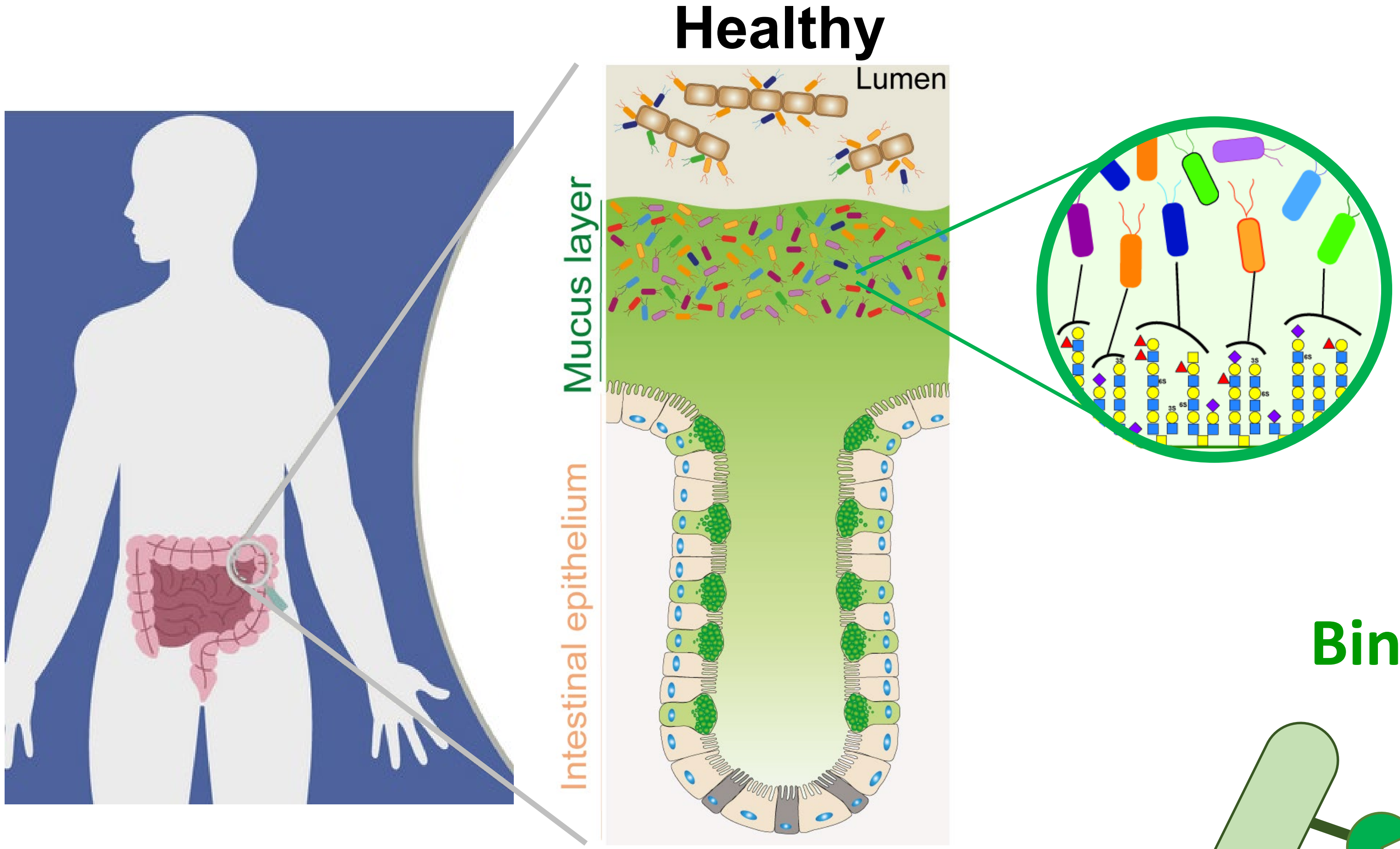


Microbiome and mucus barrier function linked to diseases



Inflammatory bowel diseases
Colorectal cancer
Obesity

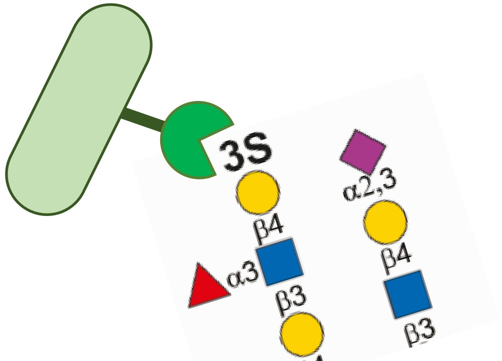
Microbiota-mucin interactions are key in gut colonization

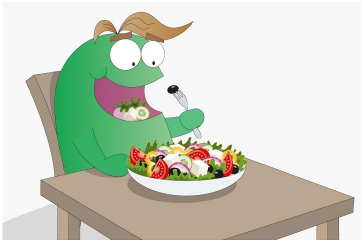


Interactions microbiota-mucin

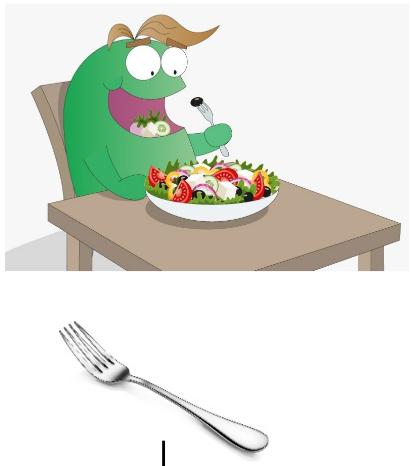
Binding

Degradation

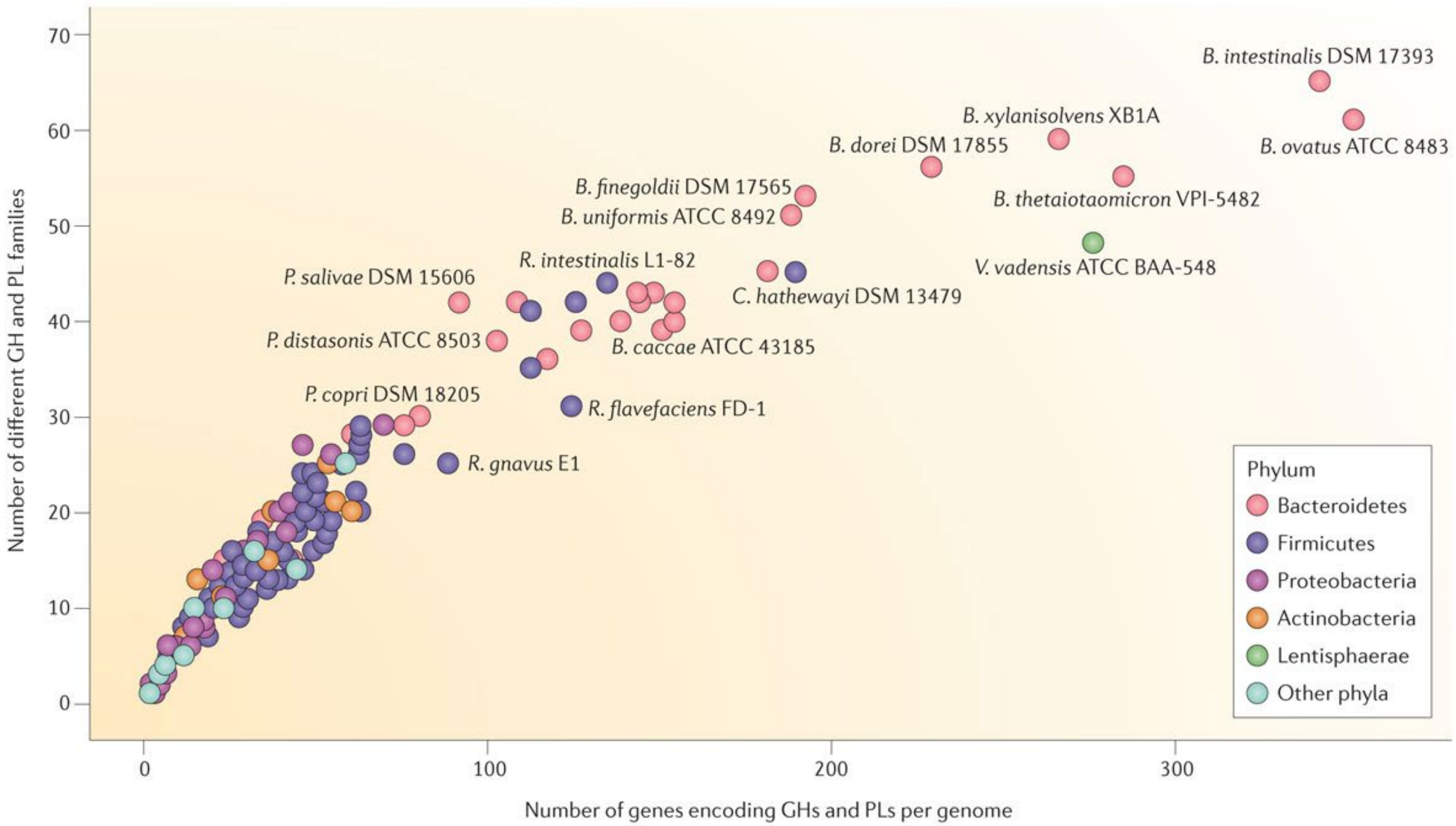




Gut microbiota enzymes



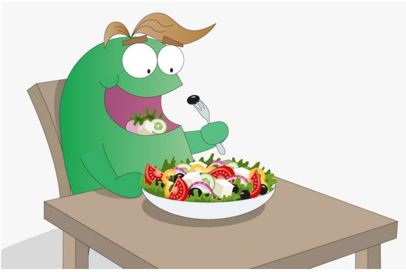
Enzymes



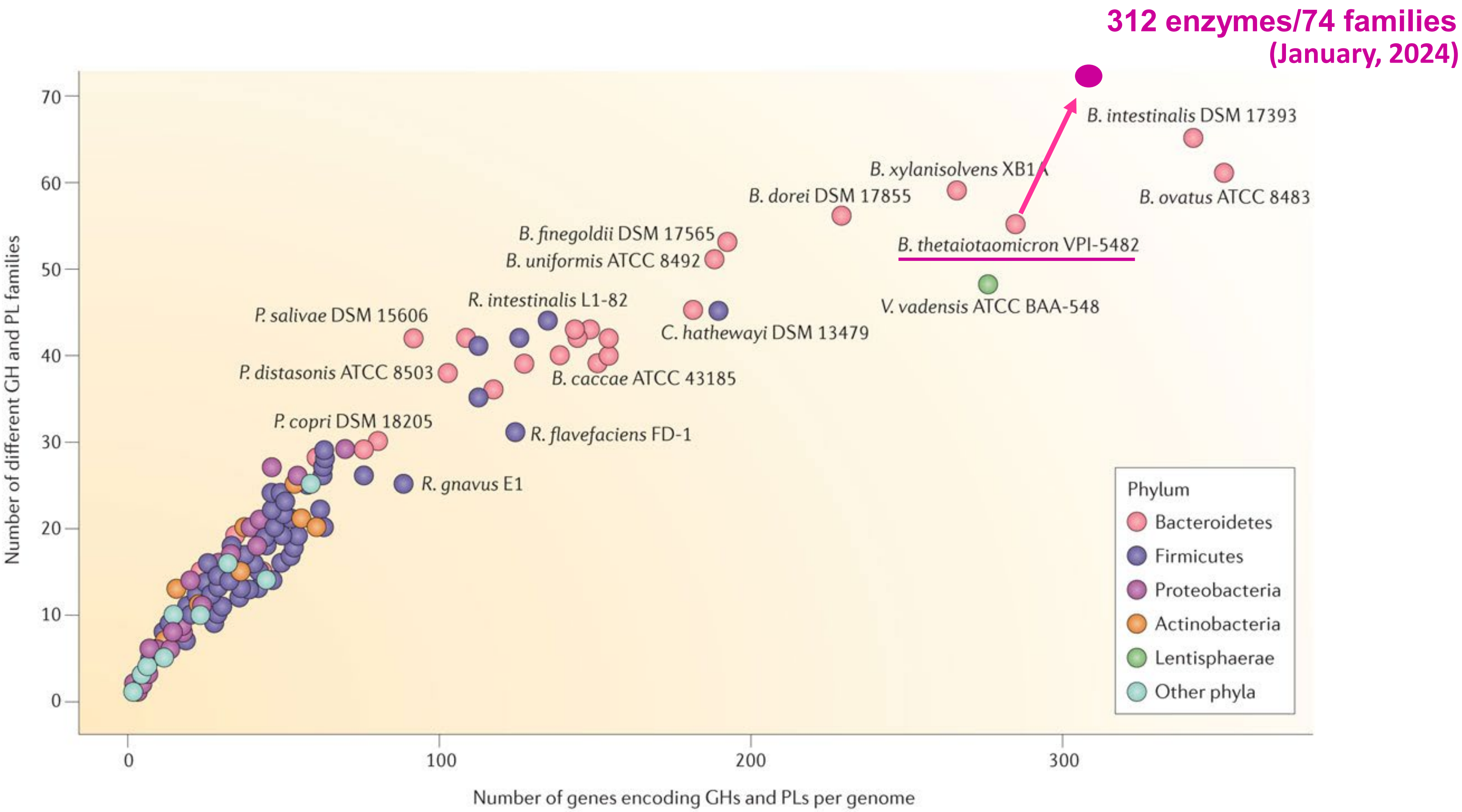
Kaoutari *et al.* (2013) Nat Rev Microbiol

Gut microbiota enzymes

Bacteroides thetaiotaomicron VPI-5482



Enzymes



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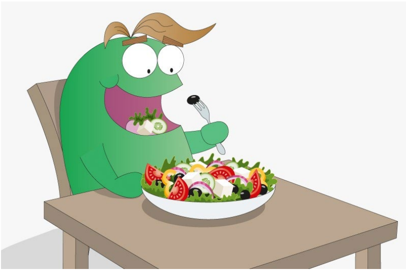
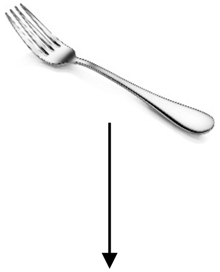
Gut microbiota enzymes





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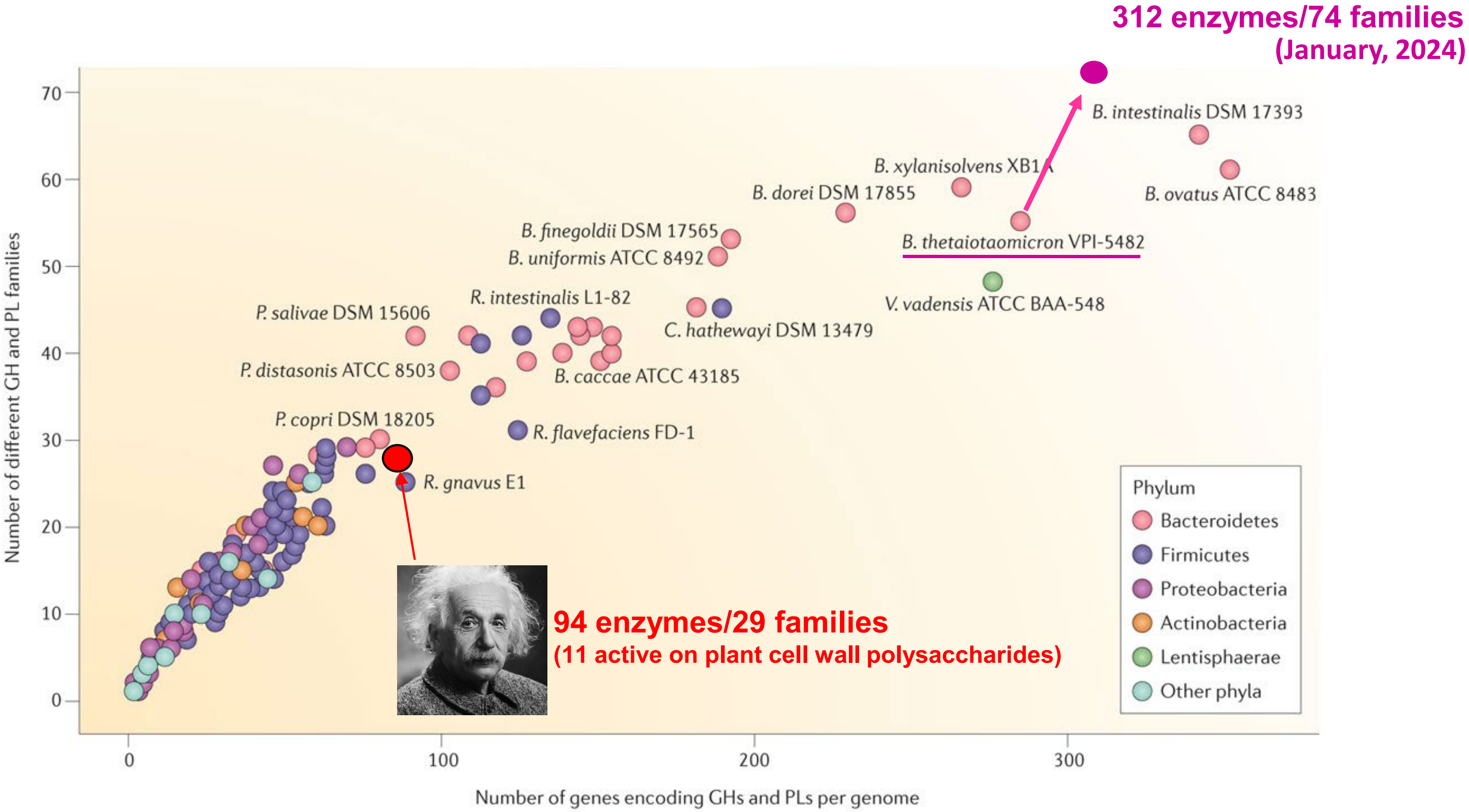

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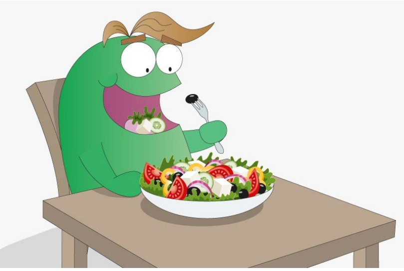
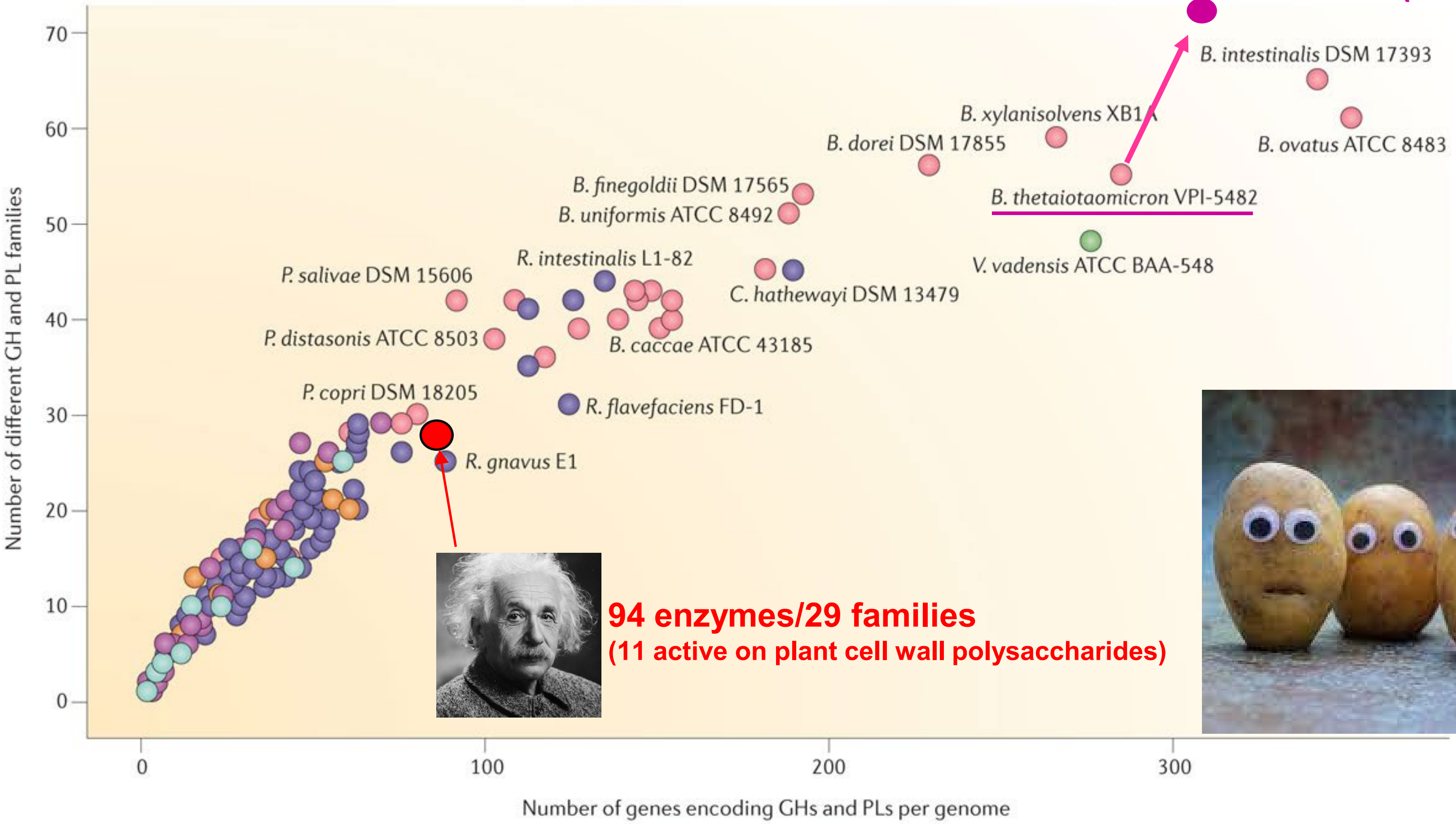


Enzymes

Bacteroides thetaiotaomicron VPI-5482

312 enzymes/74 families
(January, 2024)



94 enzymes/29 families
(11 active on plant cell wall polysaccharides)

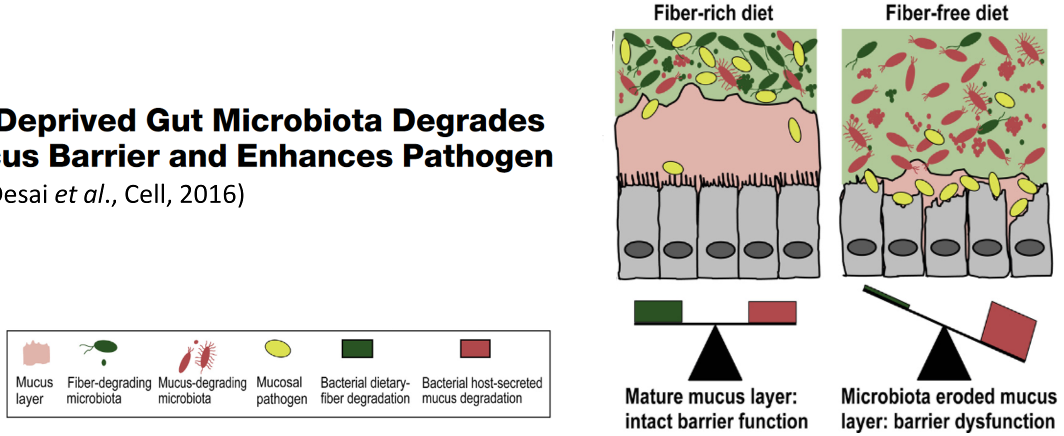
Kaoutari *et al.* (2013) Nat Rev Microbiol

Microbiota enzymes are potential drug targets

Microbiota utilization of *O*-glycans enhances pathogen susceptibility

Cell

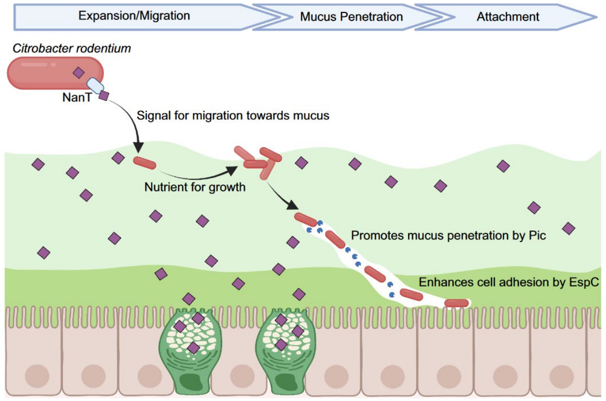
A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility (Desai *et al*., Cell, 2016)



Sialidase activity is required to pathogen infection

PNAS RESEARCH ARTICLE | MICROBIOLOGY OPEN ACCESS

Sialic acid plays a pivotal role in licensing *Citrobacter rodentium's* transition from the intestinal lumen to a mucosal adherent niche (Liang *et al*., PNAS, 2023)



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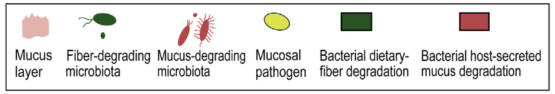
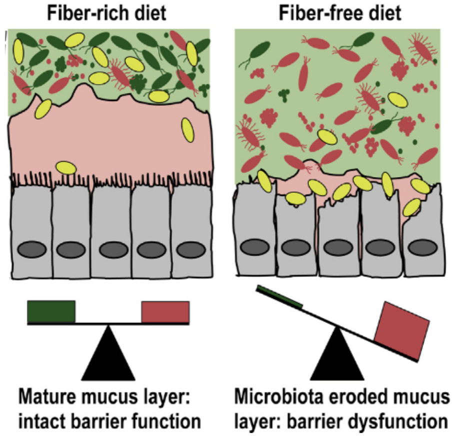


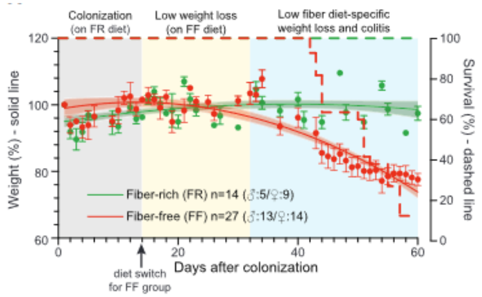


Mucin-degrading bacteria exacerbate colitis

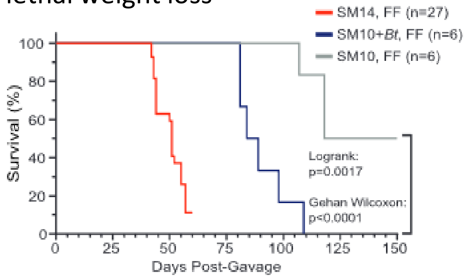
Cell Host & Microbe

Opposing diet, microbiome, and metabolite mechanisms regulate inflammatory bowel disease in a genetically susceptible host (Pereira *et al*., Cell host & microbe, 2024)

Western style diet exacerbate colitis in Il10$^{-/-}$ mice colonized with the SM14



