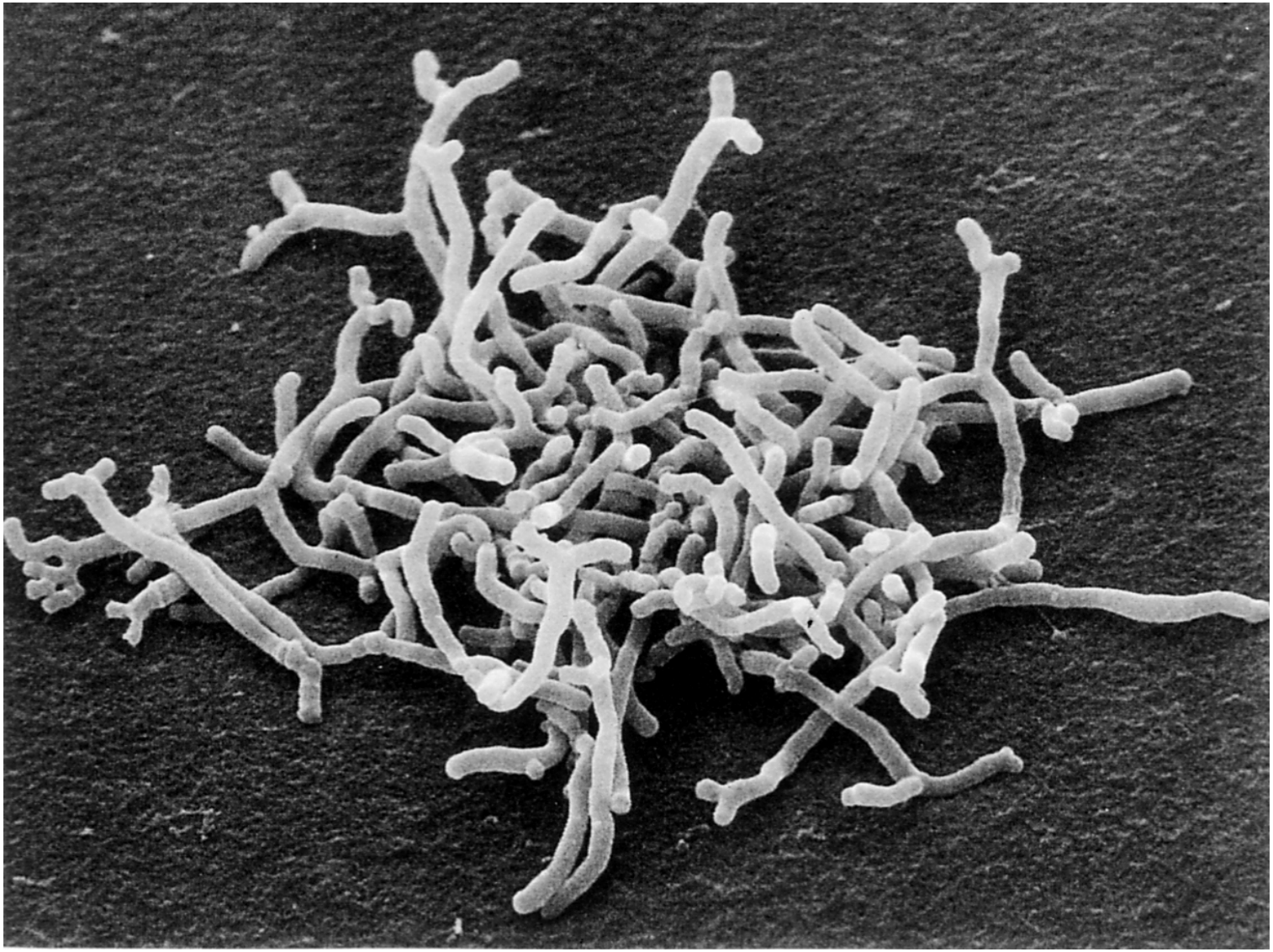
Symbiotic Relationship between Human and Bifidobacteria