B. thetaiotaomicron
accelerats lethal weight loss



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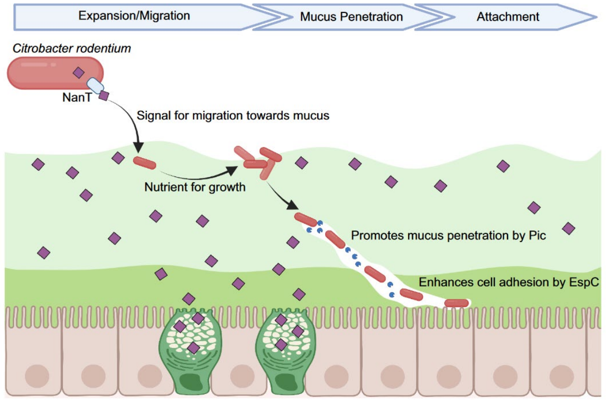
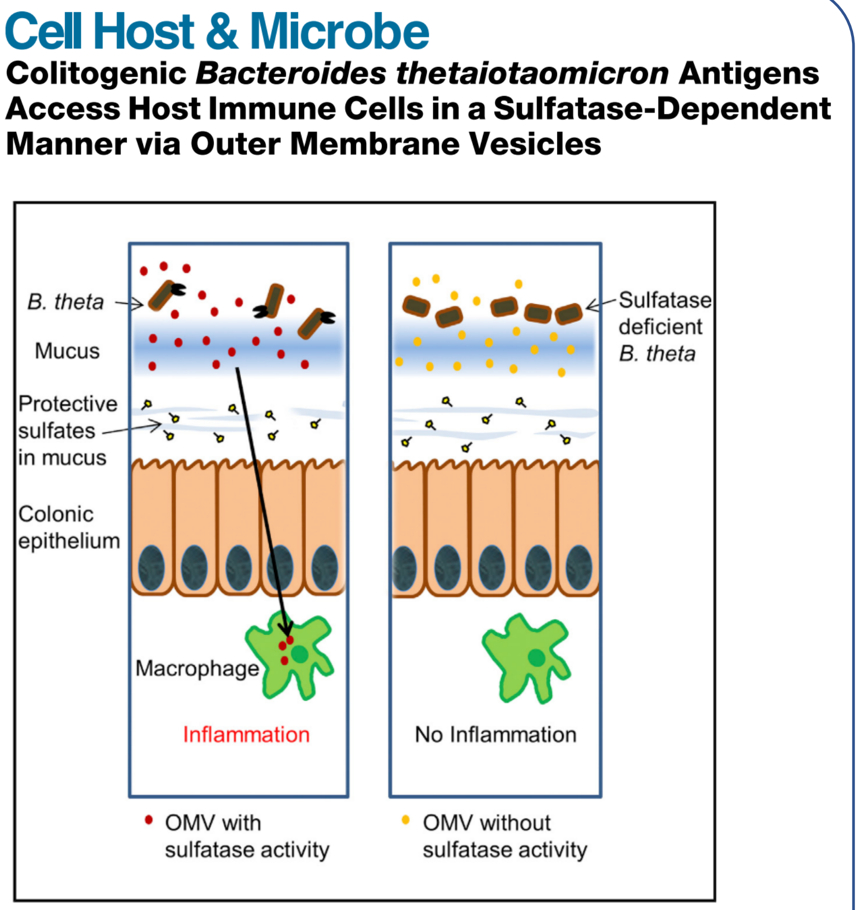
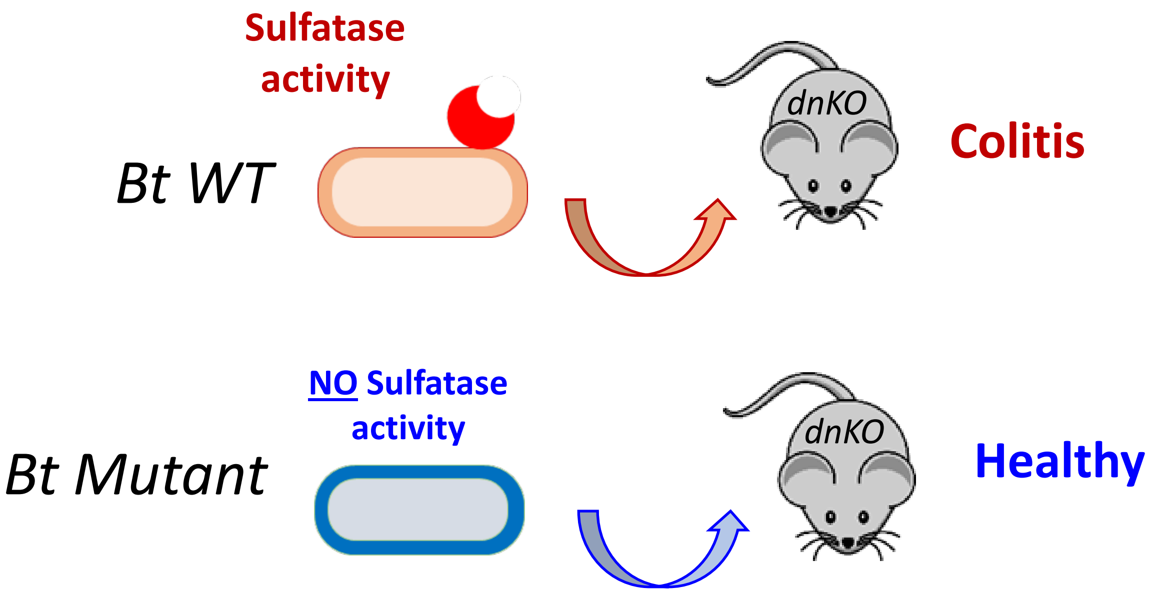


Microbiota enzymes are potential drug targets



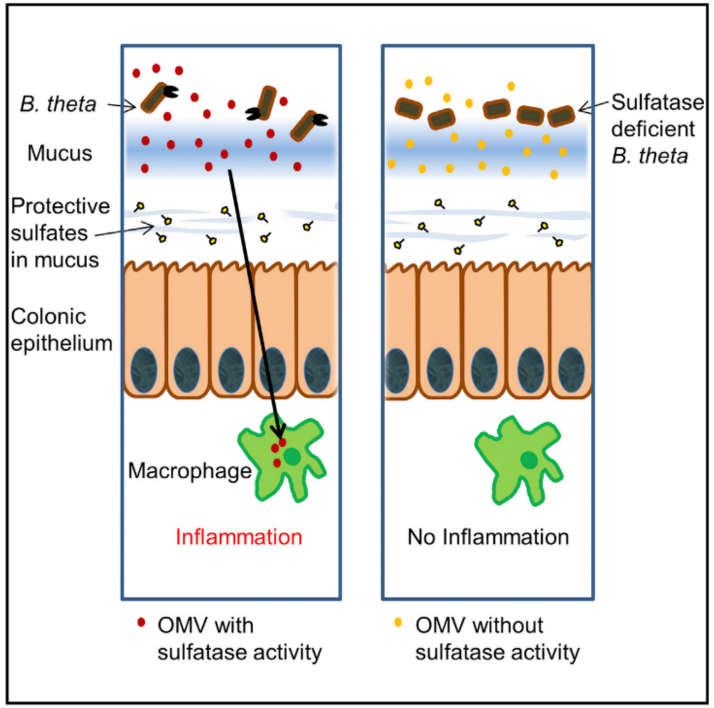
(Hickey *et al*., Cell Host Microbe, 2015)



Microbiota enzymes are potential drug targets

Cell Host & Microbe

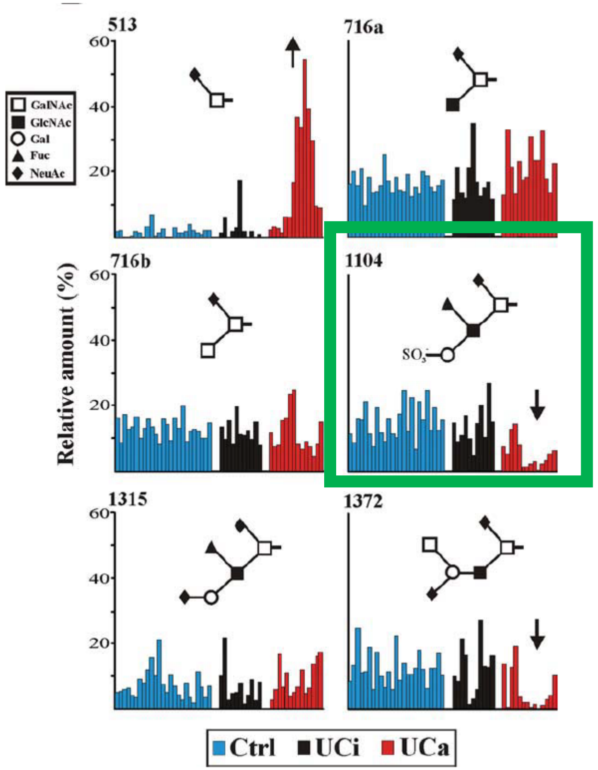
Colitogenic *Bacteroides thetaiotaomicron* Antigens Access Host Immune Cells in a Sulfatase-Dependent Manner via Outer Membrane Vesicles



(Hickey *et al*., Cell Host Microbe, 2015)

Patients with active **ulcerative colitis** have less complex *O*-glycans

↓ **Sulfated oligos**



(Larsson *et al.*, Inflamm Bowel Dis. 2011)

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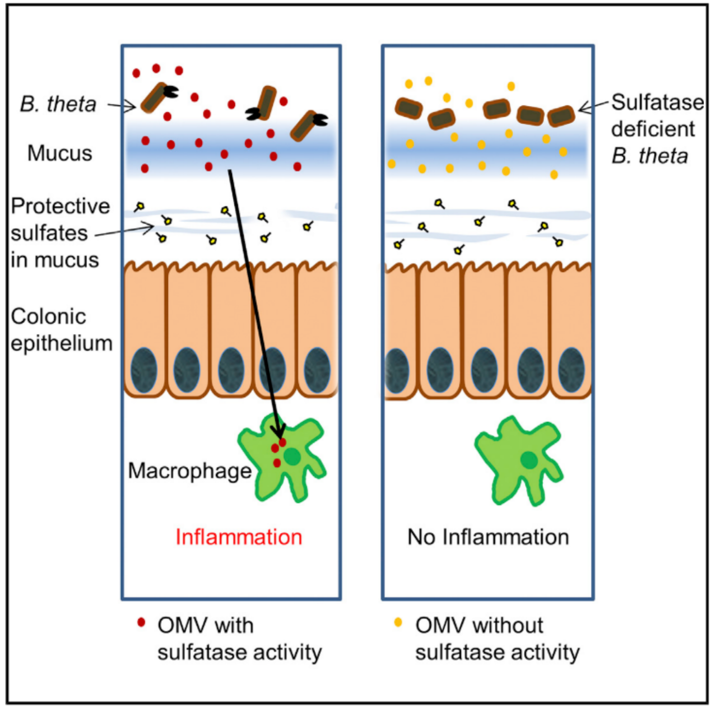

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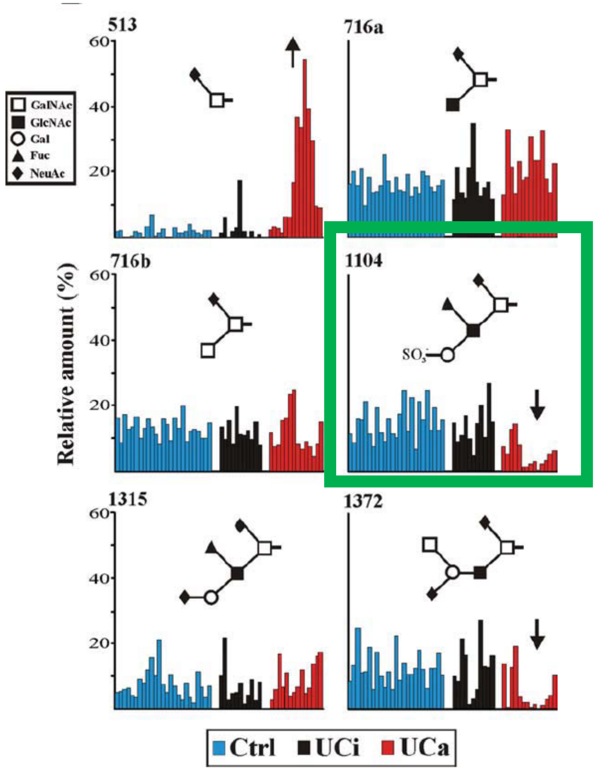

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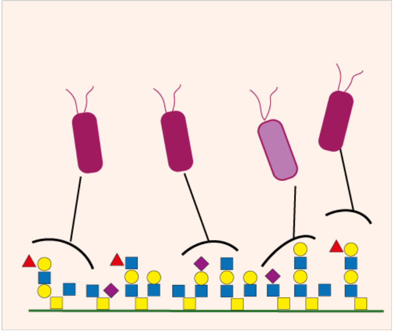
Disease



↑ Mucin degradation

Microbiota enzymes are potential drug targets

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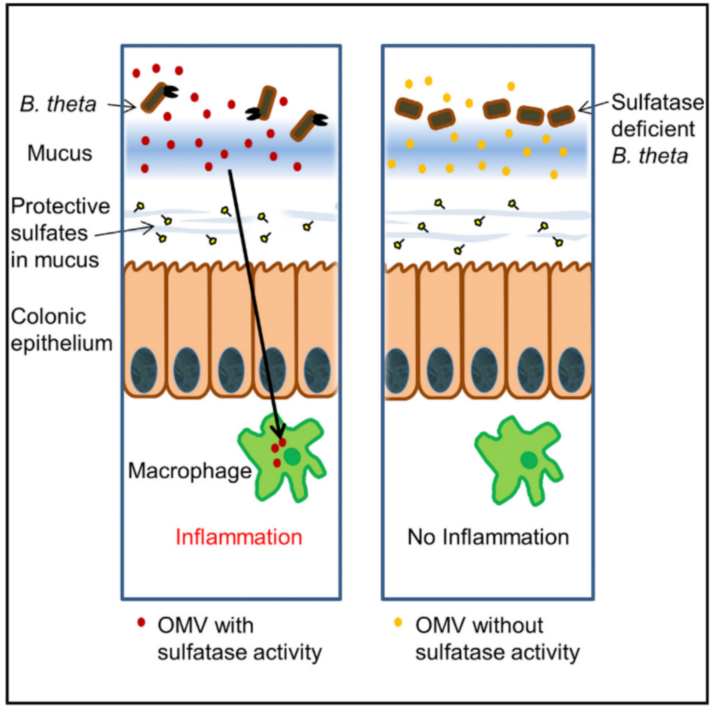

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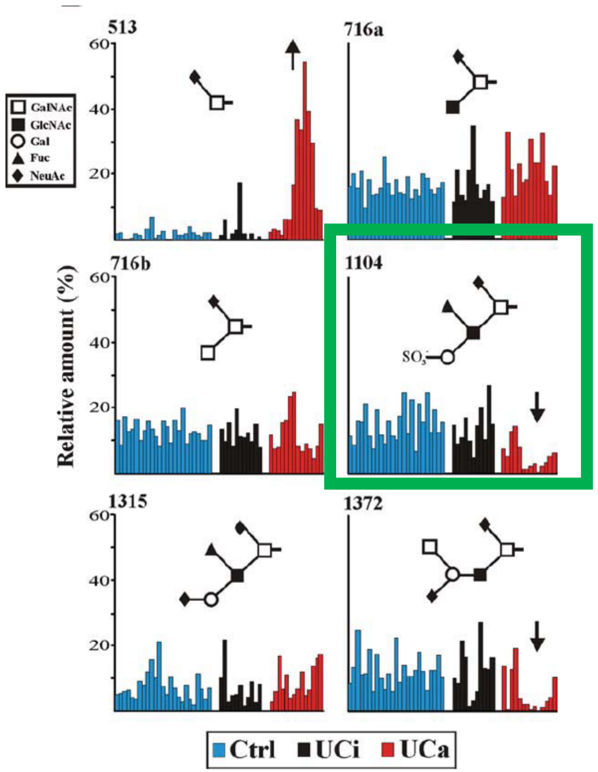
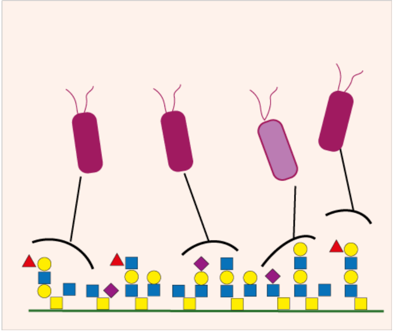


(Larsson *et al*., Inflamm Bowel Dis. 2011)

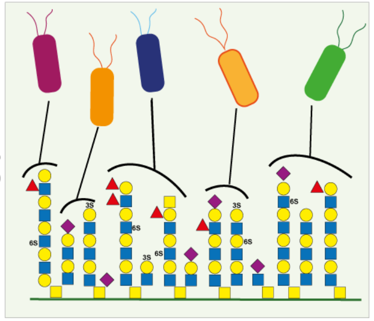
Disease



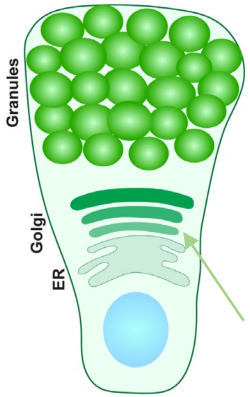
↑ Mucin degradation

STOP

Healthy



Mucin *O*-glycosylation



<u>*O*-glycans</u>

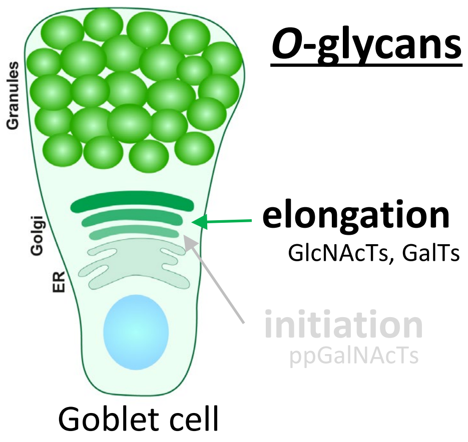
initiation
ppGalNAcTs

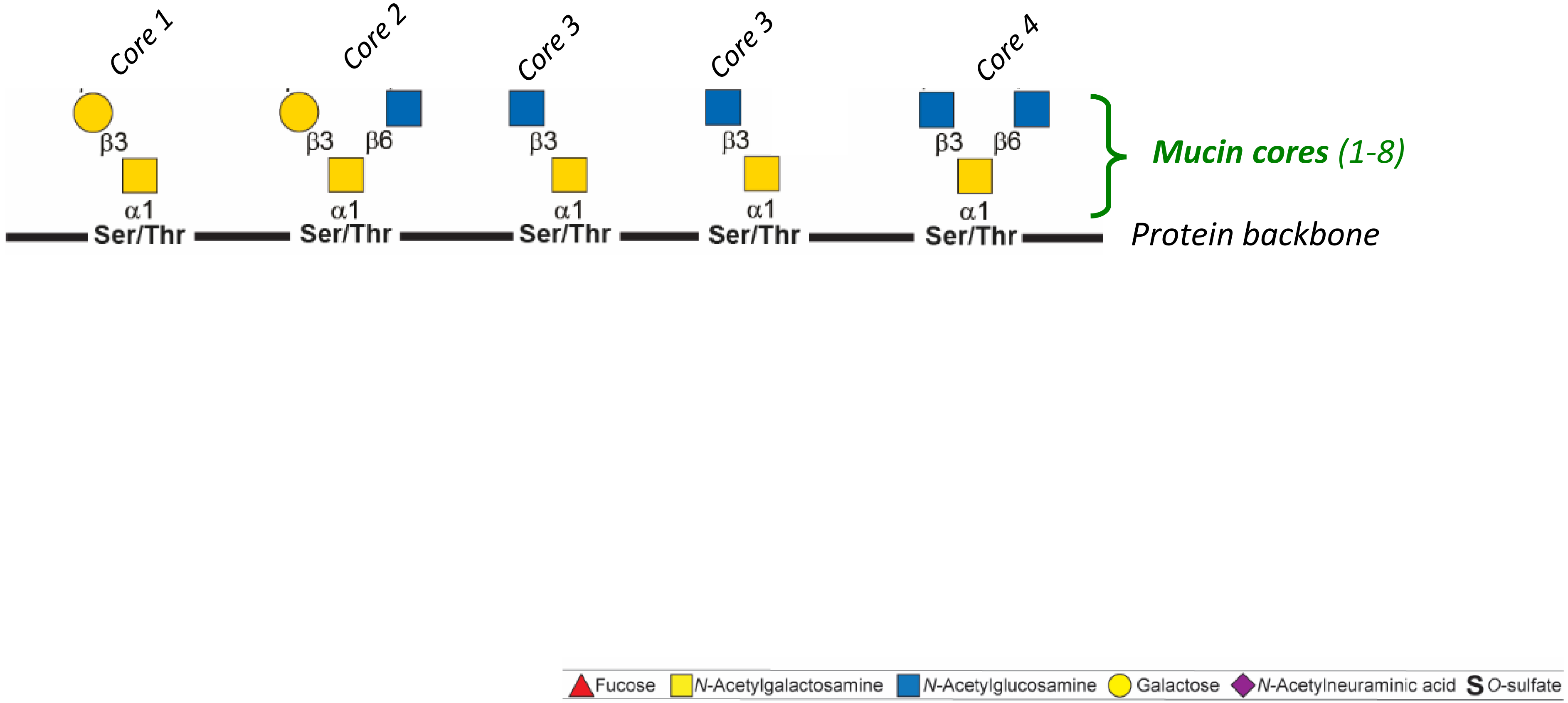
Goblet cell

Protein backbone

Fucose | *N*-Acetylgalactosamine | *N*-Acetylglucosamine | Galactose | *N*-Acetylneuraminic acid | **S** *O*-sulfate

Mucin *O*-glycosylation





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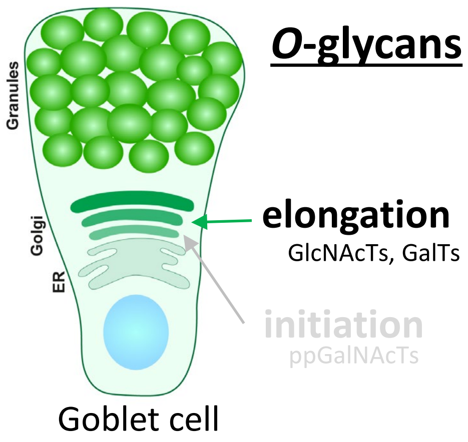

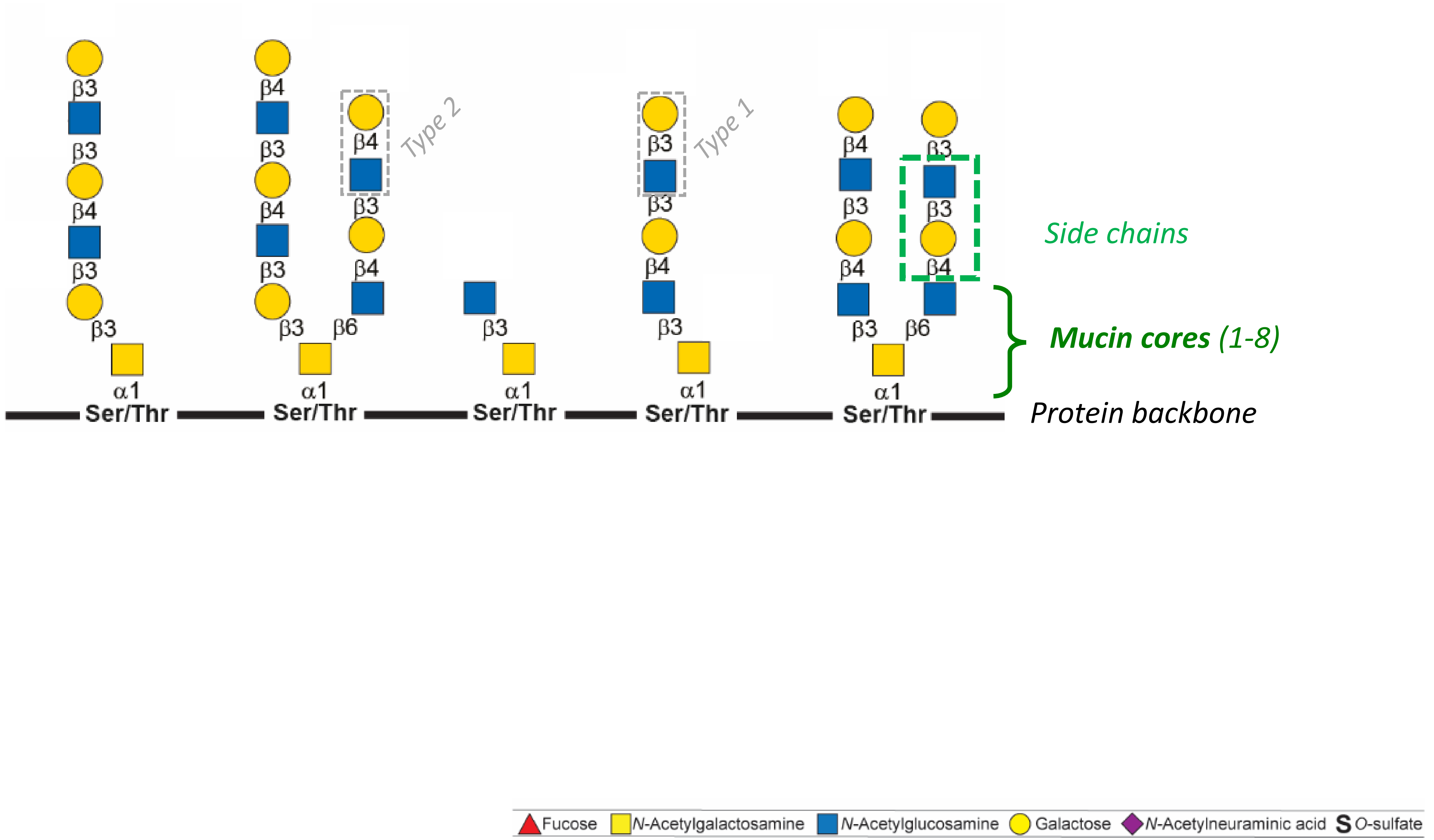

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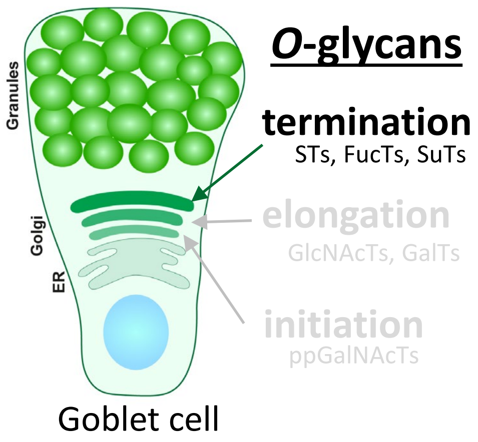

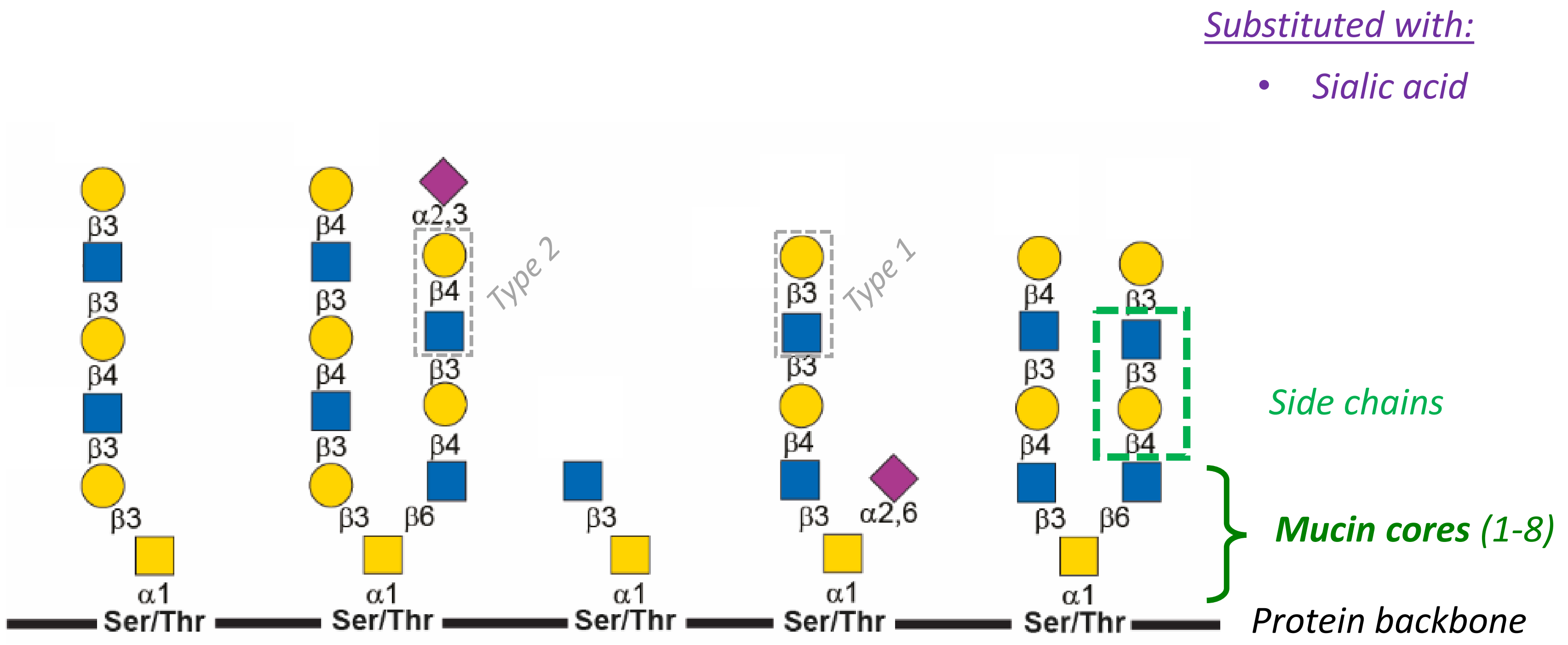

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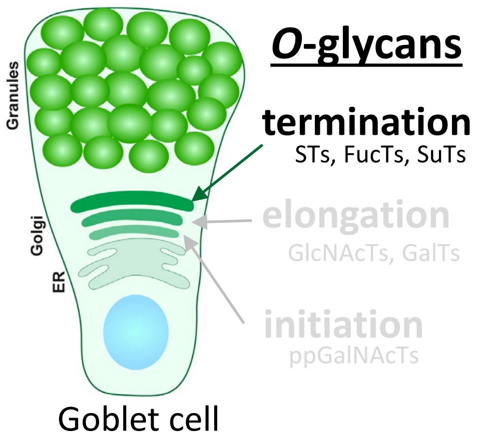

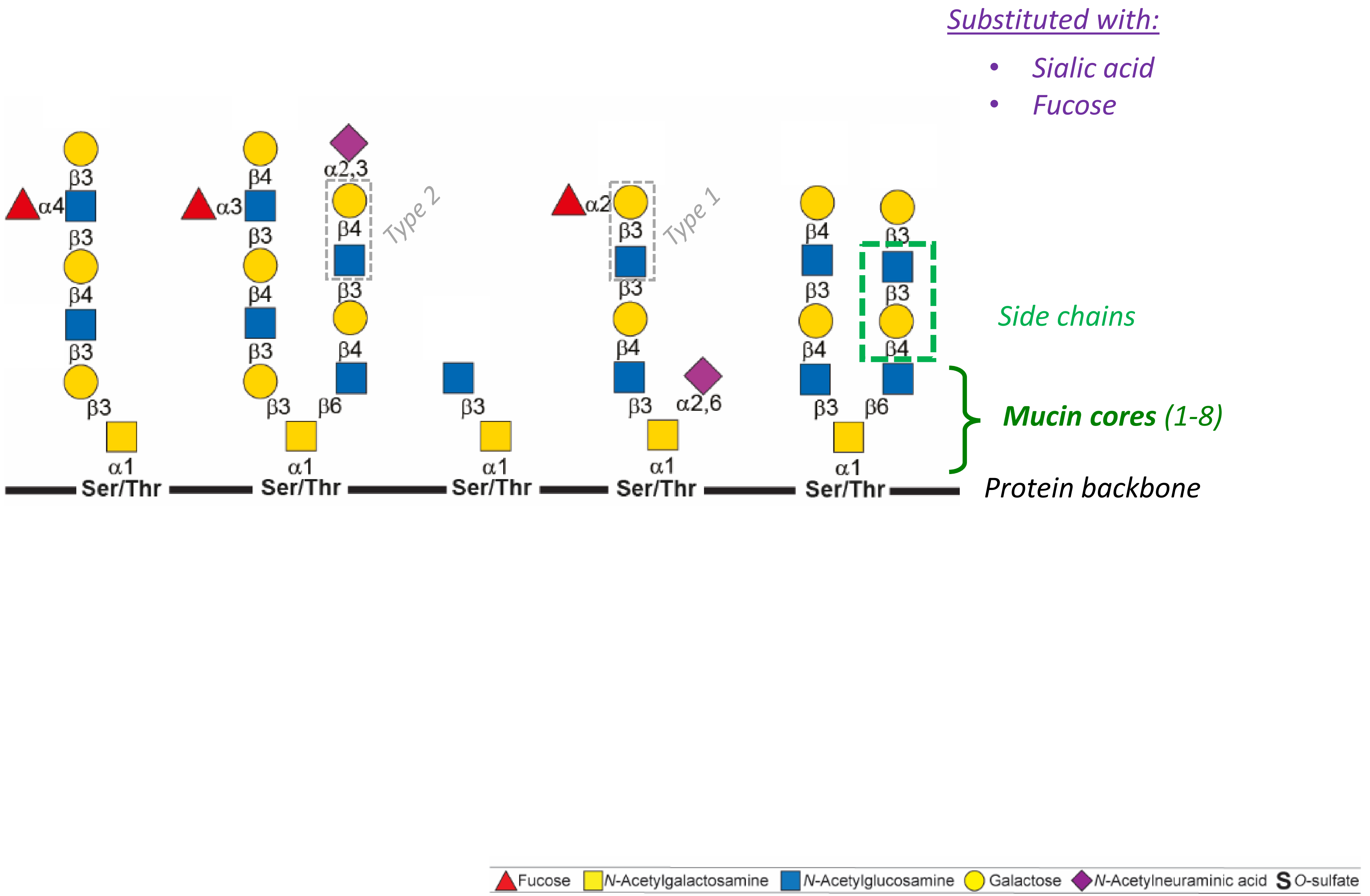

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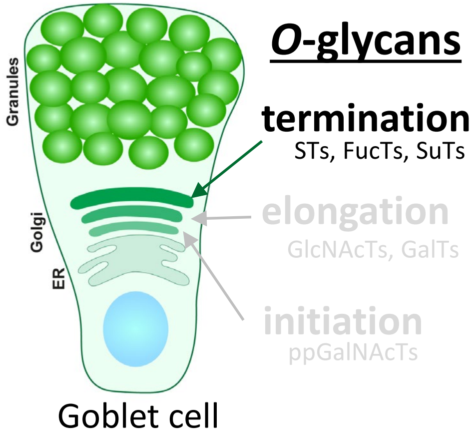
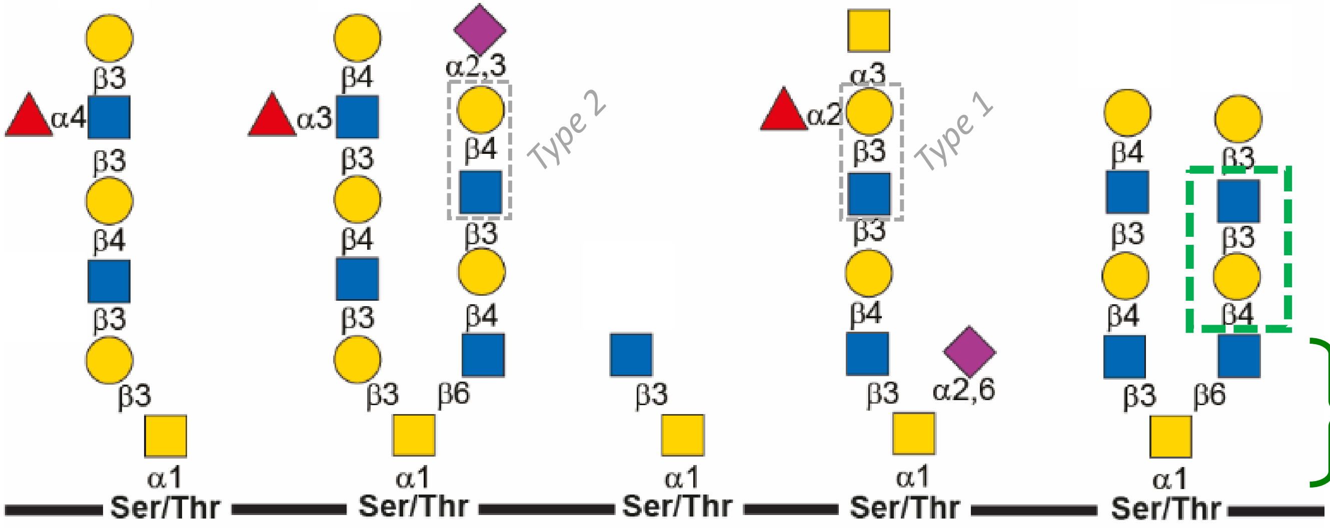




Substituted with:

- *Sialic acid*
- *Fucose*

(Blood groups)

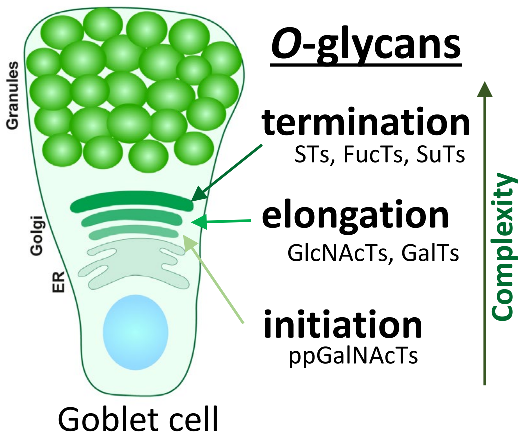
Side chains

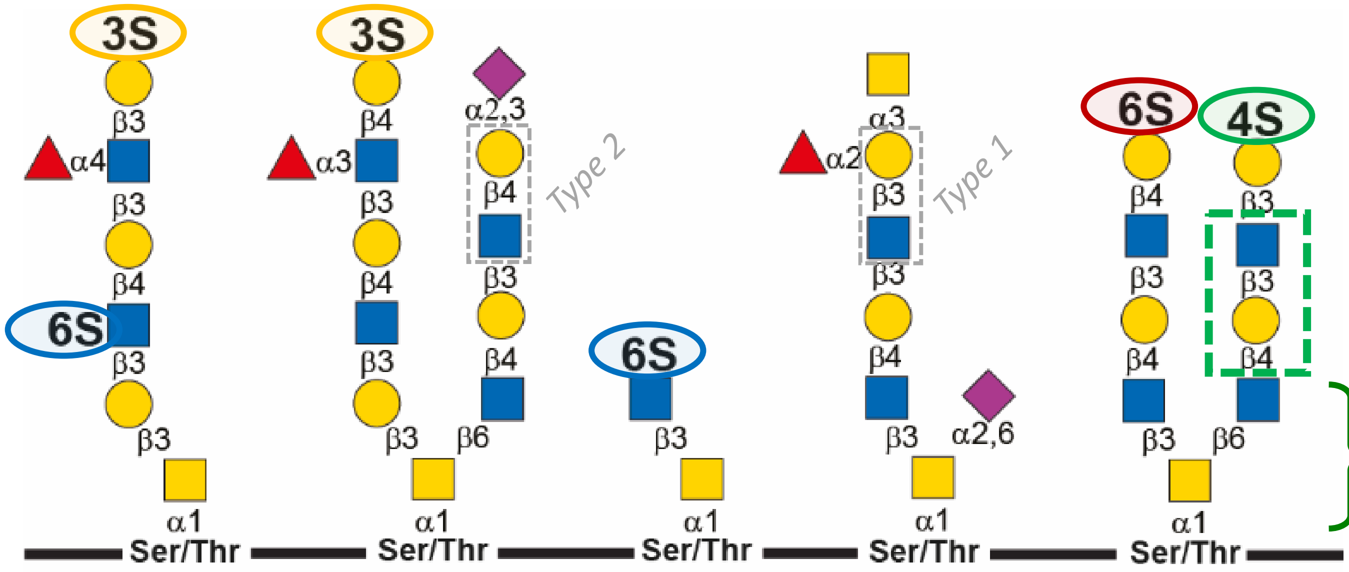
***Mucin cores** (1-8)*

Protein backbone

Fucose N-Acetylgalactosamine N-Acetylglucosamine Galactose N-Acetylneuraminic acid S O-sulfate

Mucin *O*-glycosylation





Substituted with:

- *Sialic acid*
- *Fucose*
  (Blood groups)
- *Sulfate*
  3S/4S/6S-Gal
  6S-GlcNAc

\+

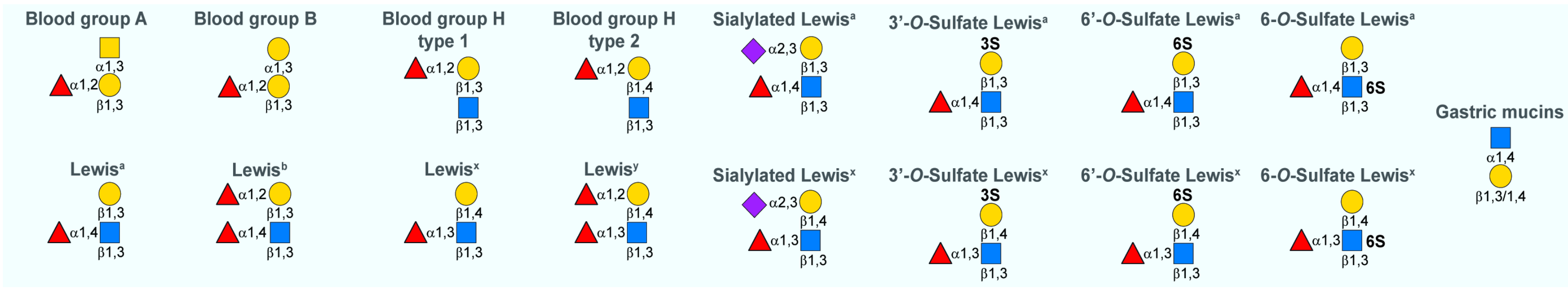
Side chains

\+

***Mucin cores** (1-8)*

Protein backbone

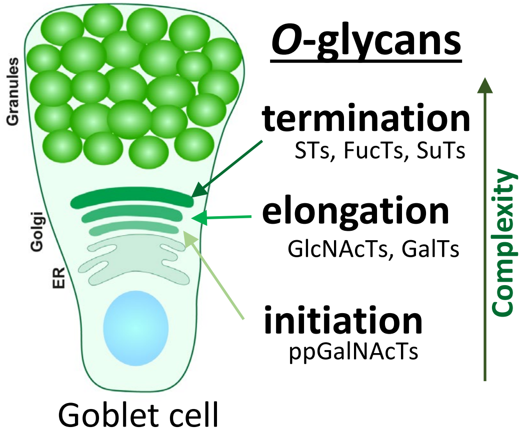
Terminal mucin *O*-glycan epitopes

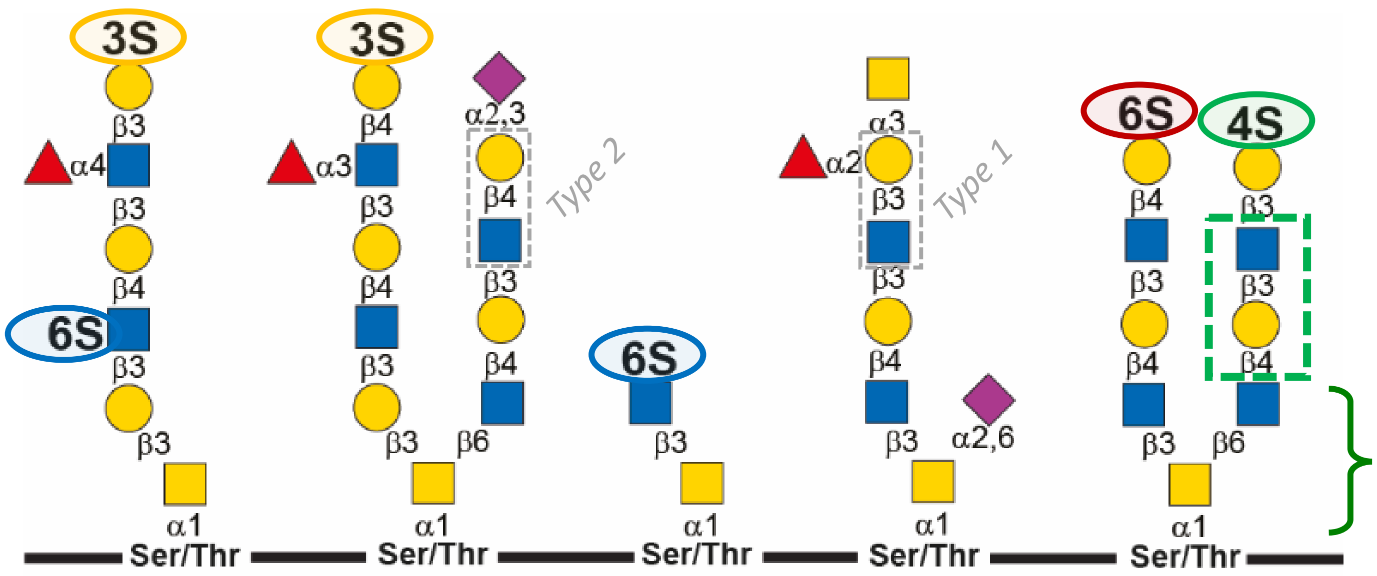


=

> 100 different *O*-glycans

Mucin *O*-glycosylation





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  (Blood groups)
- *Sulfate*
  3S/4S/6S-Gal
  6S-GlcNAc

\+

Side chains

\+

***Mucin cores** (1-8)*

Protein backbone

Glycosylation is variable:

- between species  vs 
- along the gastrointestinal tract  vs 

=

> 100 different *O*-glycans

Fucose | *N*-Acetylgalactosamine | *N*-Acetylglucosamine | Galactose | *N*-Acetylneuraminic acid | **S** *O*-sulfate

Mucin *O*-glycosylation is variable

Previous studies:

Porcine **gastric** mucins

Commercially available

Mucin *O*-glycosylation is variable

Previous studies:

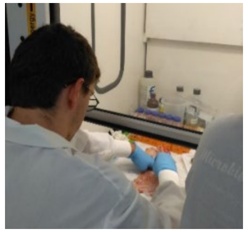

Porcine **gastric** mucins

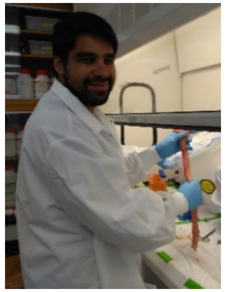
Commercially available

Present:


Porcine **colonic** mucins

in house purification




Mucin *O*-glycosylation is variable

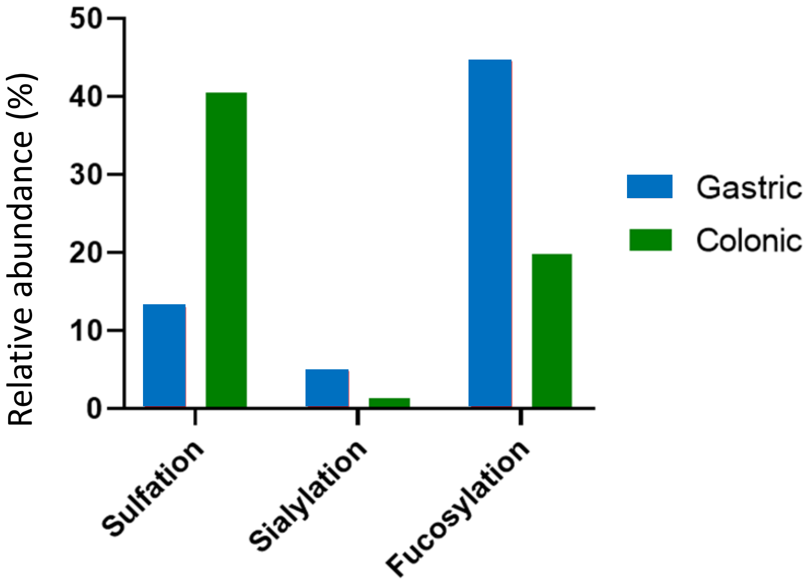
Previous studies:


Porcine **gastric** mucins

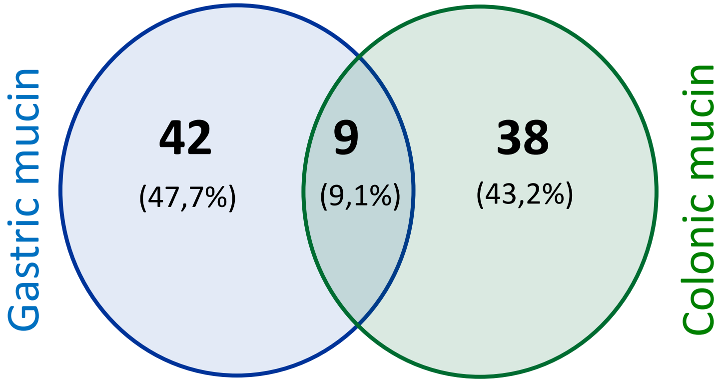
Present:


Porcine **colonic** mucins

Mucin modifications



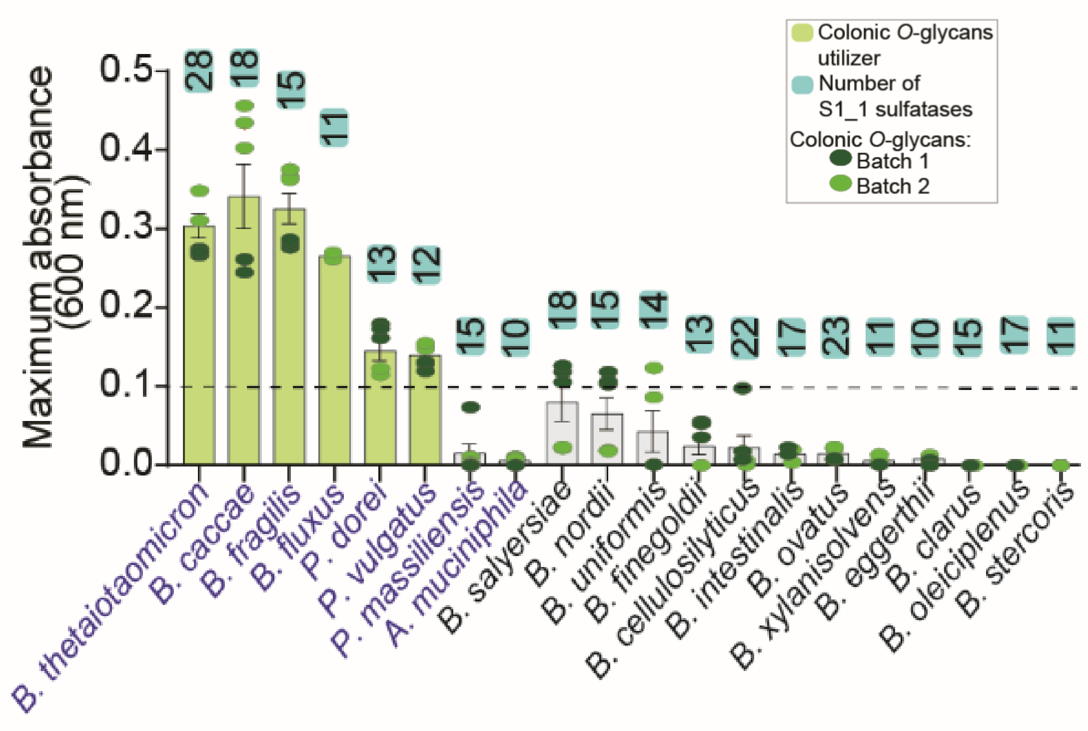
Detected glycans



Raba *et al.* iScience, 2024, Jun 21

Microbiota members can degrade *O*-glycans

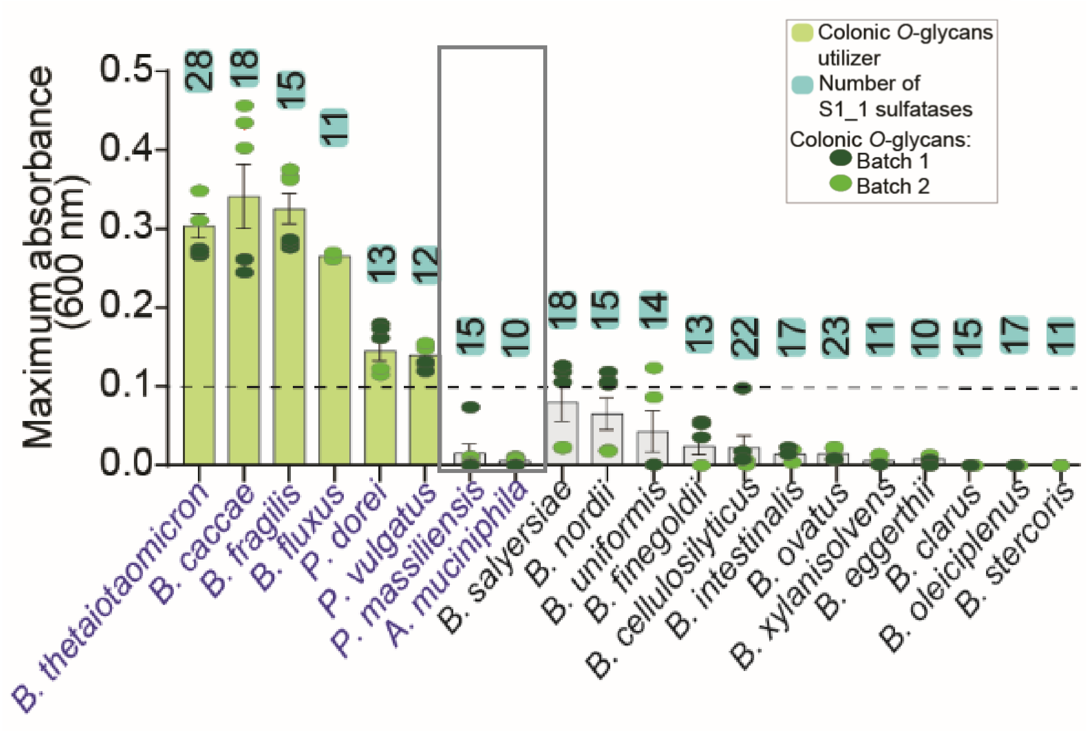
Bacterial growth on porcine colonic mucin O-glycans

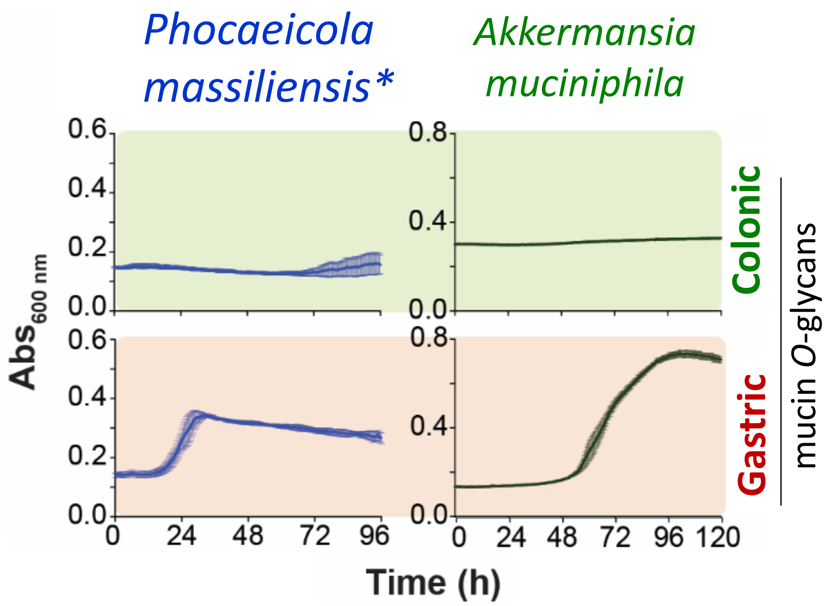


Luis AS *et al.* Nature. 2021 Oct;598(7880):332-337

Microbiota members can degrade *O*-glycans

Bacterial growth on porcine colonic mucin O-glycans





Previously Bacteroides massiliensis

Luis AS *et al.* Nature. 2021 Oct;598(7880):332-337

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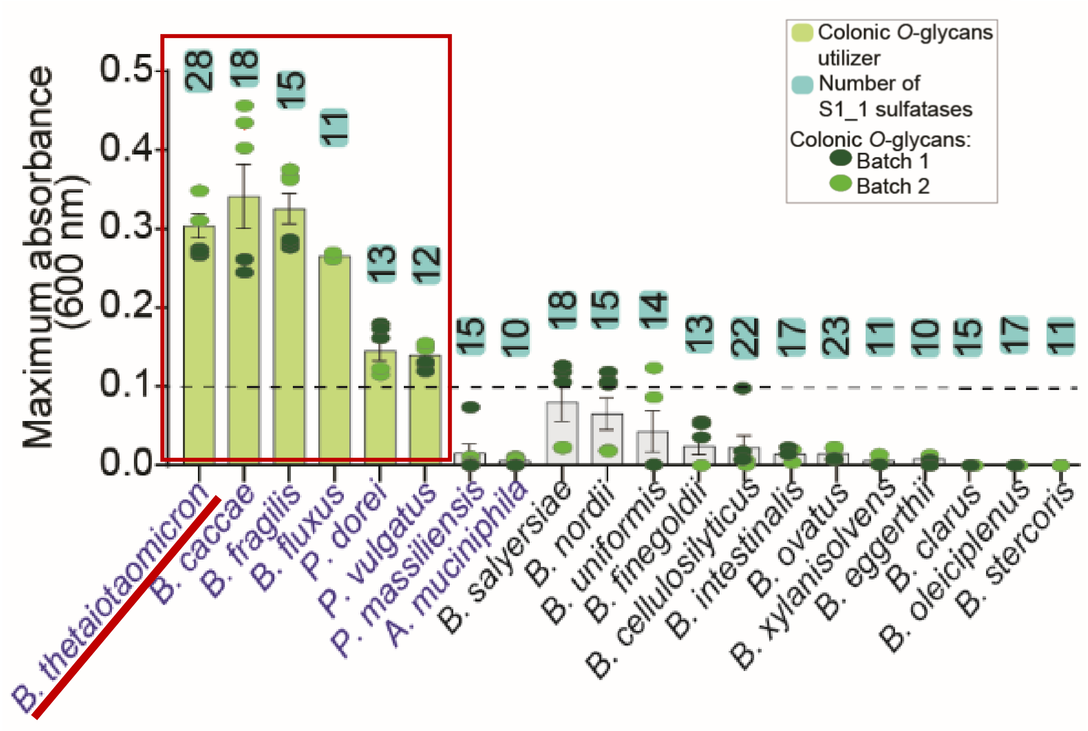
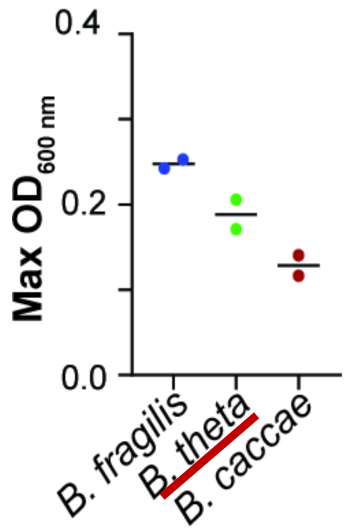


Luis AS *et al.* Nature. 2021 Oct;598(7880):332-337

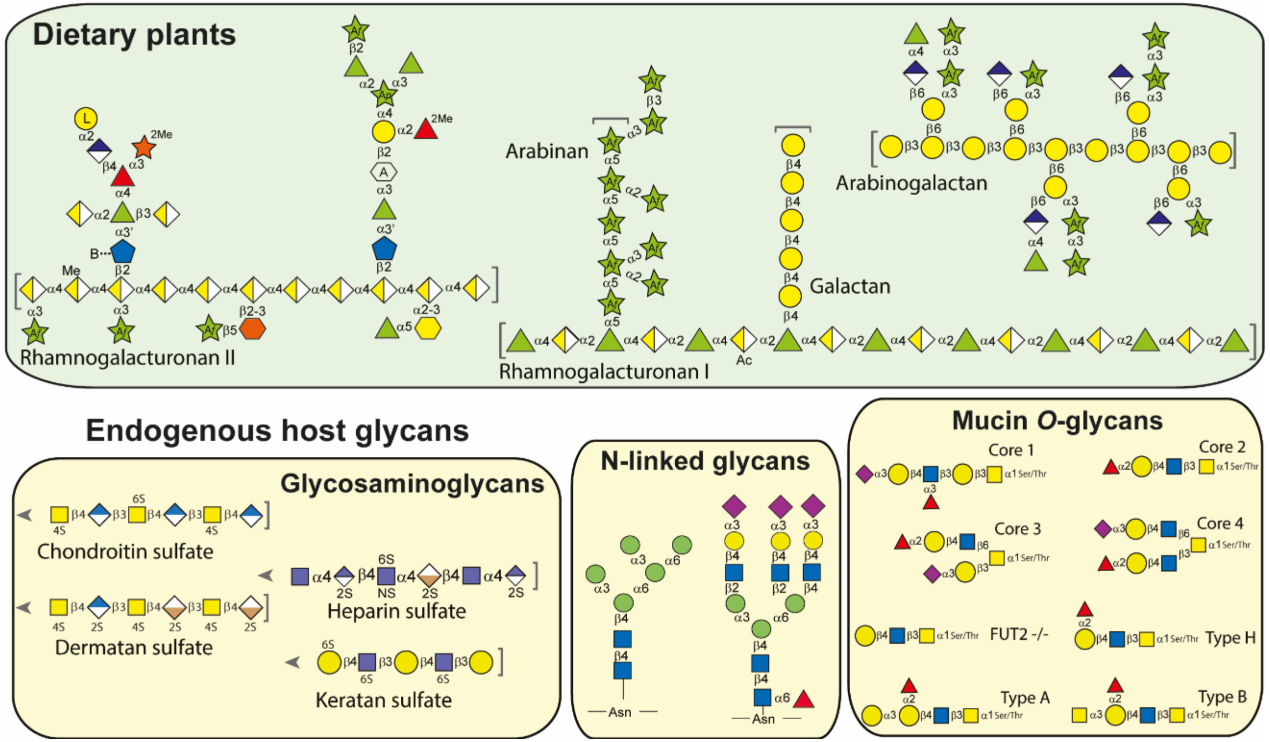
Bacterial growth on human colonic mucin O-glycans



Unpublished

Bacteroides thetaiotaomicron (B. theta)

- Commensal (gram-negative anaerobe)
- one of the most common bacteria of the human microbiota
- capable of metabolizing a very diverse range of polysaccharides (plant cell wall and host glycans)



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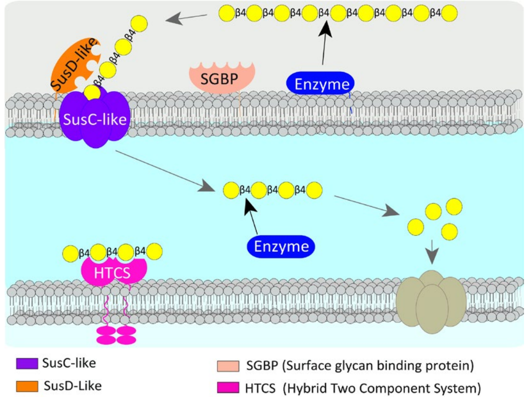
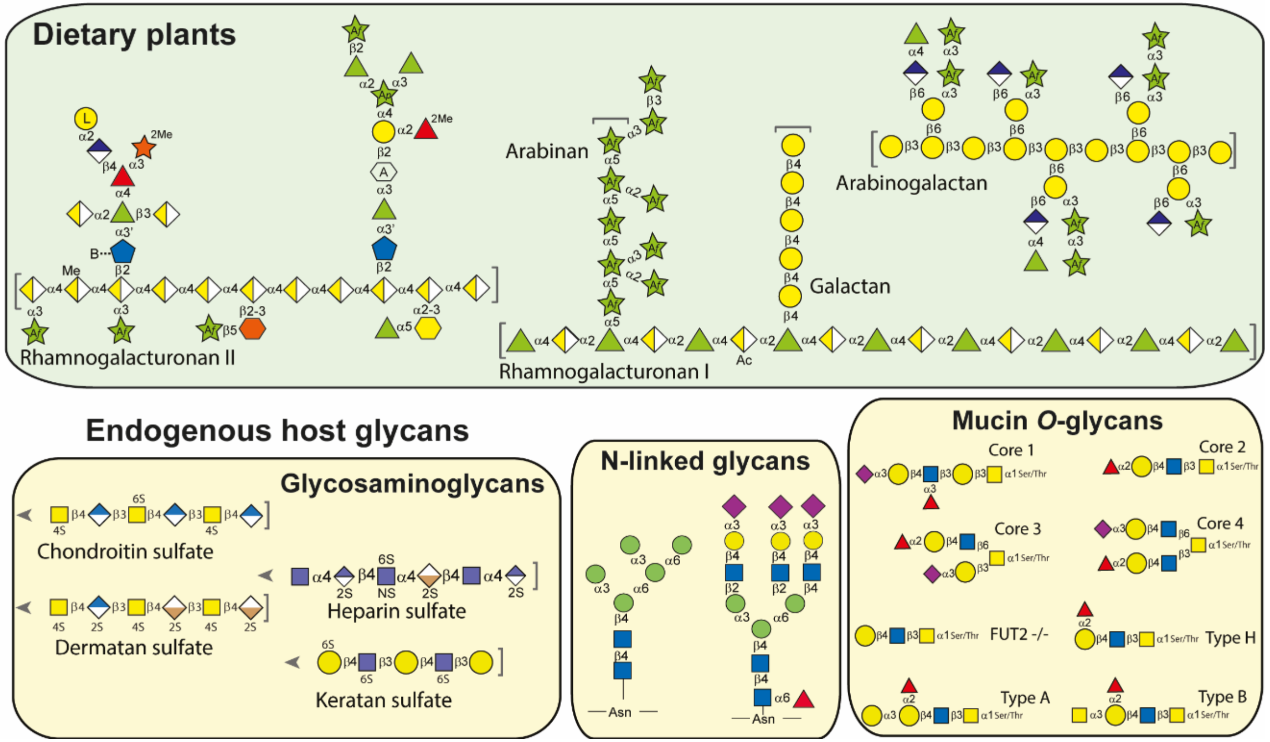
polysaccharide utilization loci (PUL)

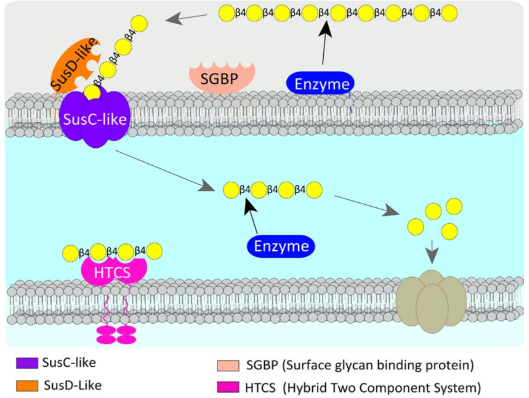




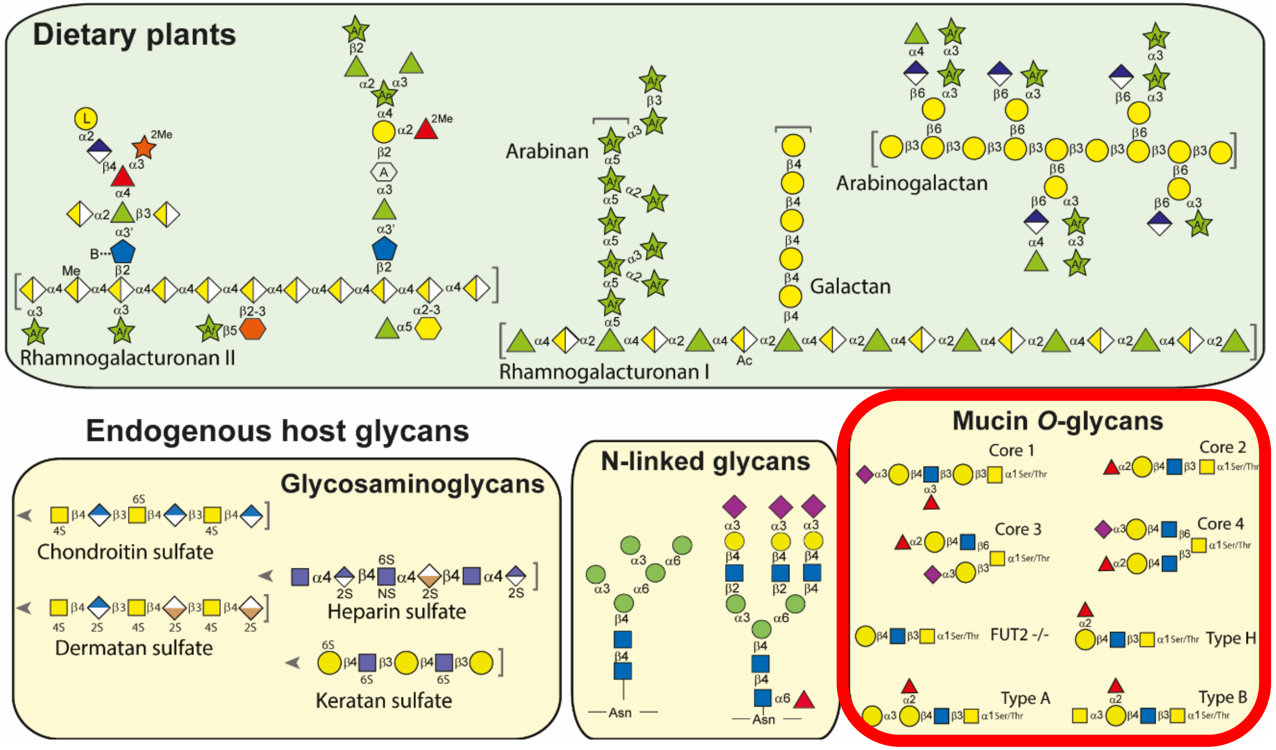
Bacteroides thetaiotaomicron (B. theta)

- Commensal (gram-negative anaerobe)
- one of the most common bacteria of the human microbiota
- capable of metabolizing a very diverse range of polysaccharides (plant cell wall and host glycans)

- all known **polysaccharide utilization loci (PUL)** are characterized (exception: *O*-glycans)



Cuskin *et al*., Nature, 2015
Ndeh *et al*., Nature, 2017
Cartmell *et al*., PNAS, 2017
Temple *et al*., JBC, 2017
Cartmell *et al*., Nat Microbiol, 2018
Luis *et al*., Nat Microbiol, 2018
Briliūtė *et al*., Nat Microbiol, 2019
Ndeh *et al*., Nat Microbiol, 2020



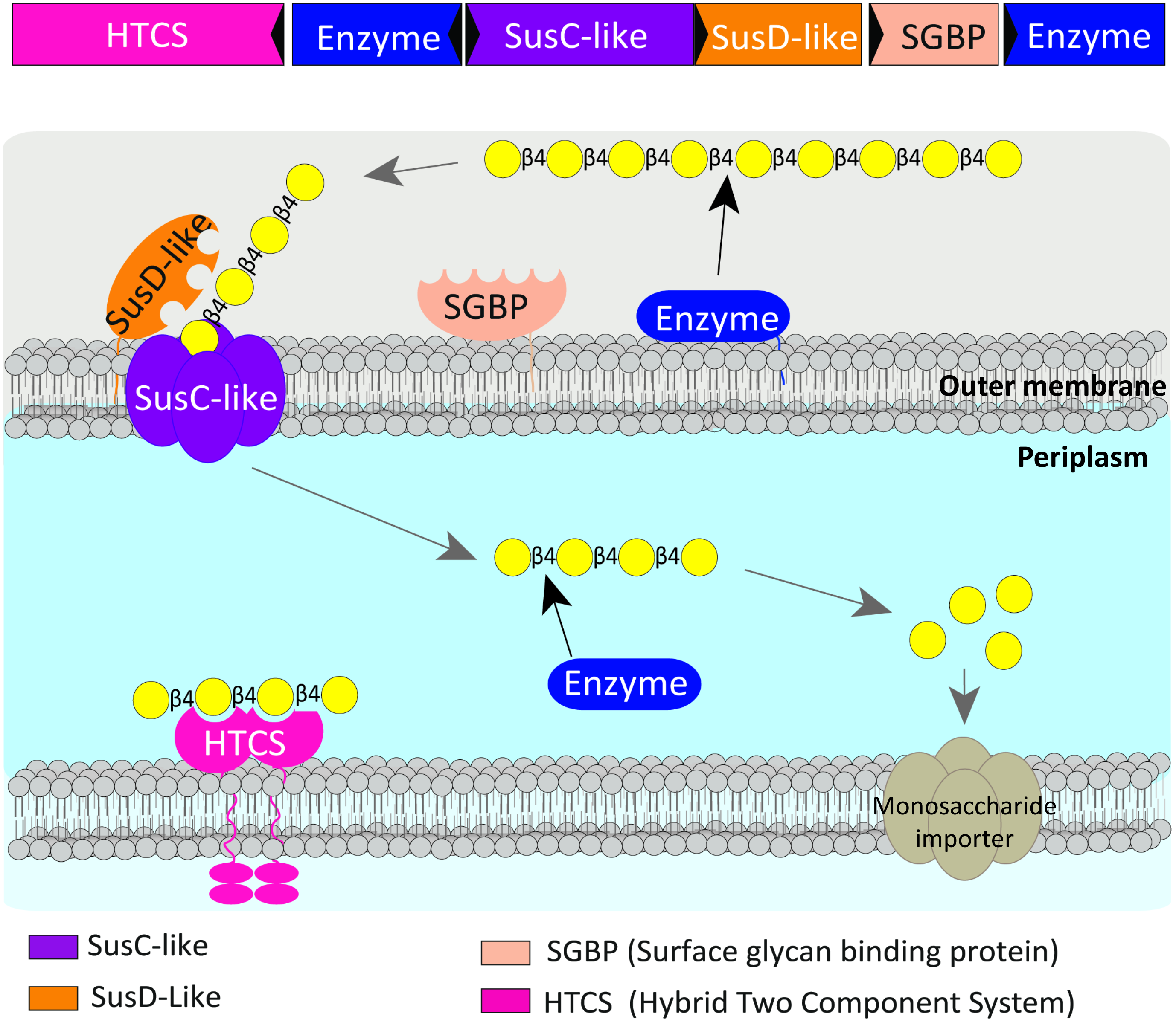
Mechanism of degradation: **Unknown**

Polysaccharide utilization loci (PUL)

<u>PUL</u> encodes genes to:
- Degrade
- Uptake
- Regulate

↓

Digestion of complex glycans



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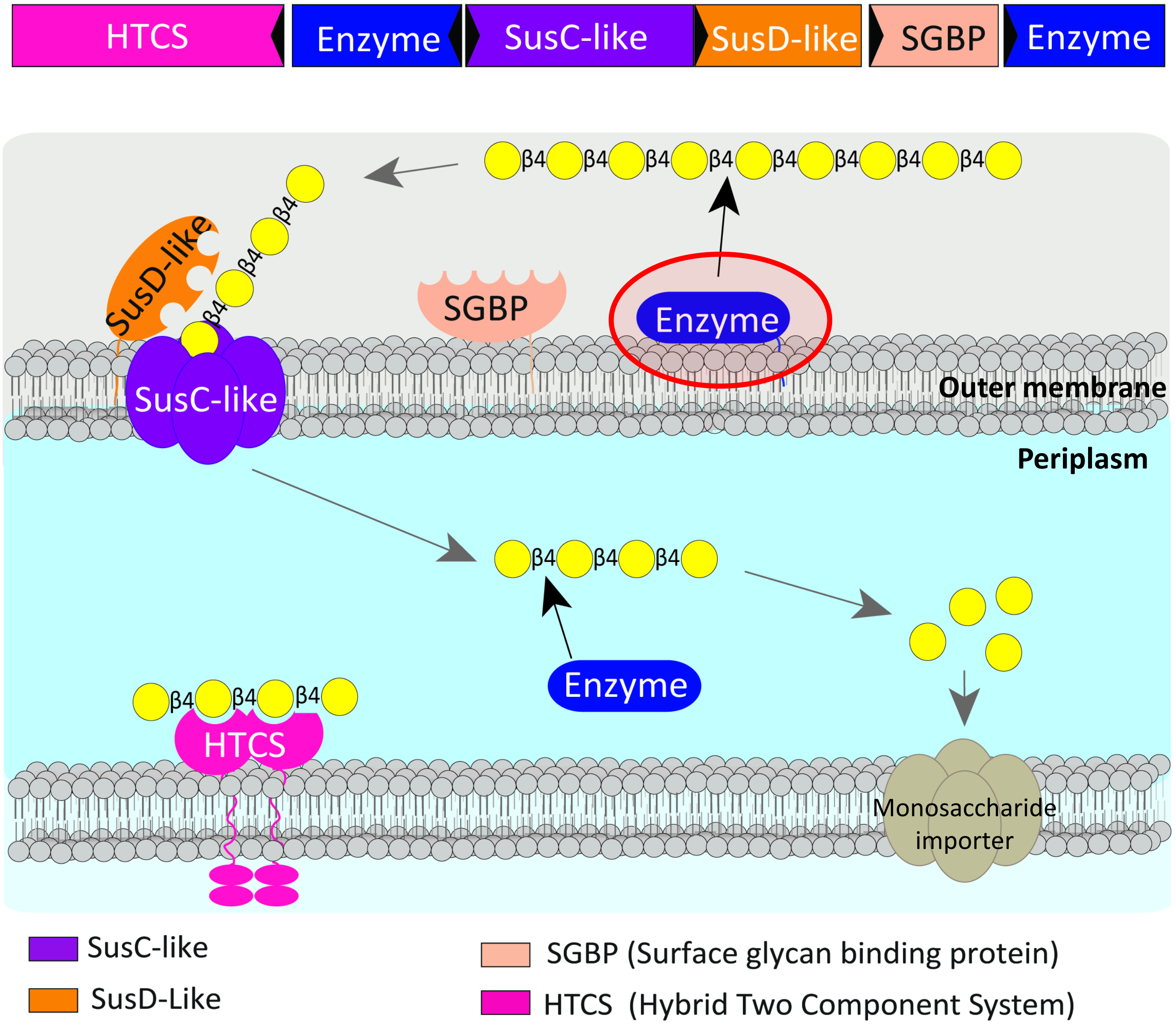


Degradation is initiated by a key enzyme

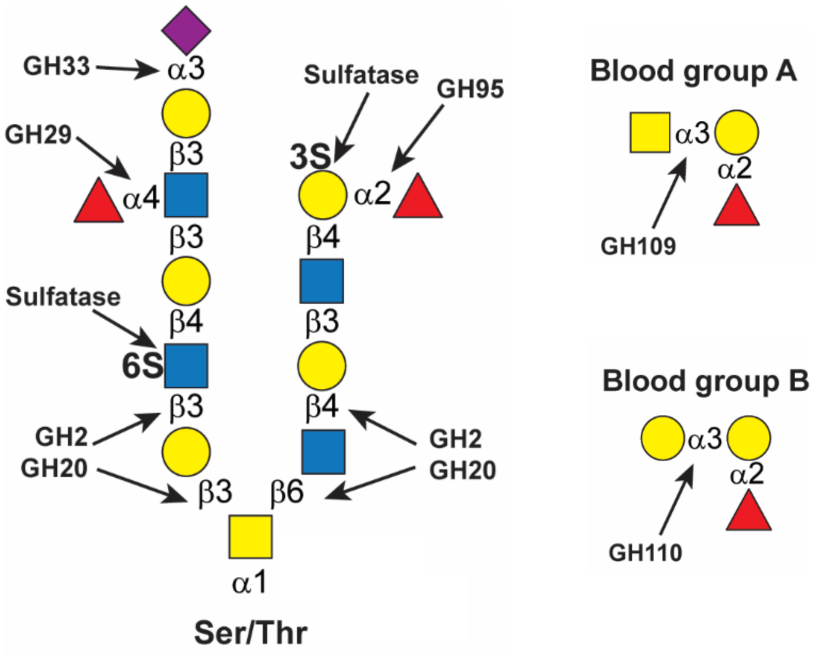
Published studies:

- Single enzyme
- Cell surface protein
- Endo-active enzyme

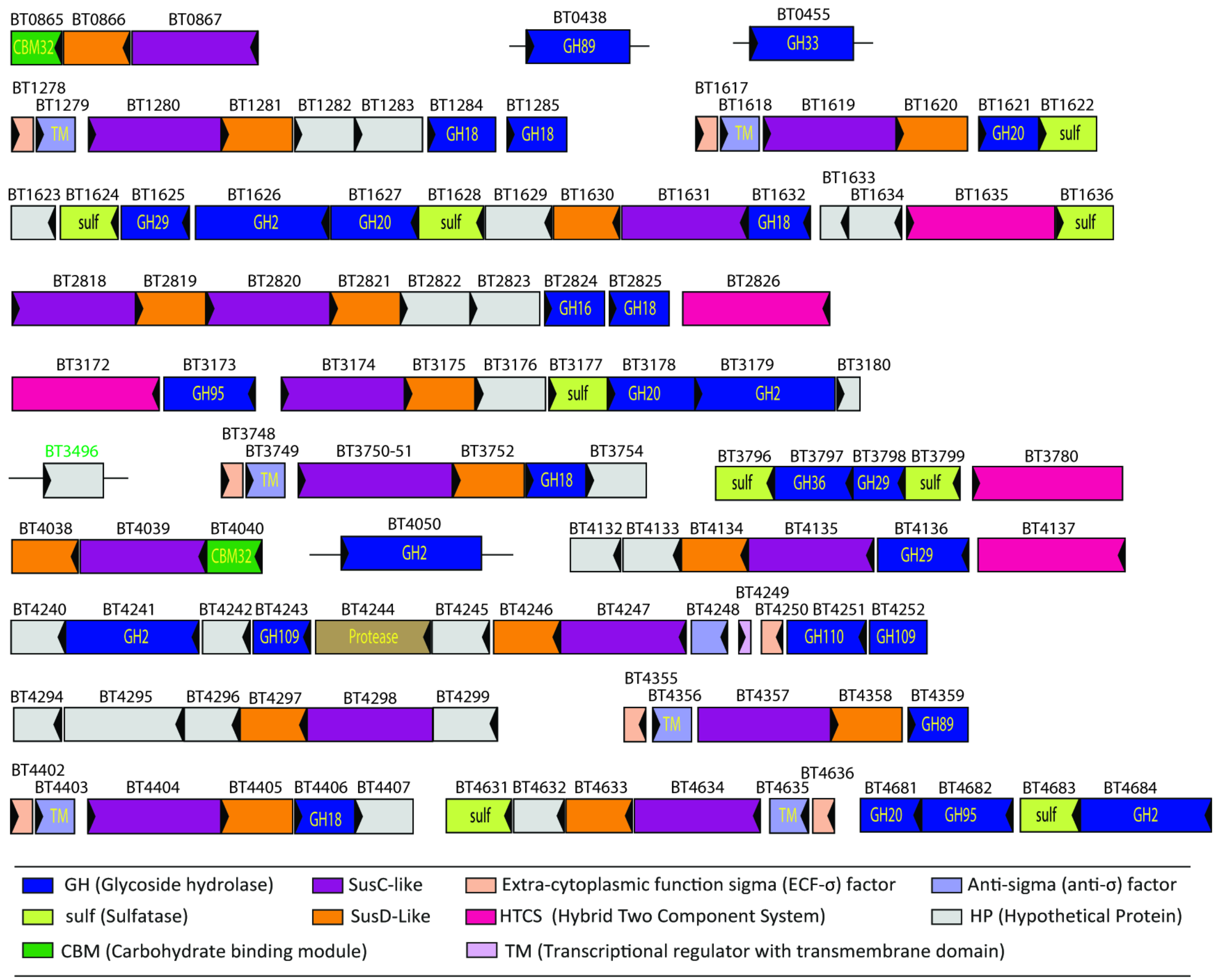
Larsbrink *et al*., Nature, 2015
Cuskin *et al*., Nature, 2015
Ndeh *et al*., Nature, 2017
Cartmell *et al*., PNAS, 2017
Tamura *et al*., Cell reports, 2017
Temple *et al*., JBC, 2017
Cartmell *et al*., Nat Microbiol, 2018
Luis *et al*., Nat Microbiol, 2018
Briliūtė *et al*., Nat Microbiol, 2019

B. theta upregulates multiple O-glycans PULs

PULs encode several predicted mucin *O*-glycans active enzymes (Glycoside hydrolases and Sulfatases)



Examples of putative O-glycans PULs



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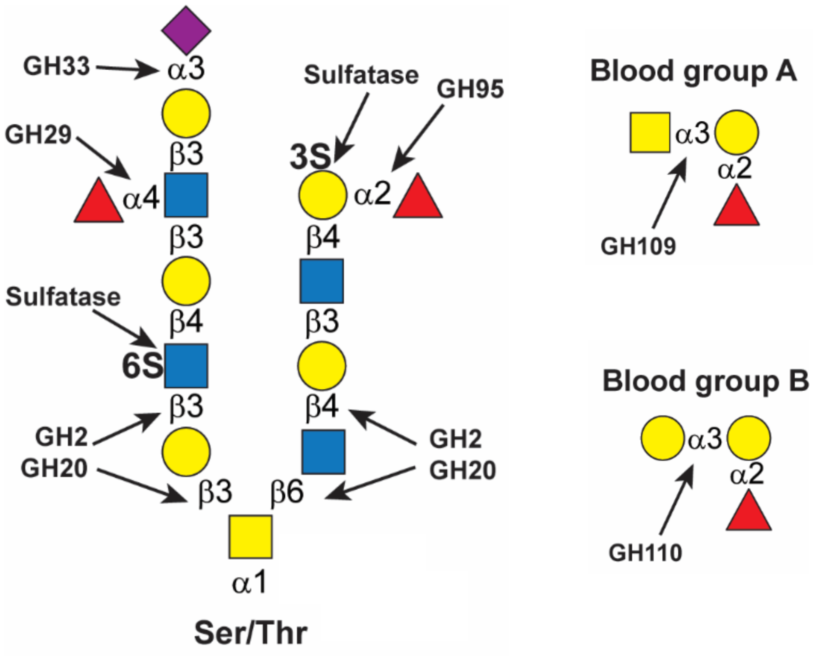

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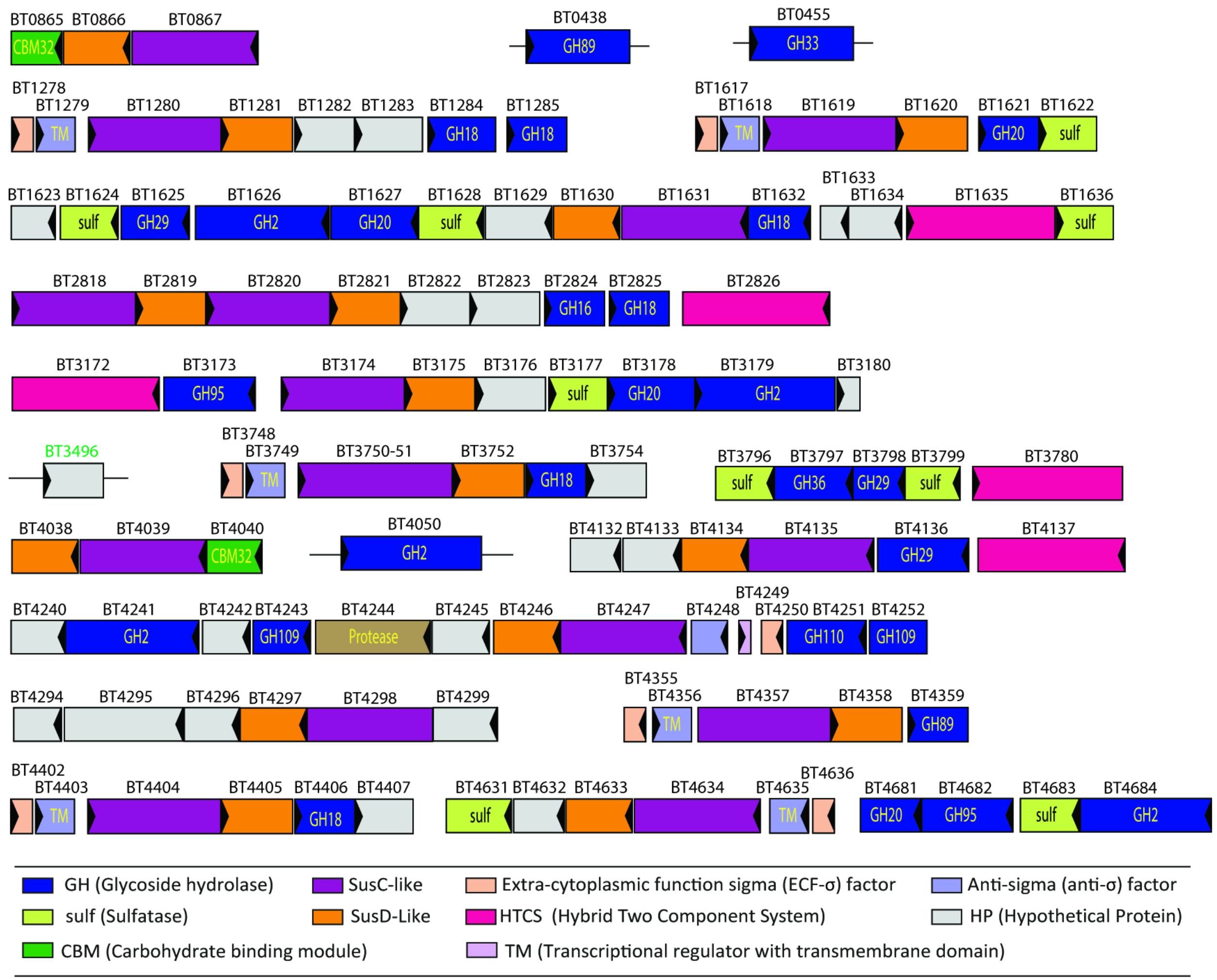
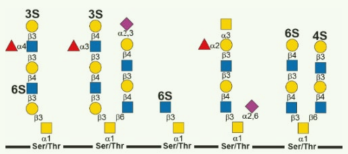


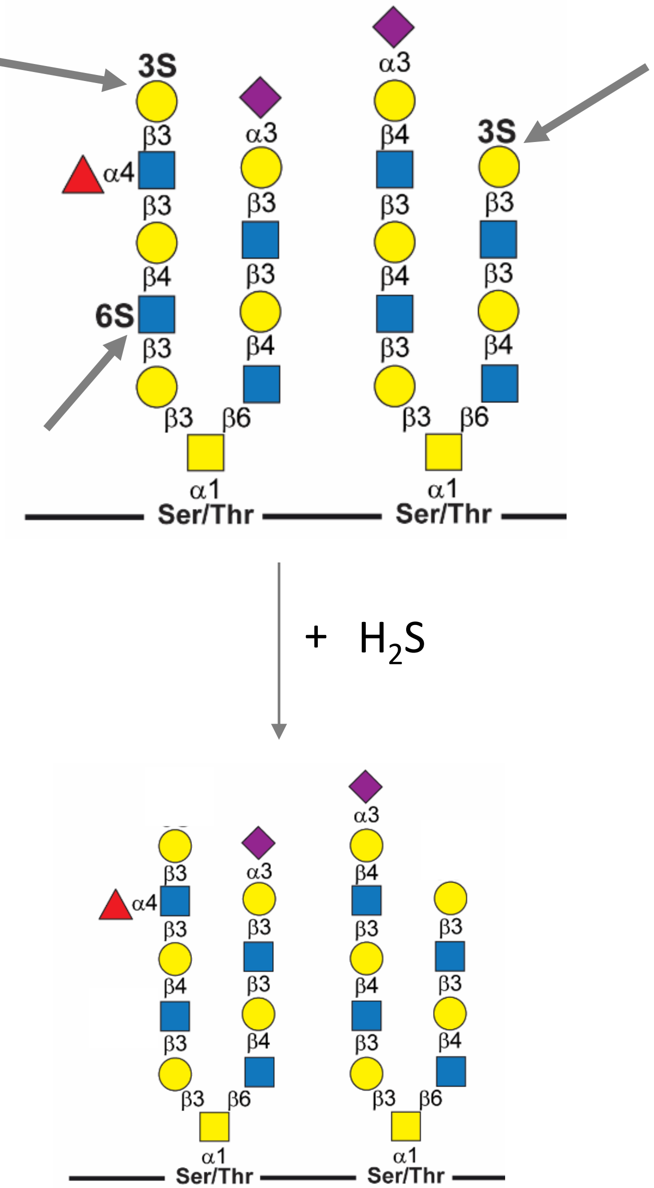


How *B. theta* utilizes *O*-glycans?

Which are the key enzymes?

Mucin utilization – Key enzymes

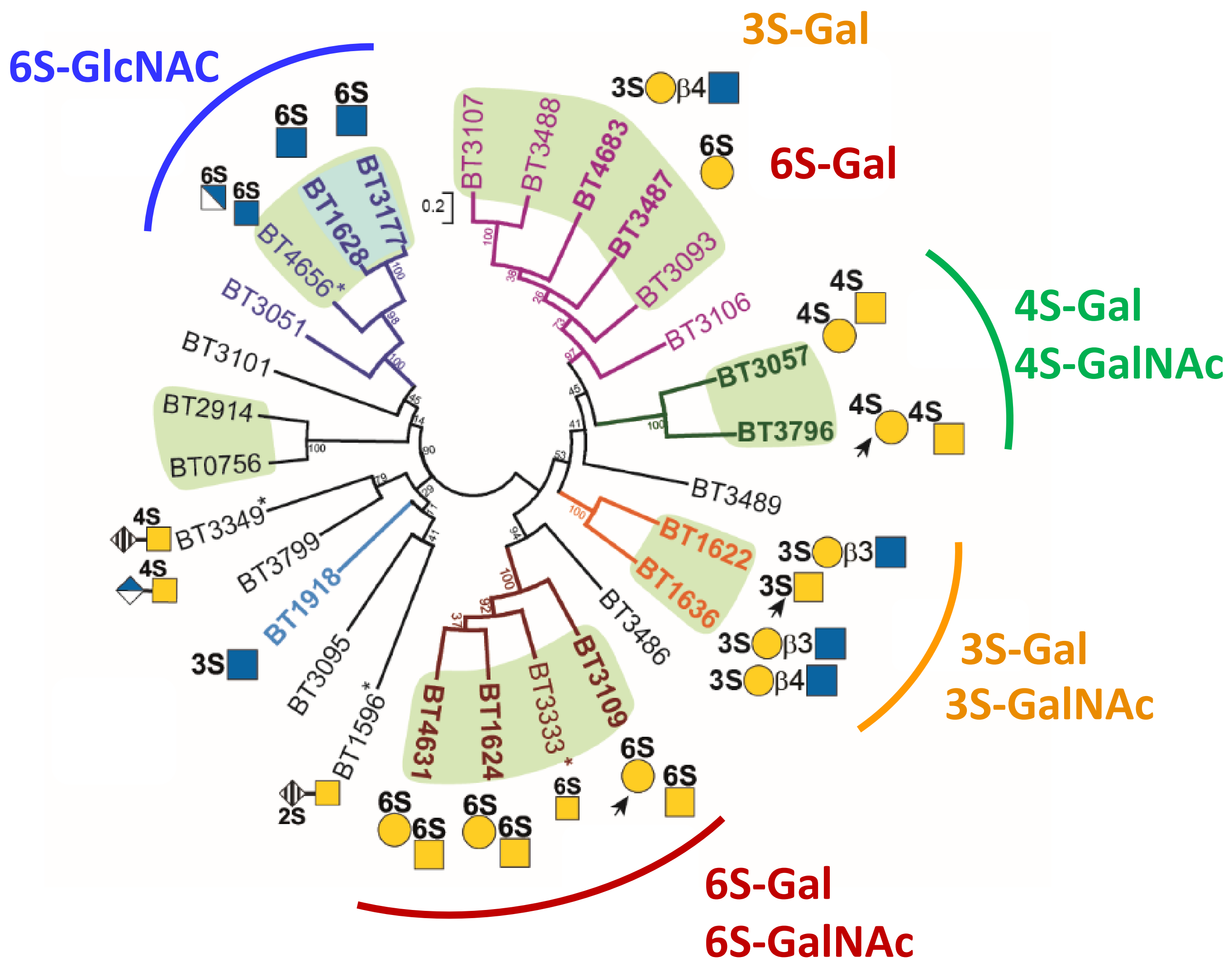
1. Sulfatases



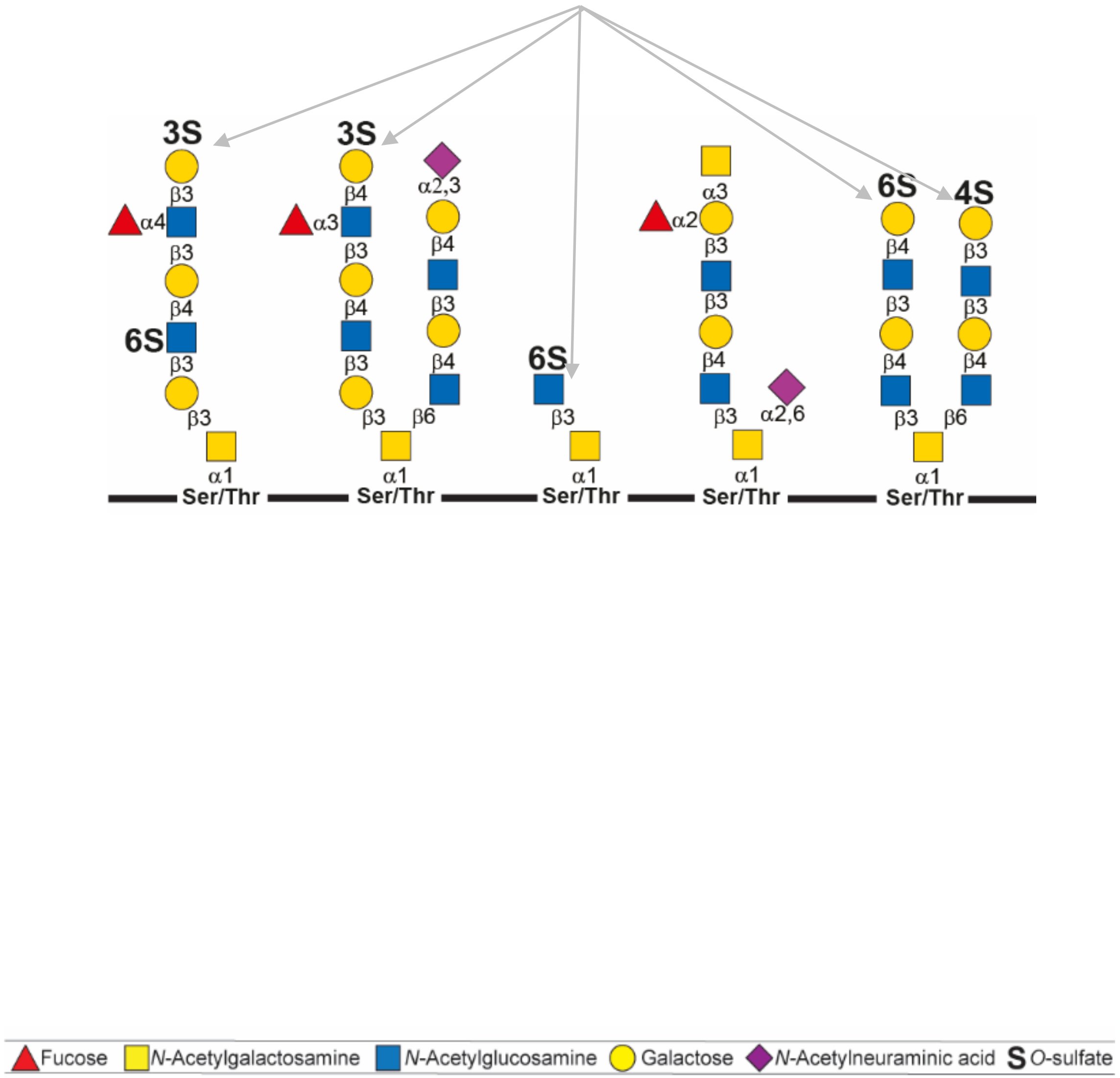
Luis AS *et al.* Nature. 2021 Oct;598(7880):332-337

B. theta sulfatases display different substrate specificity

11 sulfatases active on *O*-glycan linkages



Enzymes target all the S-linkages found in mucins



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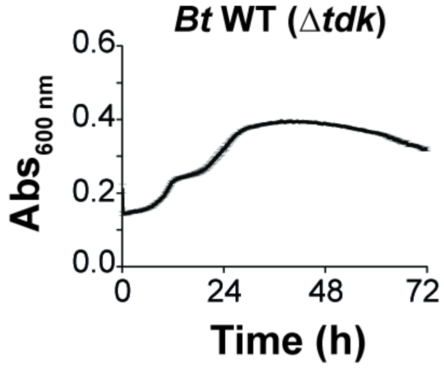
Sulfatases are required to utilization of sulfated *O*-glycans

Growth curves in colonic mucin O-glycans



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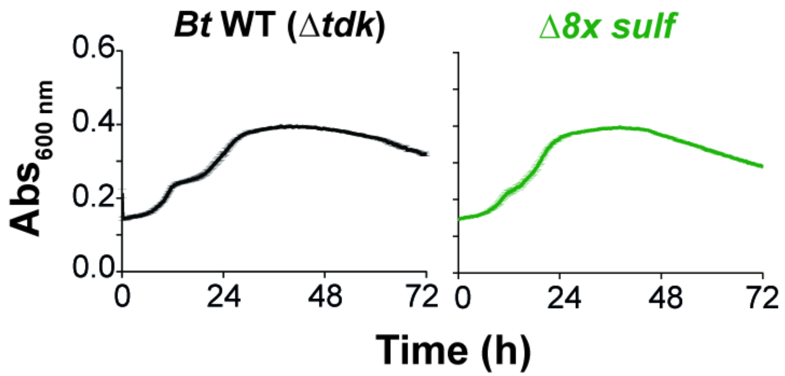

Δ*8x sulf* (*Δbt1622 + Δbt4683 + Δbt1624 + Δbt3109 + Δbt4631 + Δbt1628 + Δbt3177 + Δbt3051*)

3S-Gal/GalNAc ***6S-Gal/GalNAc*** ***6S-GlcNAc***

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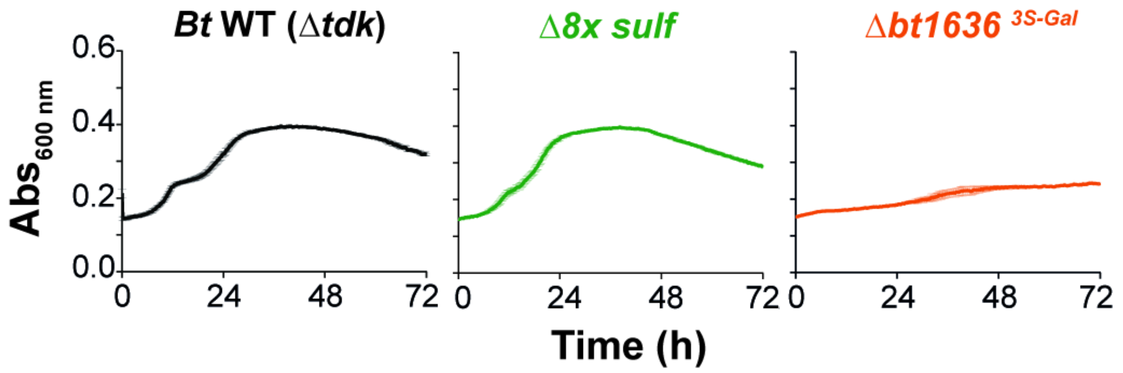

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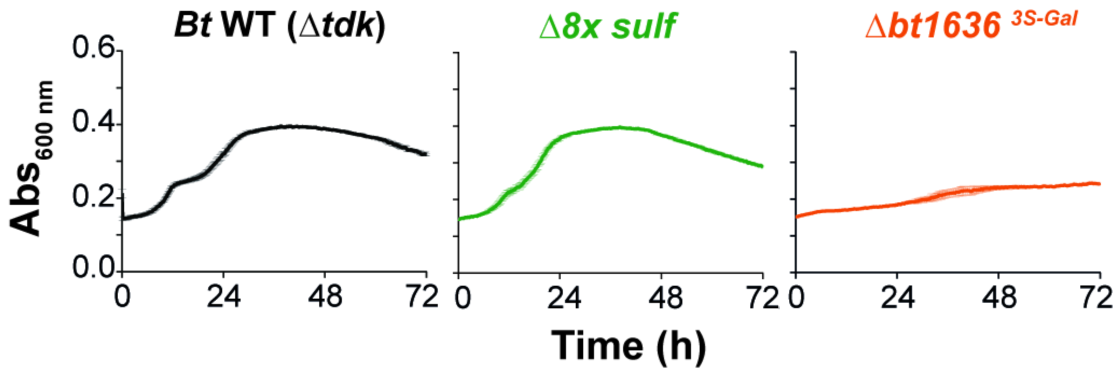

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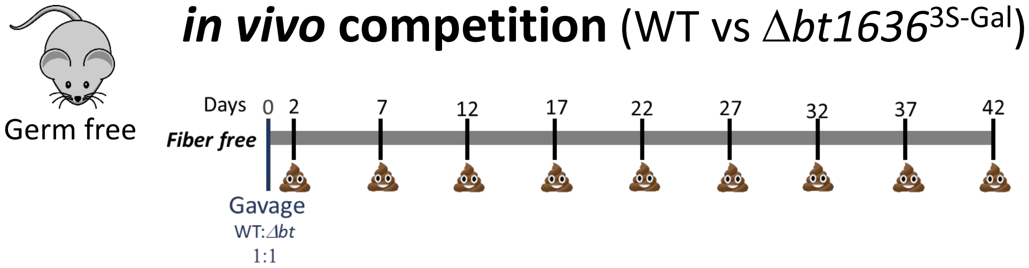
3S-Gal/GalNAc — 6S-Gal/GalNAc — 6S-GlcNAc

3S-Gal sulfatases are important fitness factors in vivo

***in vivo* competition** (WT vs Δ*bt1636*$^{3S\text{-}Gal}$)



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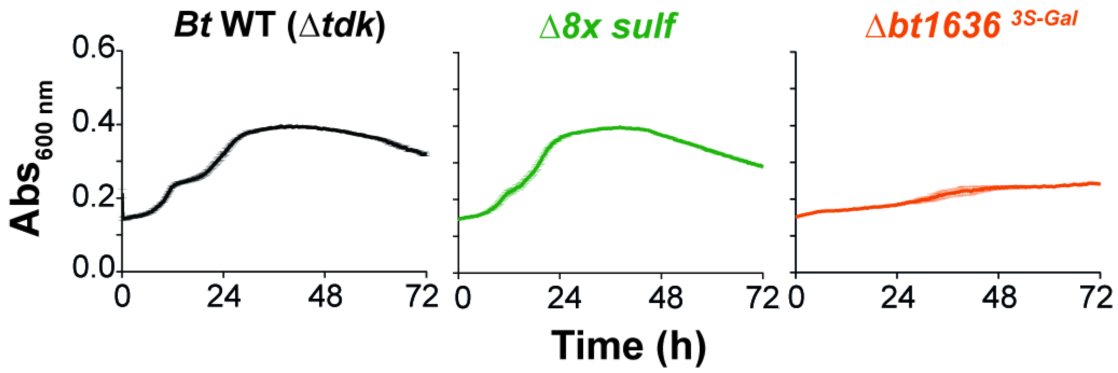

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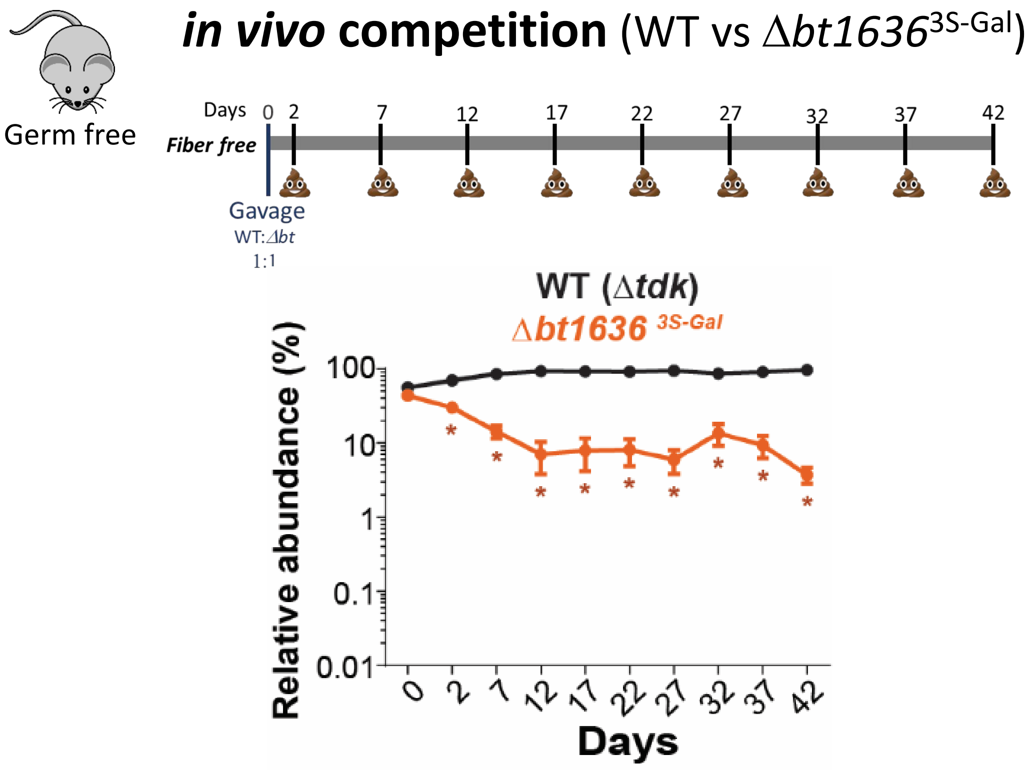

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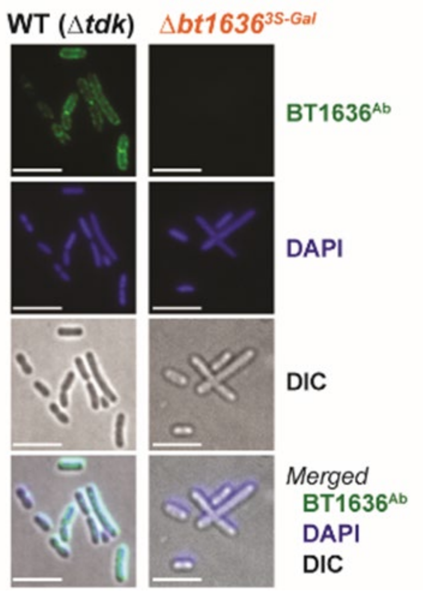
Mucin utilization – Key enzymes

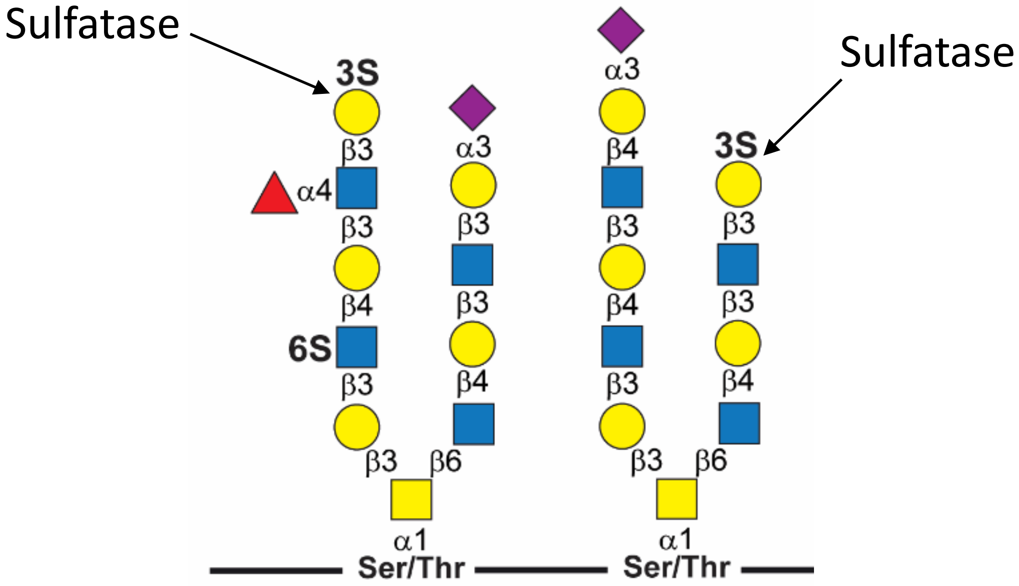
1. Sulfatases

- Exo-active

- BT1636 is a key enzyme
(*B. theta* encodes 28 sulfatases)

- Cell surface



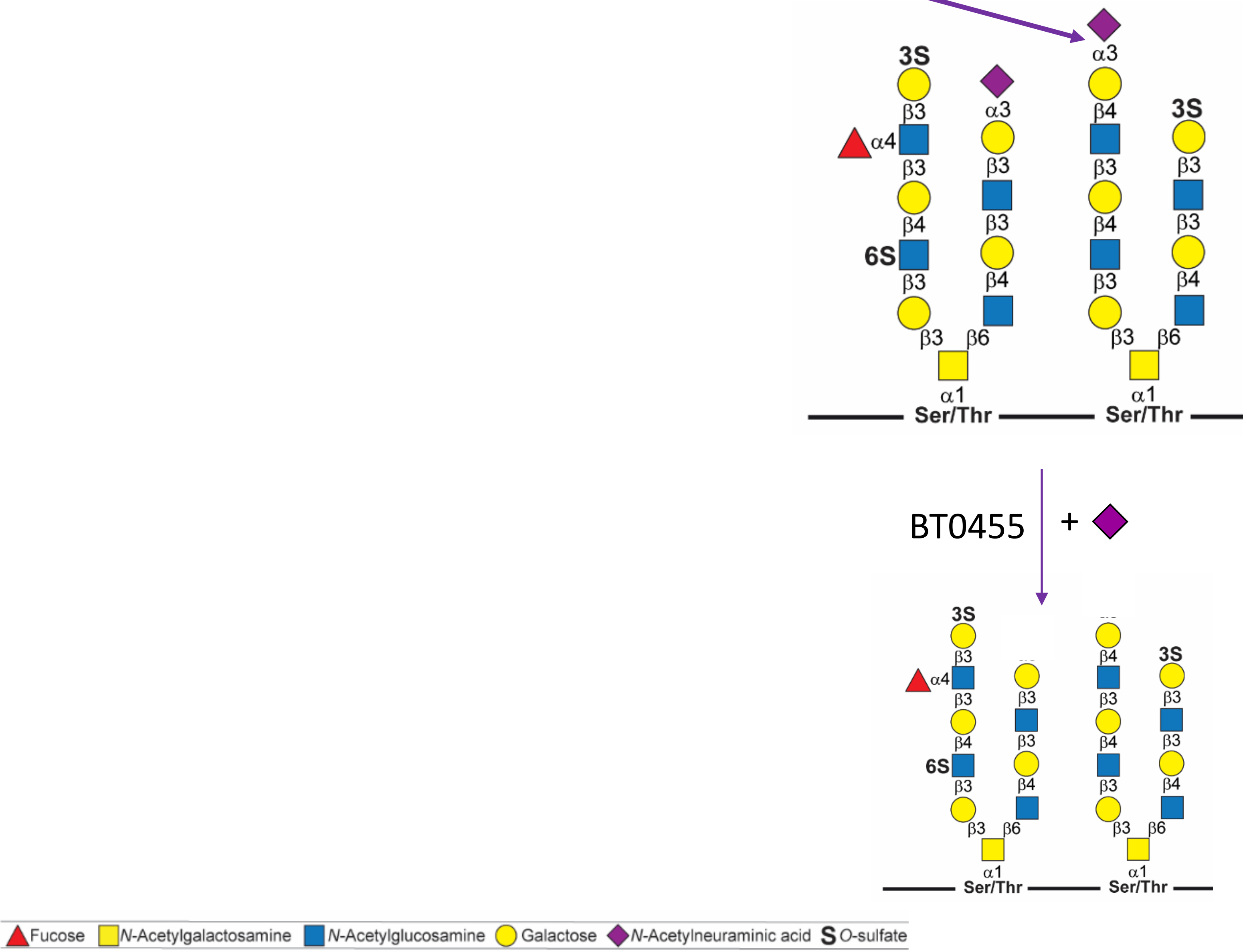


Luis AS *et al.* Nature. 2021 Oct;598(7880):332-337

Mucin utilization – Key enzymes

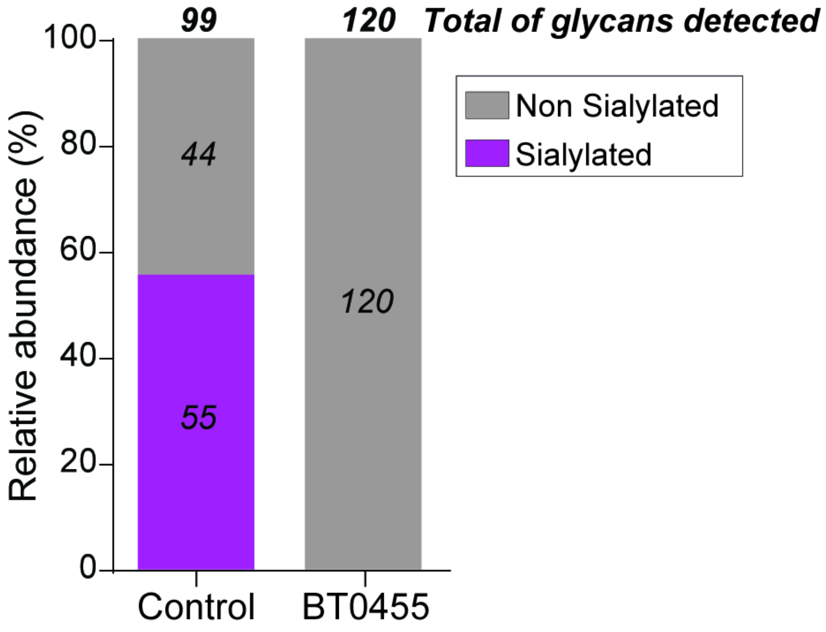
1. Sulfatases

2. Sialidase

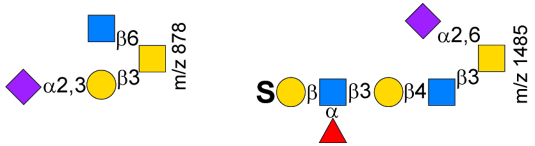


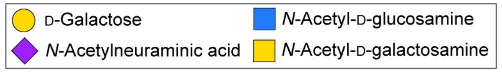
Sialidase is essential in gut colonization

Sialidase activity on colonic O-glycans *(LC-MS/MS)*



O-glycans cleaved by BT0455





Unpublished

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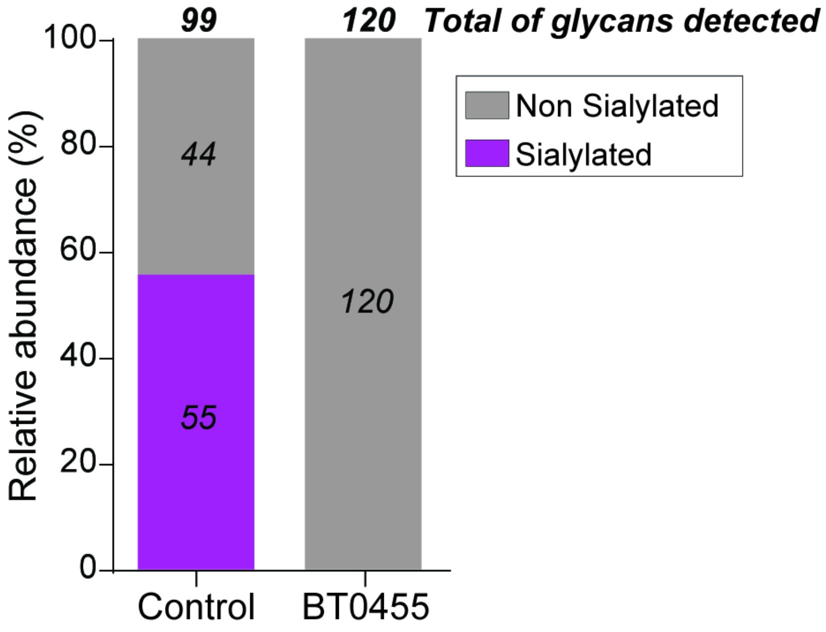

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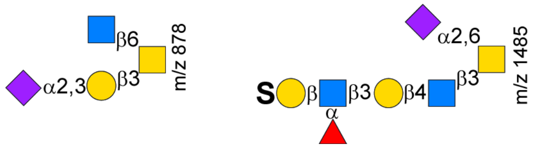
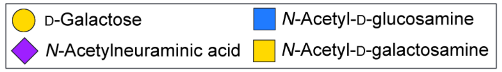
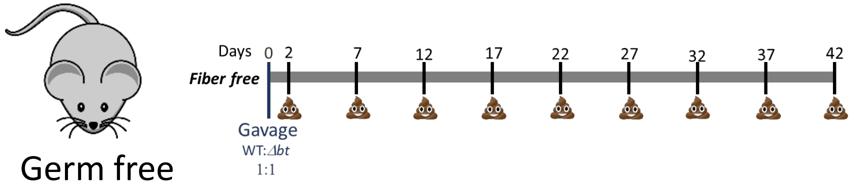




Unpublished

***in vivo* competition** (WT vs Δ*bt0455*)



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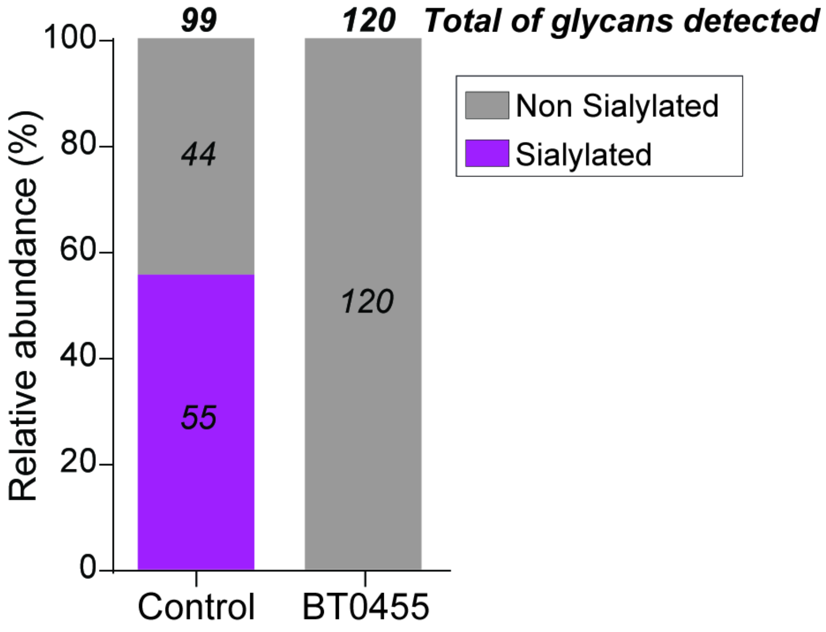

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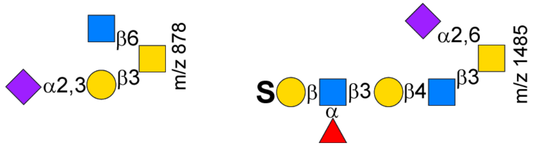

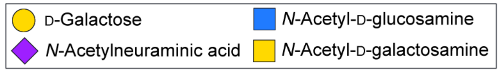

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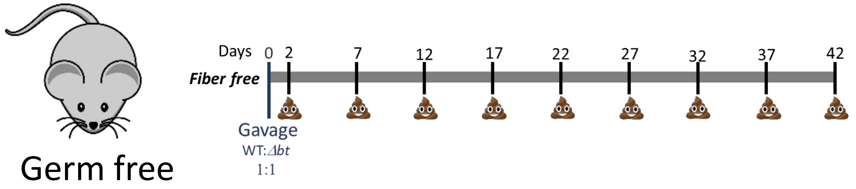
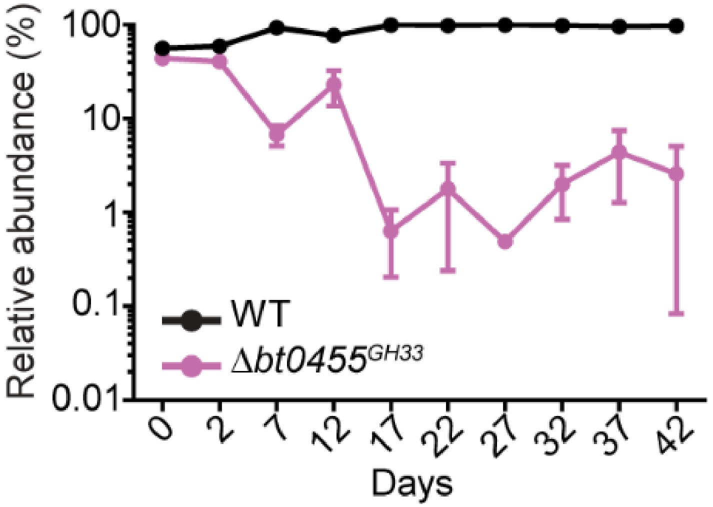




Mucin utilization – Key enzymes

1. Sulfatases

- Exo-active

- BT1636 is a key enzyme
(*B. theta* encodes 28 sulfatases)

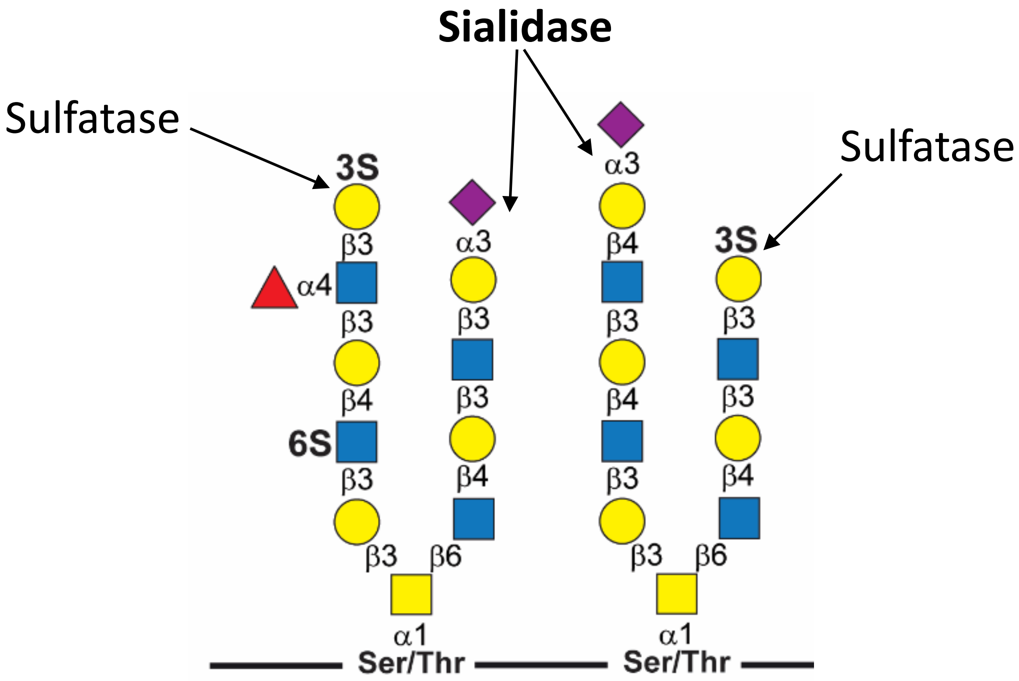
- Cell surface

2. Sialidase

- Exo-active

- Essential fitness factor *in vivo*

- Outer membrane
(Briliūtė *et al.*, Nat Microbiol, 2019)

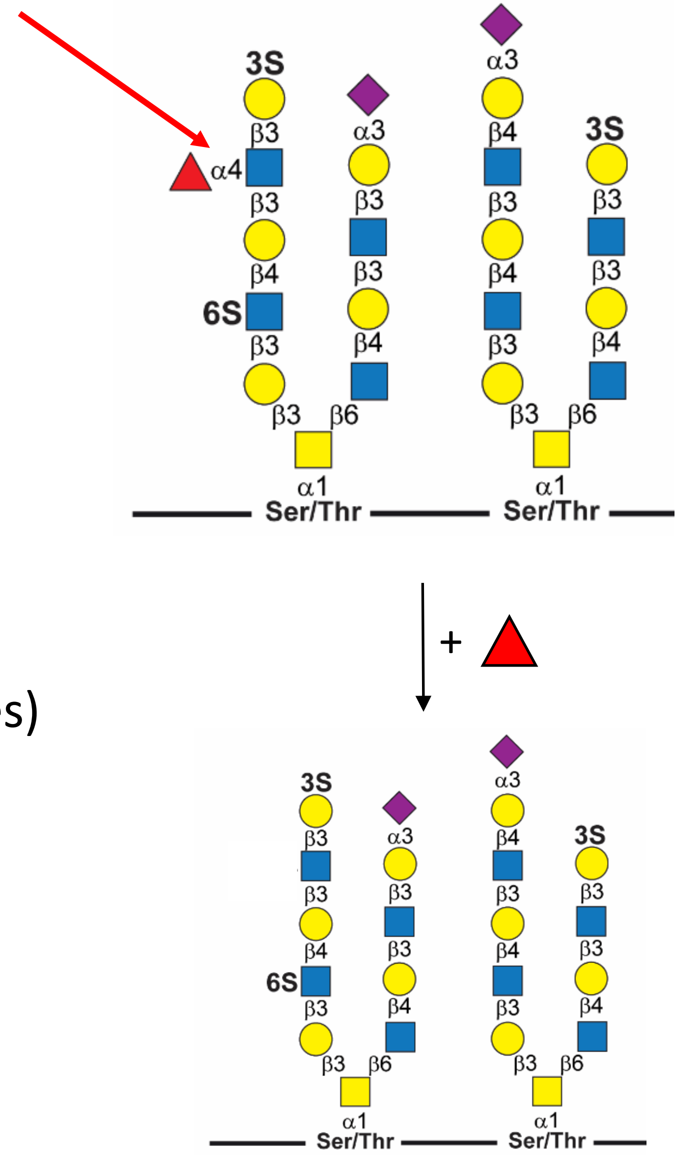


Mucin utilization – Key enzymes

1. Sulfatases

2. Sialidase

3. Fucosidases

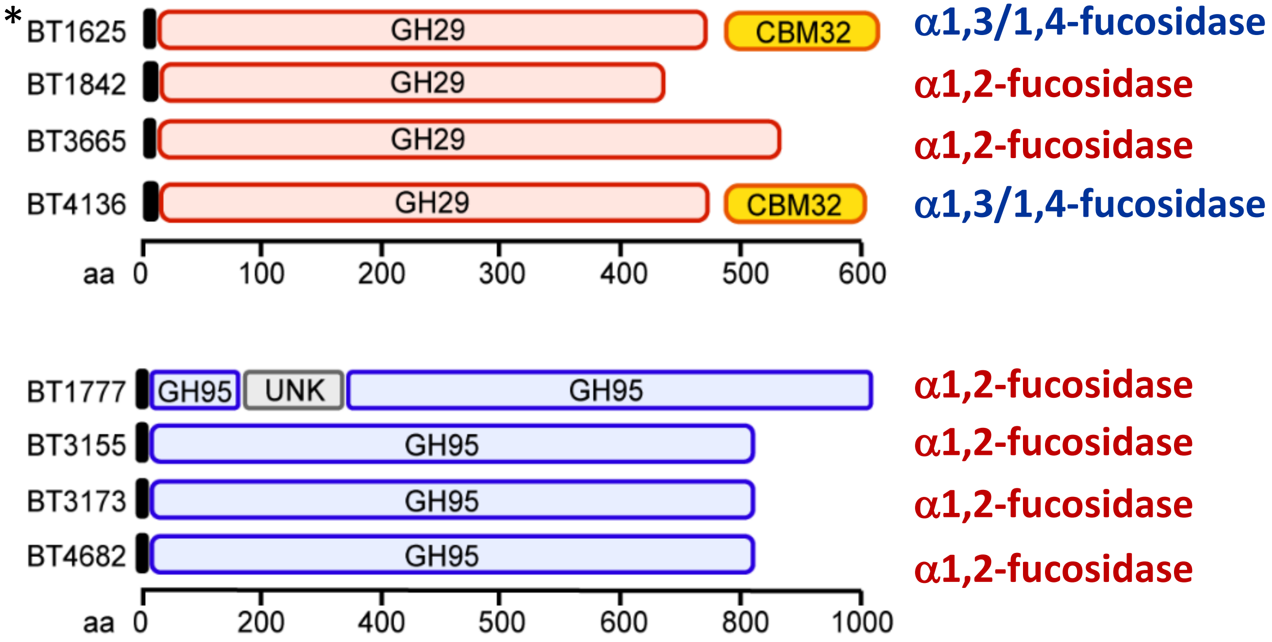


B. theta encodes:

- **GH95** (α1,2-fucosidases)
- **GH29** (α1,2/1,3/1,4-fucosidases)

Unpublished

B. theta fucosidases substrate specificity



GH29

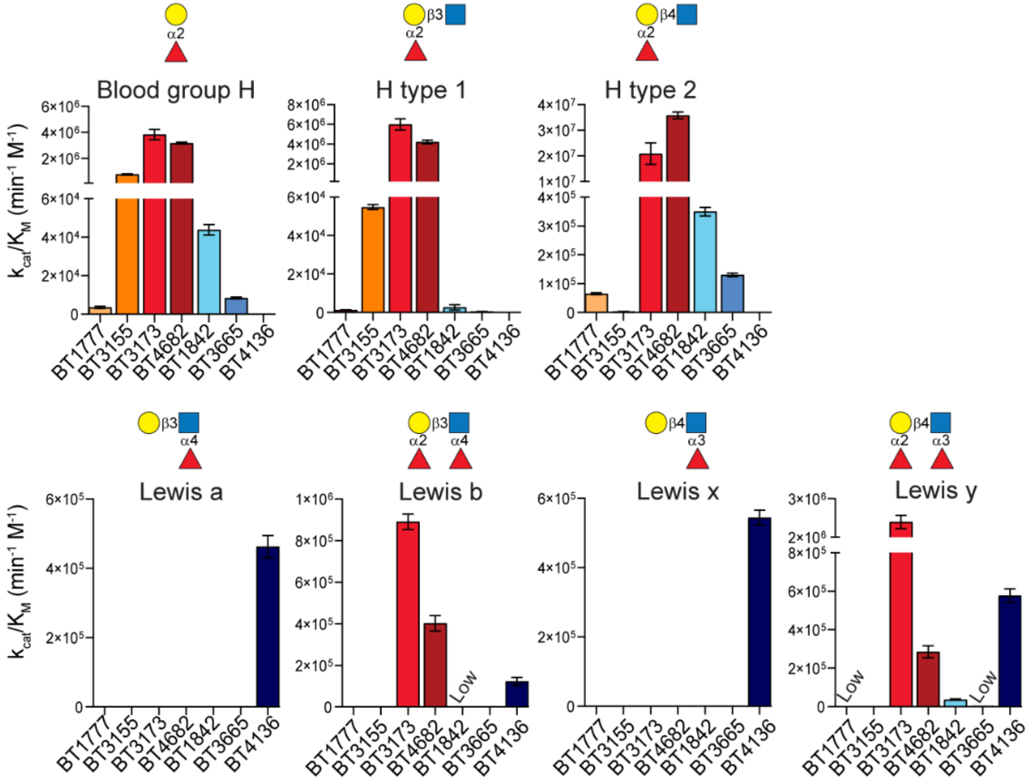
	BT1625	BT1842	BT3665	BT4136
BT1625	100			
BT1842	26.79	100		
BT3665	16.48	23.46	100	
BT4136	82.28	28.46	17.20	100

CBM32

	BT1625	BT4136
BT1625	100	
BT4136	74.78	100

GH95

	BT1777	BT3155	BT3173	BT4682
BT1777	100			
BT3155	32.71	100		
BT3173	42.78	34.77	100	
BT4682	41.92	33.78	74.02	100



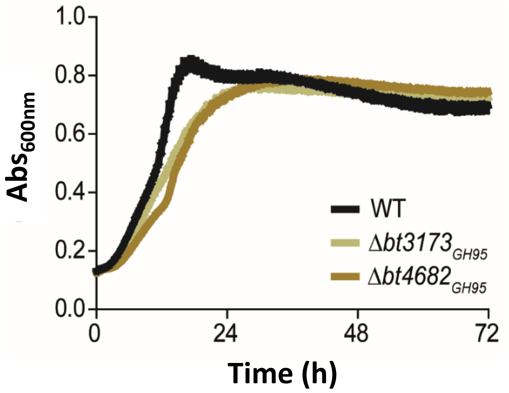
Fucose | *N*-Acetylgalactosamine | *N*-Acetylglucosamine | Galactose | *N*-Acetylneuraminic acid | S *O*-sulfate

* Enzyme kinetics published on Briliūtė *et al.*, *Nat Microbiol*, 2019

Unpublished

Fucosidases are required to mucin utilization

Growth curves on gastric mucin oligosaccharides *(1 % w/v)*



BT3173 and BT4682 - α1,2-fucosidases (GH95)

Unpublished

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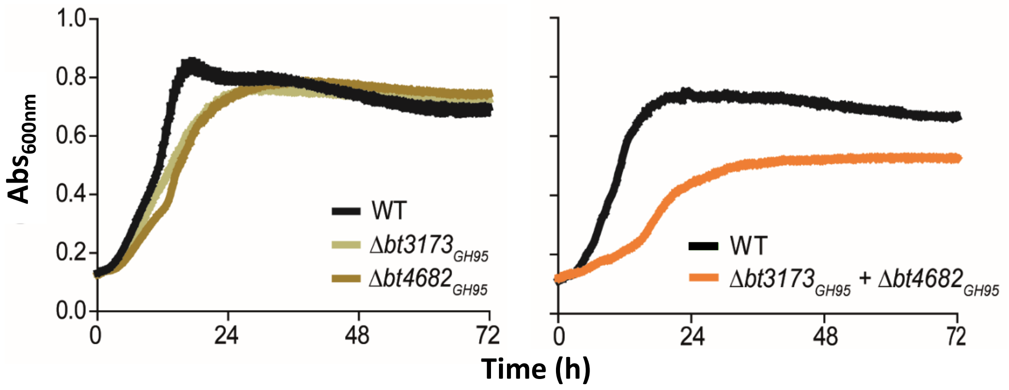

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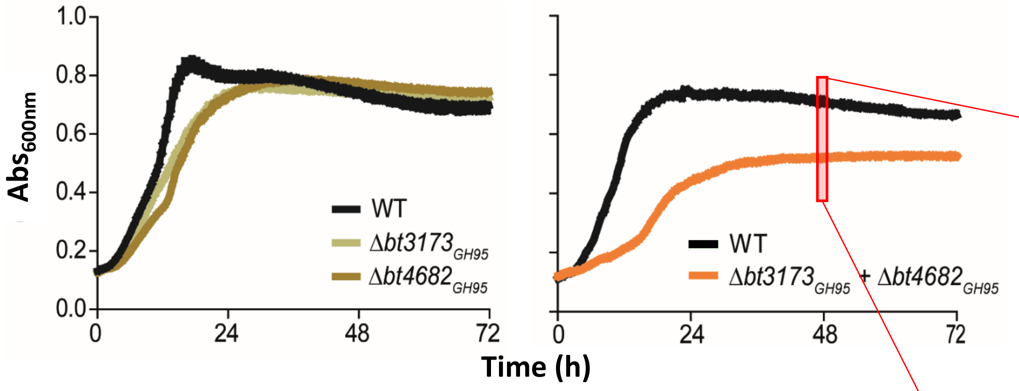
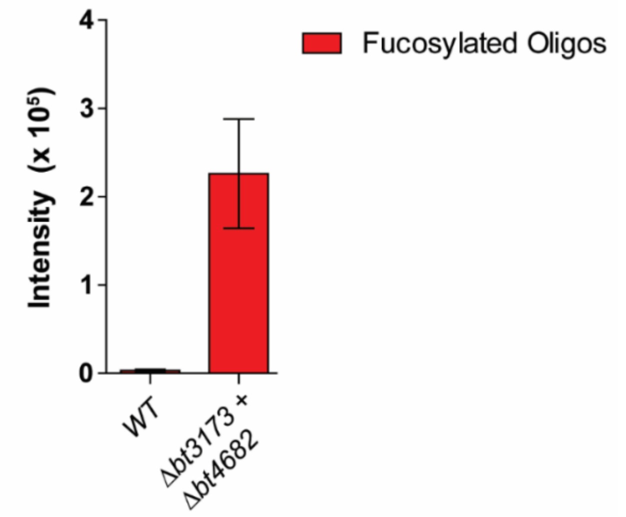
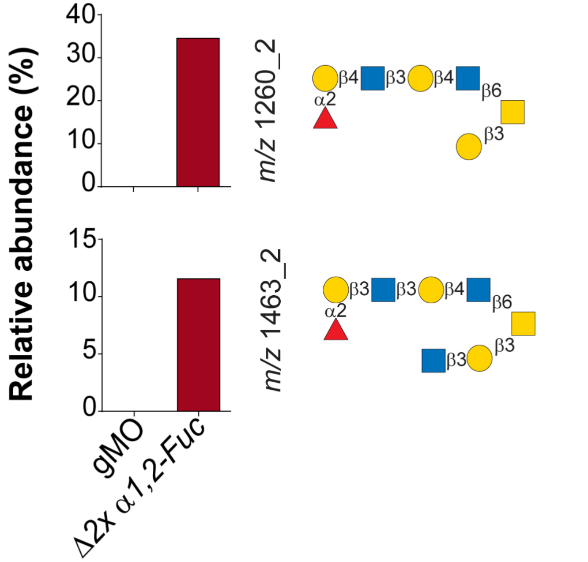


Δbt3173 + Δbt4682 is unable to utilize fucosylated oligosaccharides

Intensity of oligosaccharides detected by LC-MS/MS in culture supernatant





gMO (gastric mucin oligosaccharides)

BT3173 and BT4682 - α1,2-fucosidases (GH95)

Unpublished

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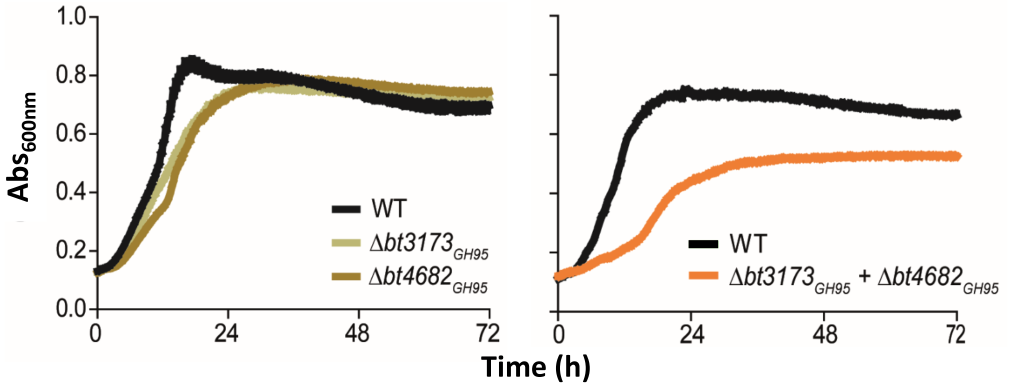
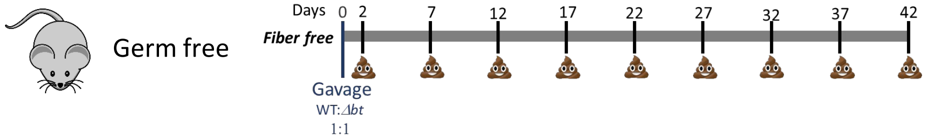
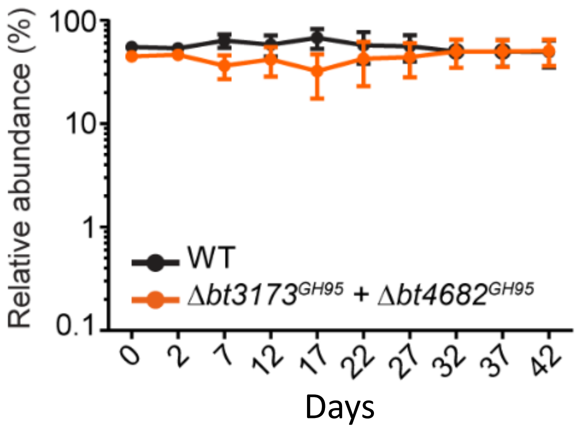


***in vivo* competition** (WT vs Δ *Fuc*)





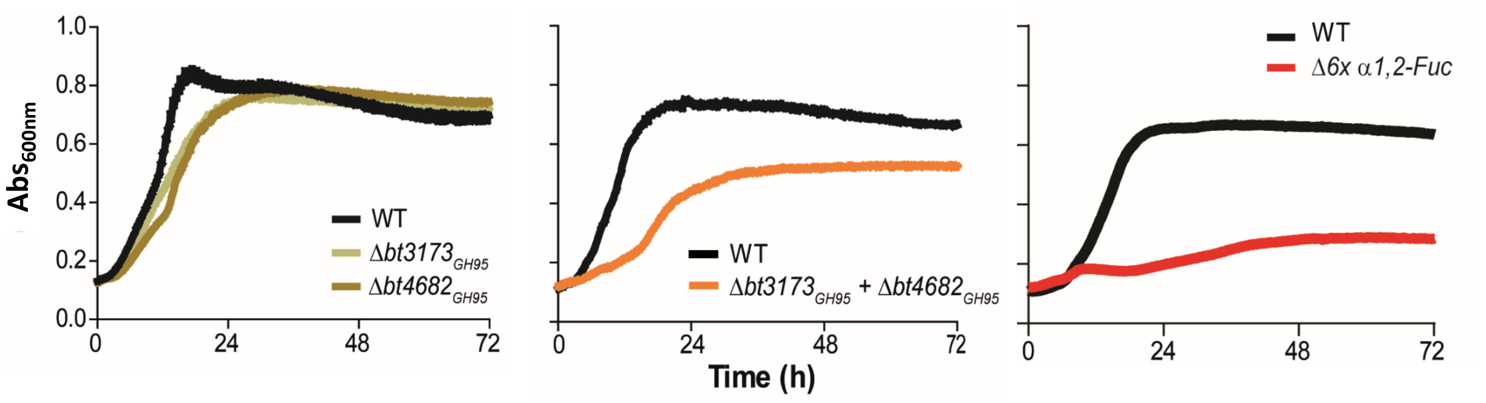
Δbt3173 + Δbt4682 are not key in vivo fitness factors

BT3173 and BT4682 - α1,2-fucosidases (GH95)

Unpublished

Fucosidases are required to mucin utilization

Growth curves on gastric mucin oligosaccharides *(1 % w/v)*



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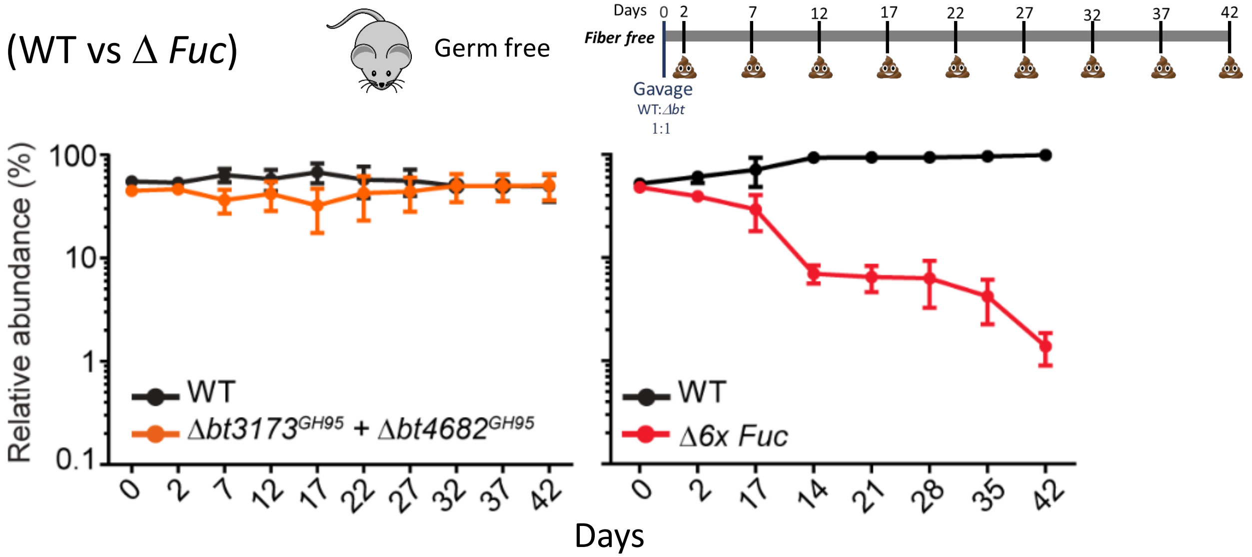


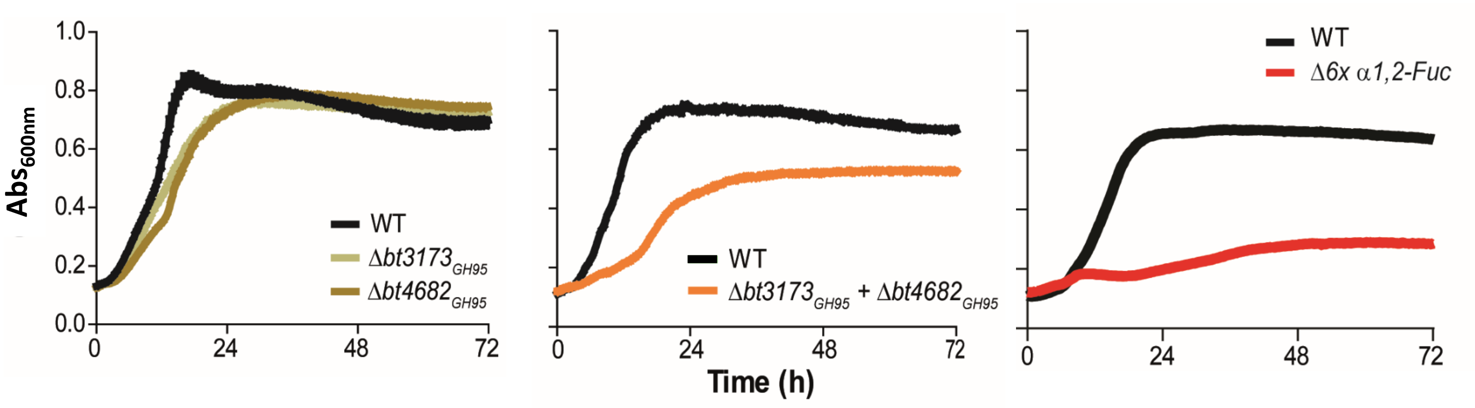
Δ6x α1,2-Fuc (Δ*bt3173*$_{GH95}$ + Δ*bt4682*$_{GH95}$ + Δ*bt1777*$_{GH95}$ + Δ*bt3155*$_{GH95}$ + Δ*bt3665*$_{GH29}$ + Δ*bt1842*$_{GH29}$)

BT3173 and BT4682 - α1,2-fucosidases (GH95)

Unpublished

Fucosidases are required to mucin utilization

*Growth curves on **gastric** mucin oligosaccharides (1 % w/v)* **(α1,2-fucose)**

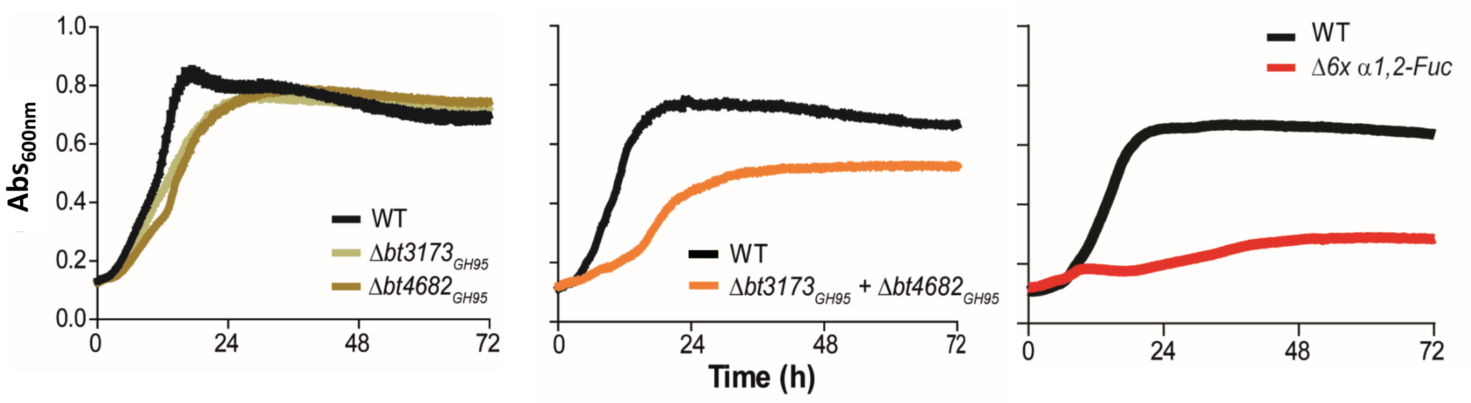


*Growth curves on **colonic** mucin oligosaccharides (1 % w/v)* **(α1,3/1,4-fucose)**

Unpublished

Fucosidases are required to mucin utilization

*Growth curves on **gastric** mucin oligosaccharides (1 % w/v)* **(α1,2-fucose)**



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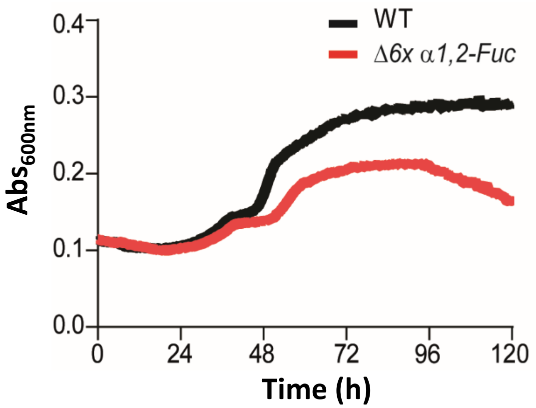


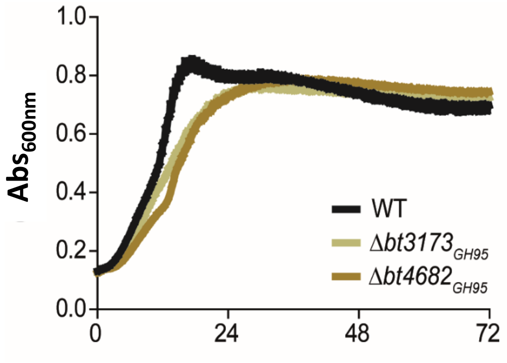
Unpublished

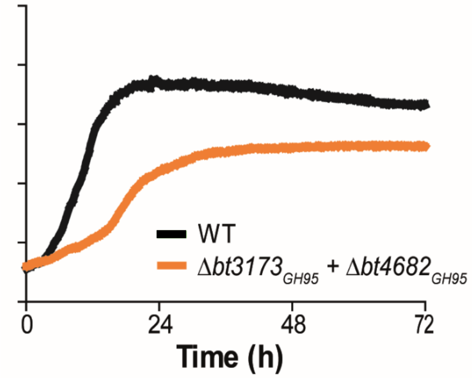
Fucosidases are required to mucin utilization

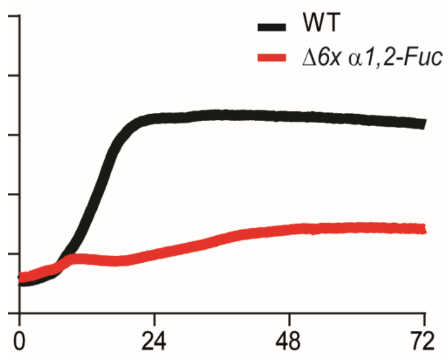
*Growth curves on **gastric** mucin oligosaccharides (1 % w/v)*

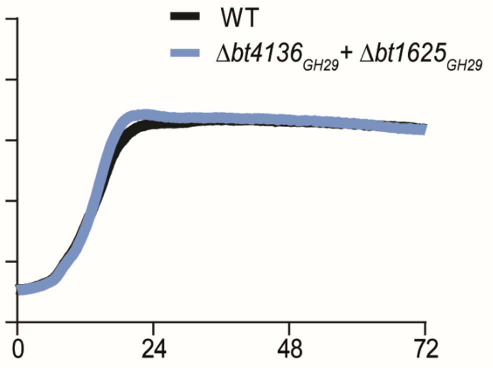
α1,3/1,4-fucosidases

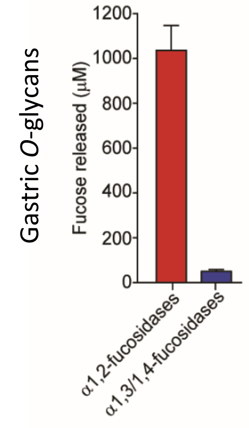
Fucosidase activity on different *O*-glycan substrates



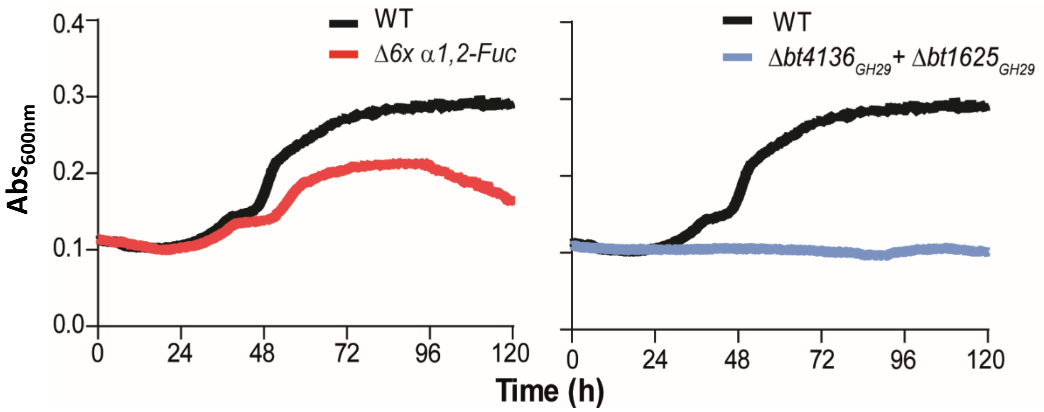


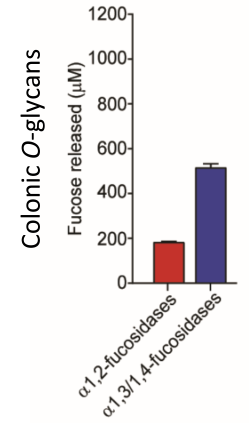






*Growth curves on **colonic** mucin oligosaccharides (1 % w/v)* **(α1,3/1,4-fucose)**





Quantification of fucose released after enzymatic treatment by HPAEC-PAD detection

Unpublished

Mucin utilization – Key enzymes

1. Sulfatases

- Exo-active

- BT1636 is a key enzyme
(*B. theta* encodes 28 sulfatases)

- Cell surface

2. Sialidase

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(Briliūtė *et al*., Nat Microbiol, 2019)

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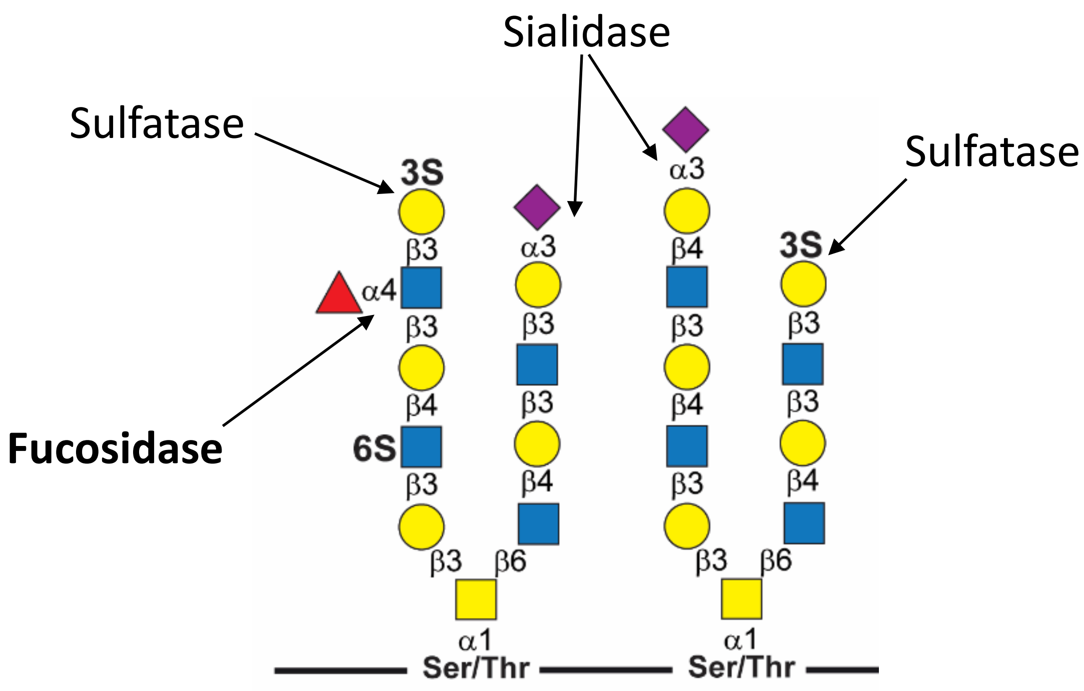


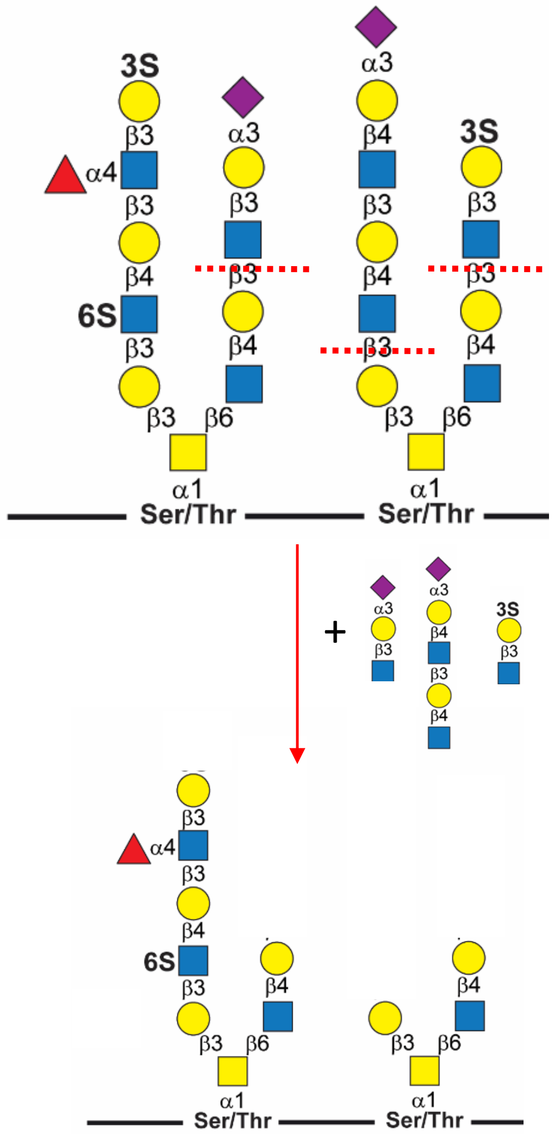
Mucin utilization – Key enzymes

1. Sulfatases

2. Sialidase

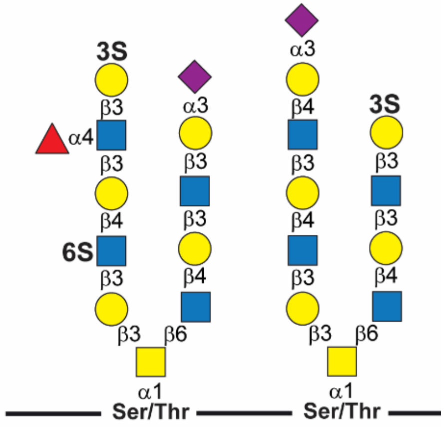
3. Fucosidases

4. Endo-active



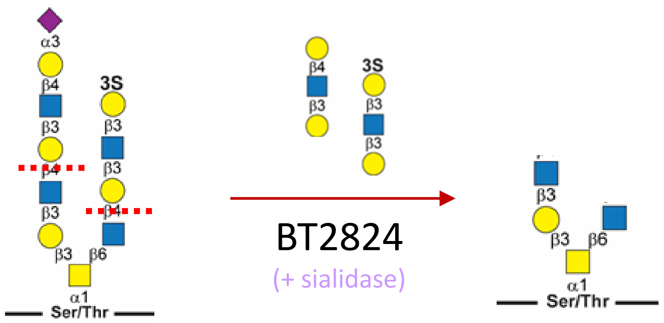
Unpublished

B. theta encodes multiple endo-active GHs



GH16

Endo-β-galactosidase



ARTICLE

https://doi.org/10.1038/s41467-020-17847-5 OPEN

Check for updates

Prominent members of the human gut microbiota express endo-acting O-glycanases to initiate mucin breakdown

Crouch et al. Nat Commun. 2020 Aug 11;11(1):4017

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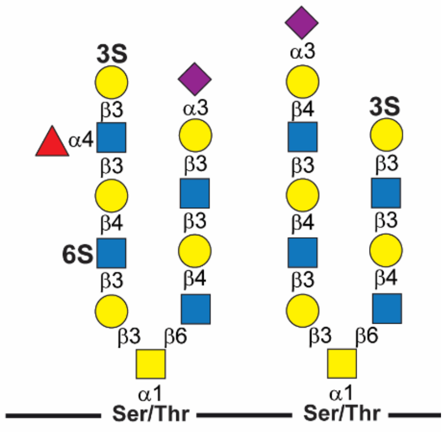

GH16
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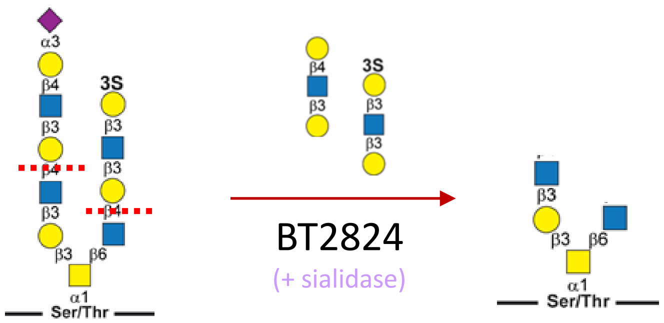

ARTICLE

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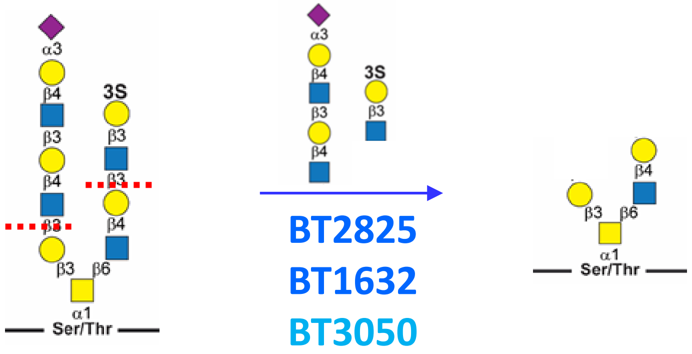
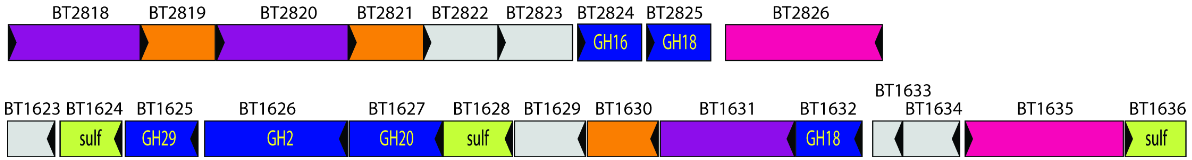
?

GH18
Endo-β-N-acetylglucosaminidase

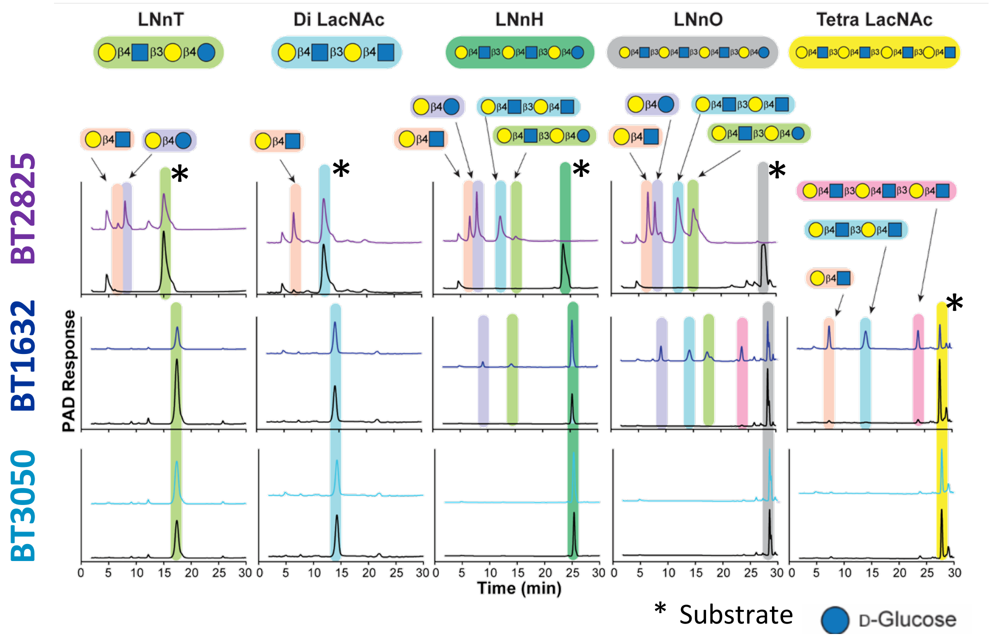




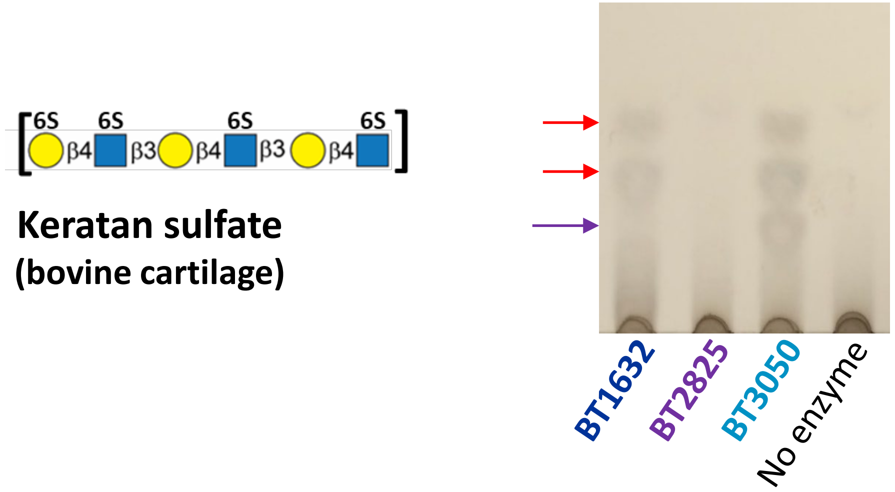
Unpublished

GH18s display distinct substrate specificities

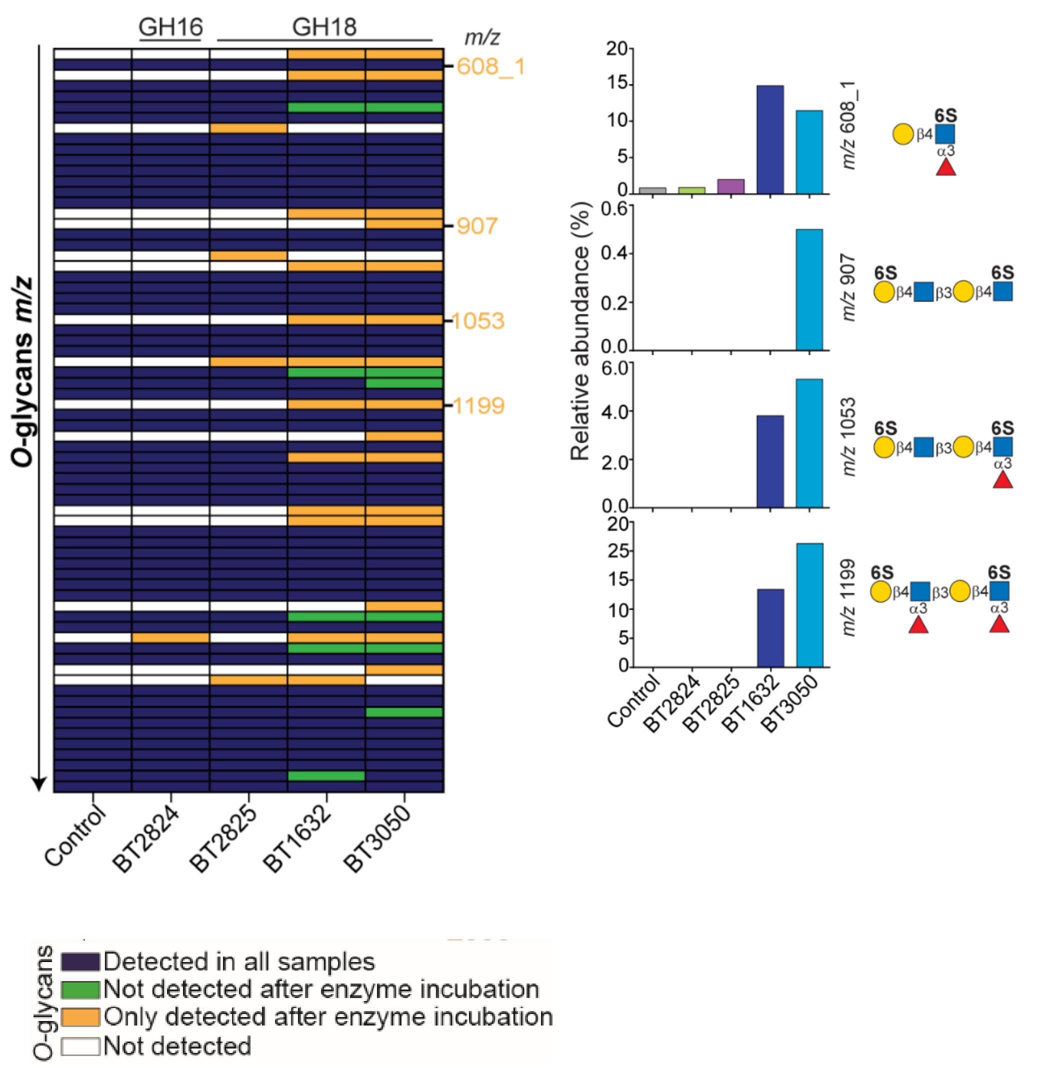
Activity against Poly-LacNAc oligosaccharides (HPAEC-PAD)



Activity against complex substrates



Activity porcine colonic O-glycans (LC-MS/MS)

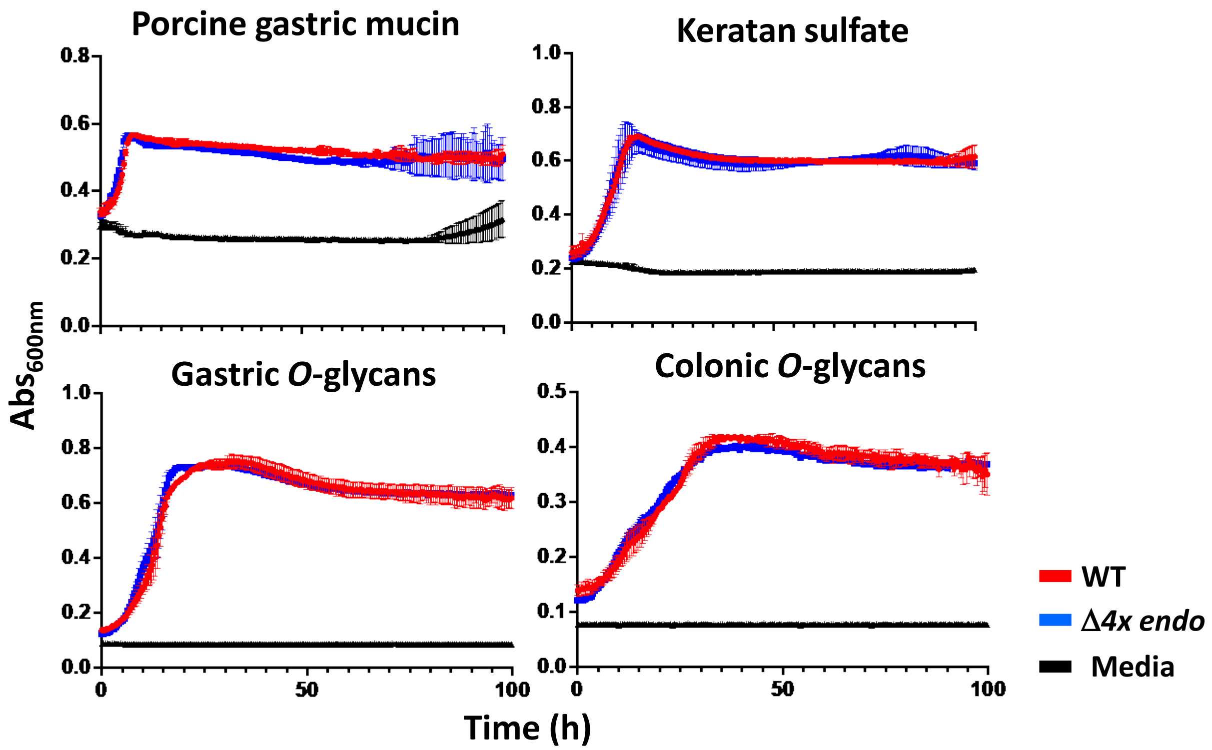


Fucose N-Acetylgalactosamine N-Acetylglucosamine Galactose N-Acetylneuraminic acid S O-sulfate

Unpublished

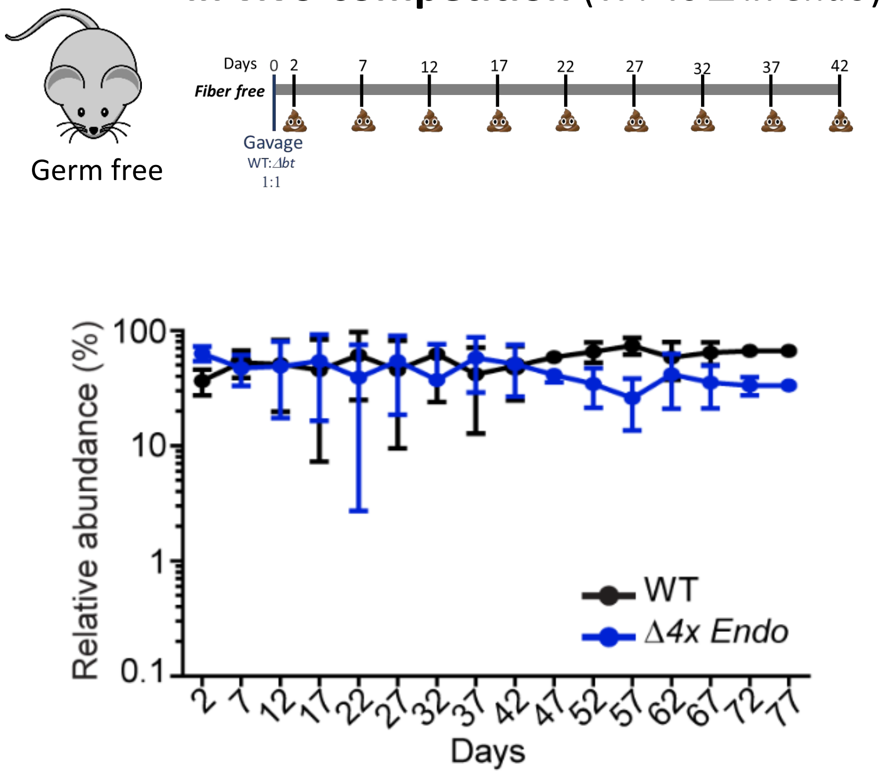
Endo enzymes do not affect mucin utilization

Growth curves



Δ*4x endo:* $\Delta bt2824_{GH16}$ + $\Delta bt2825_{GH18}$ + $\Delta bt1632_{GH18}$ + $\Delta bt3050_{GH18}$

***in vivo* competition** (WT vs Δ*4x endo*)



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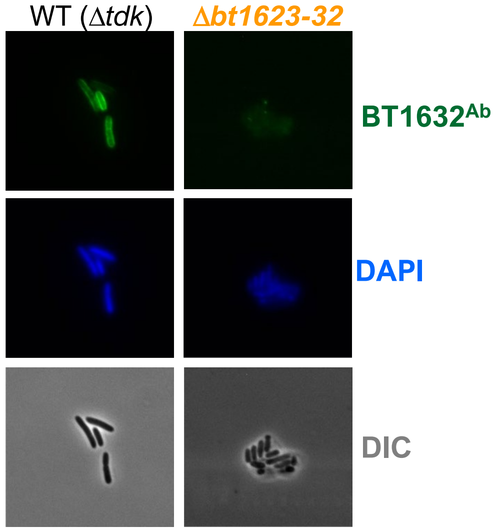
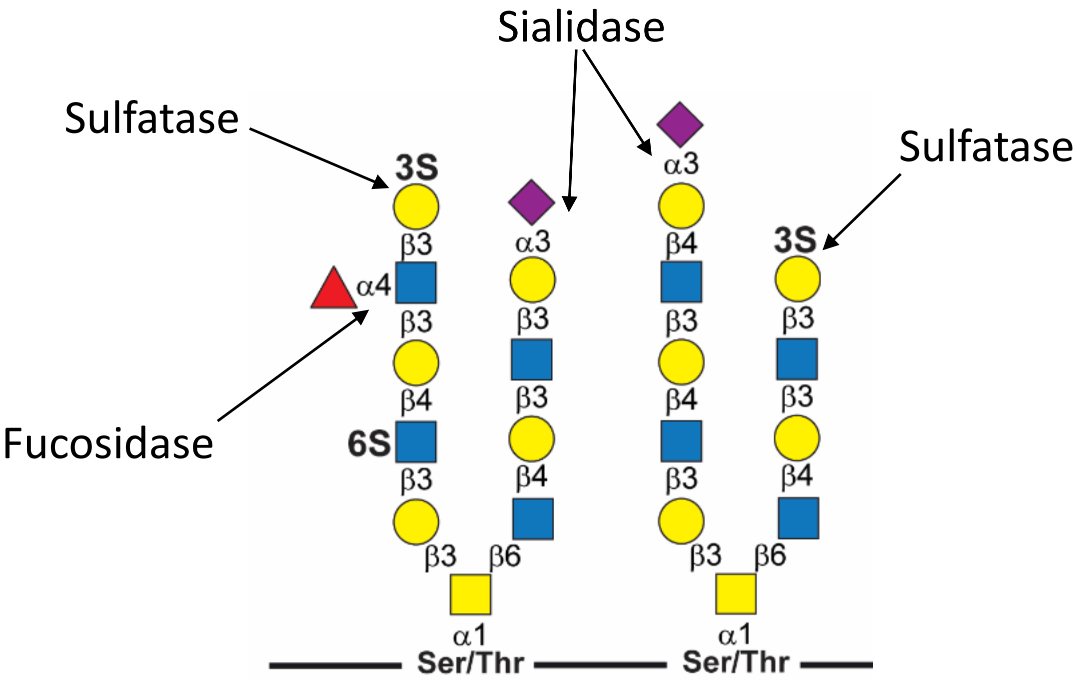
- Tested enzymes not essential for utilization of *O*-glycans or *in vivo* colonization
- Outer membrane





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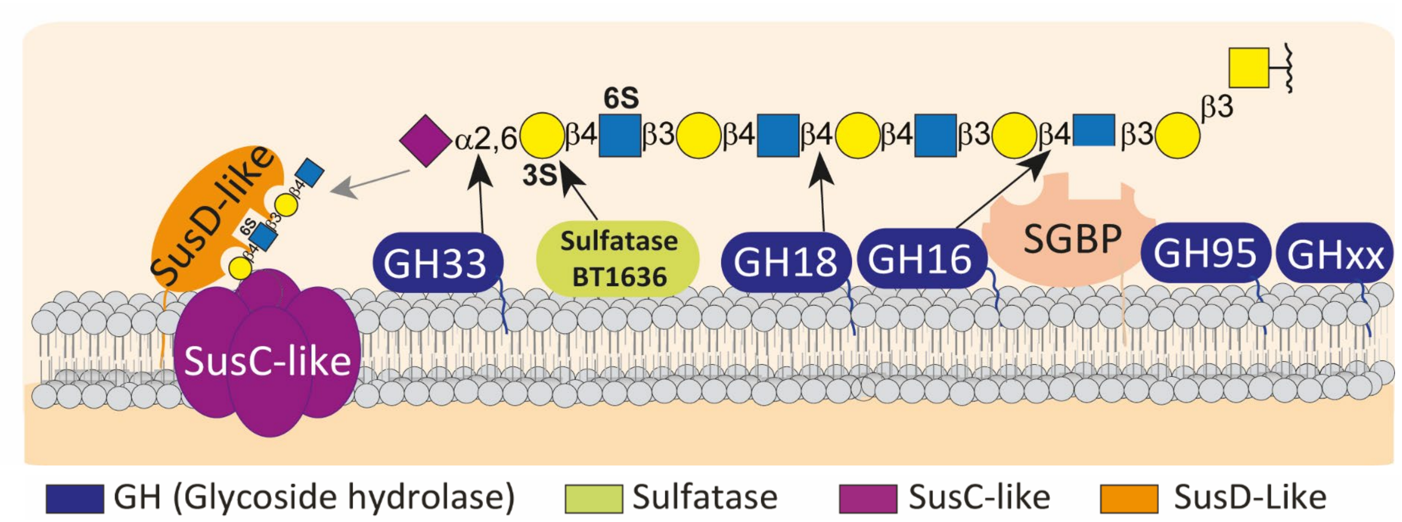
<u>Mucin *O*-glycans utilization system</u>

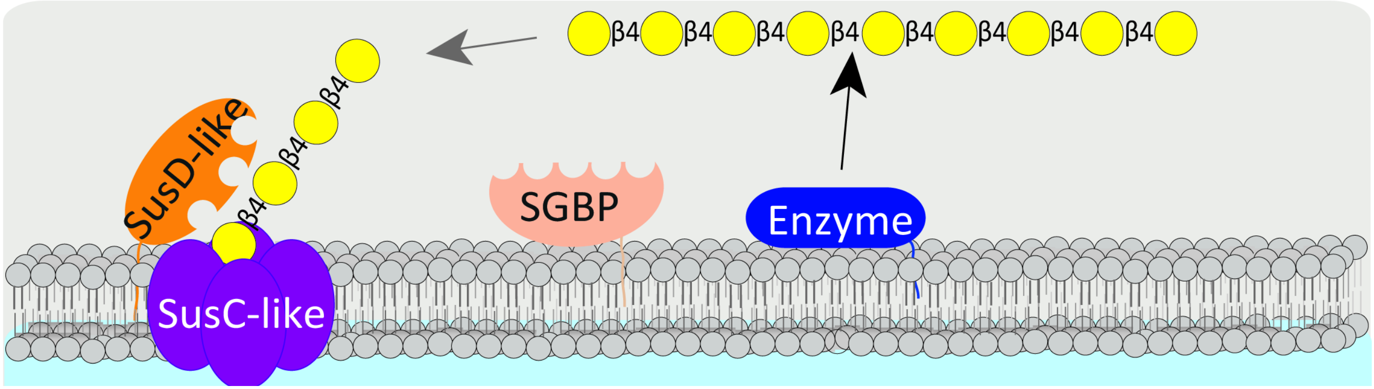


- **<u>Multiple</u> key enzymes**
- **Cell <u>surface</u> protein**
- **<u>Exo</u>-active enzyme**

Unpublished

Mucin utilization – Key enzymes

Other glycans

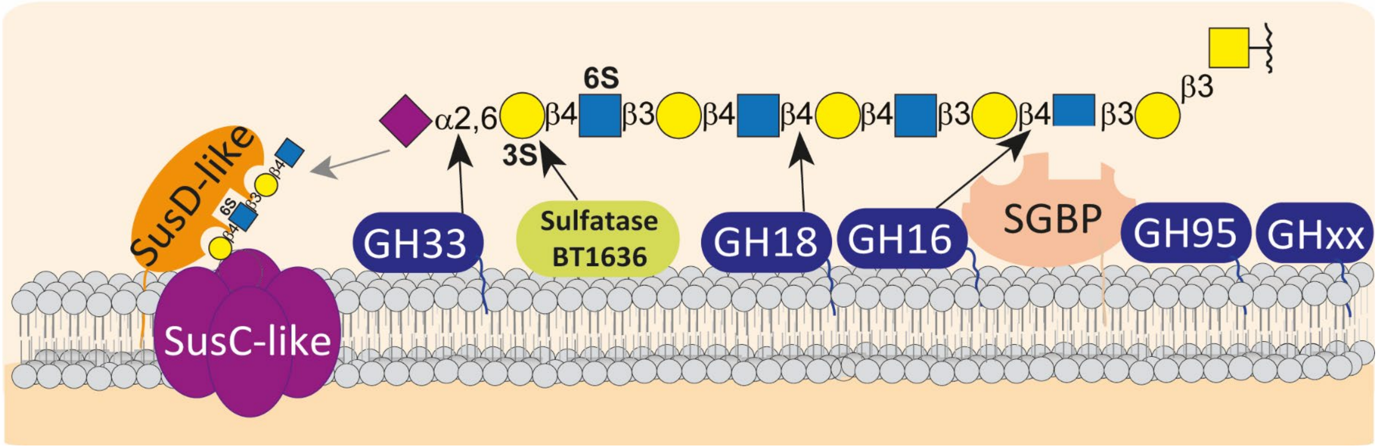


Published studies:

- Single enzyme
- Cell surface protein
- Endo-active enzyme

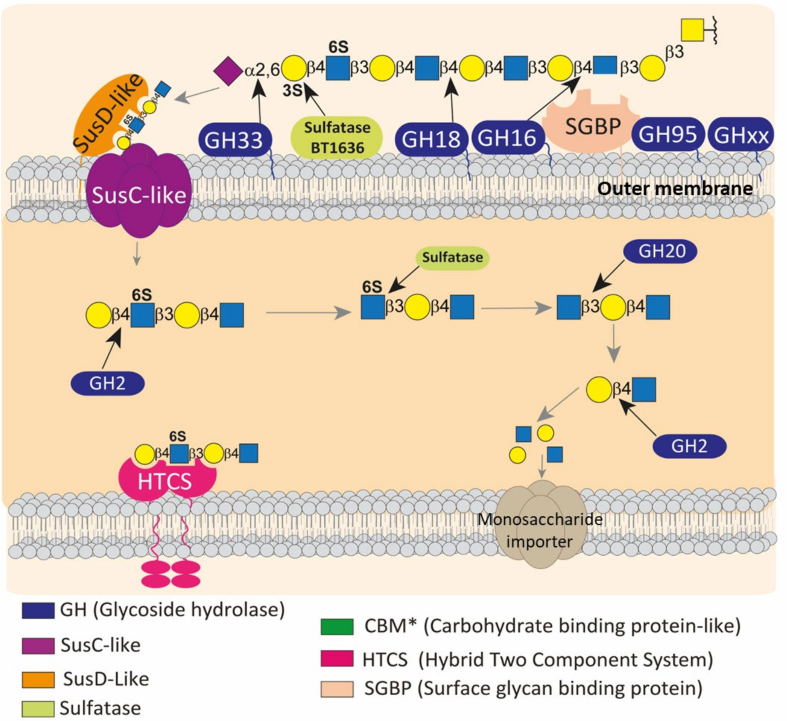
Larsbrink *et al.*, Nature, 2015
Cuskin *et al.*, Nature, 2015
Ndeh *et al.*, Nature, 2017
Cartmell *et al.*, PNAS, 2017
Tamura *et al.*, Cell reports, 2017
Temple *et al.*, JBC, 2017
Cartmell *et al.*, Nat Microbiol, 2018
Luis *et al.*, Nat Microbiol, 2018
Briliūtė *et al.*, Nat Microbiol, 2019

Mucin *O*-glycans utilization system



- **Multiple key enzymes**
- **Cell surface protein**
- **Exo-active enzyme**

Conclusions



Model of mucin *O*-glycans depolymerization

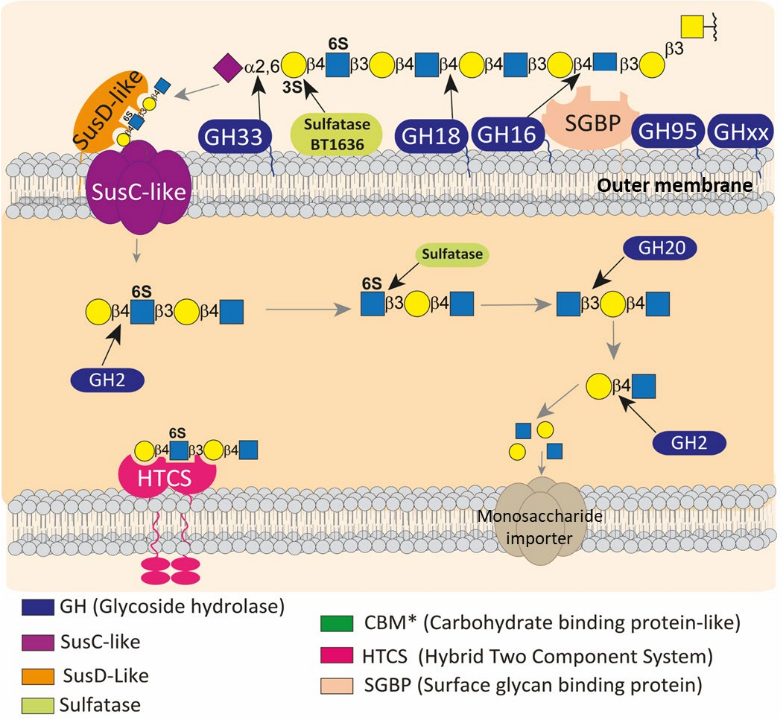
- **<u>Sequential degradation requires multiple enzymes</u>**
  30 GH
  6 sulfatases

- **<u>Initiated by Multiple</u> key enzymes**

  Cell <u>surface</u> protein

  <u>Exo</u>-active enzyme

Conclusions



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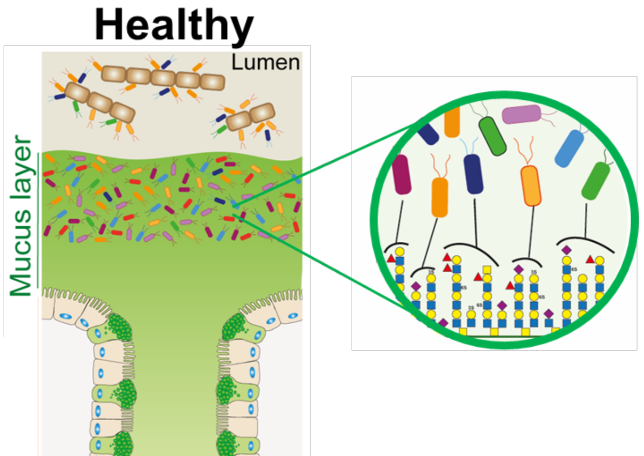
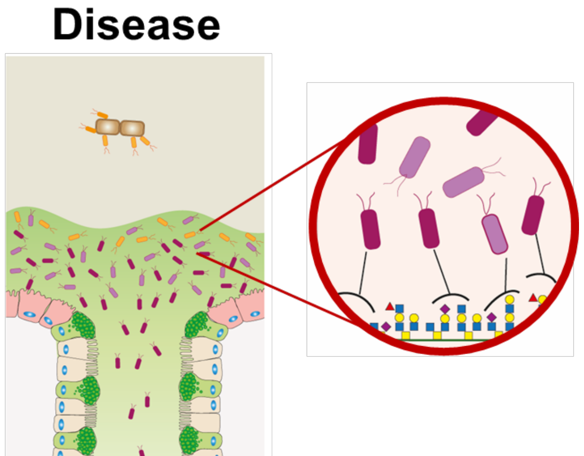
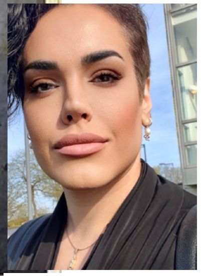




Blocking key enzymes in mucin O-glycan utilization by the microbiota can restore the mucus-barrier function in diseases

Acknowledgements

Luis's Lab



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Rhea Bains

Alan Cartmell (York Univ.)

Proteomics Core Facility
BioMS Swedish National Infrastructure for Biological Mass Spectrometry
SciLifeLab

Funding:

SSMF
Swedish Society for
Medical Research

Vetenskapsrådet

Jeanssons Stiftelser

UNIVERSITY OF GOTHENBURG
Sahlgrenska Academy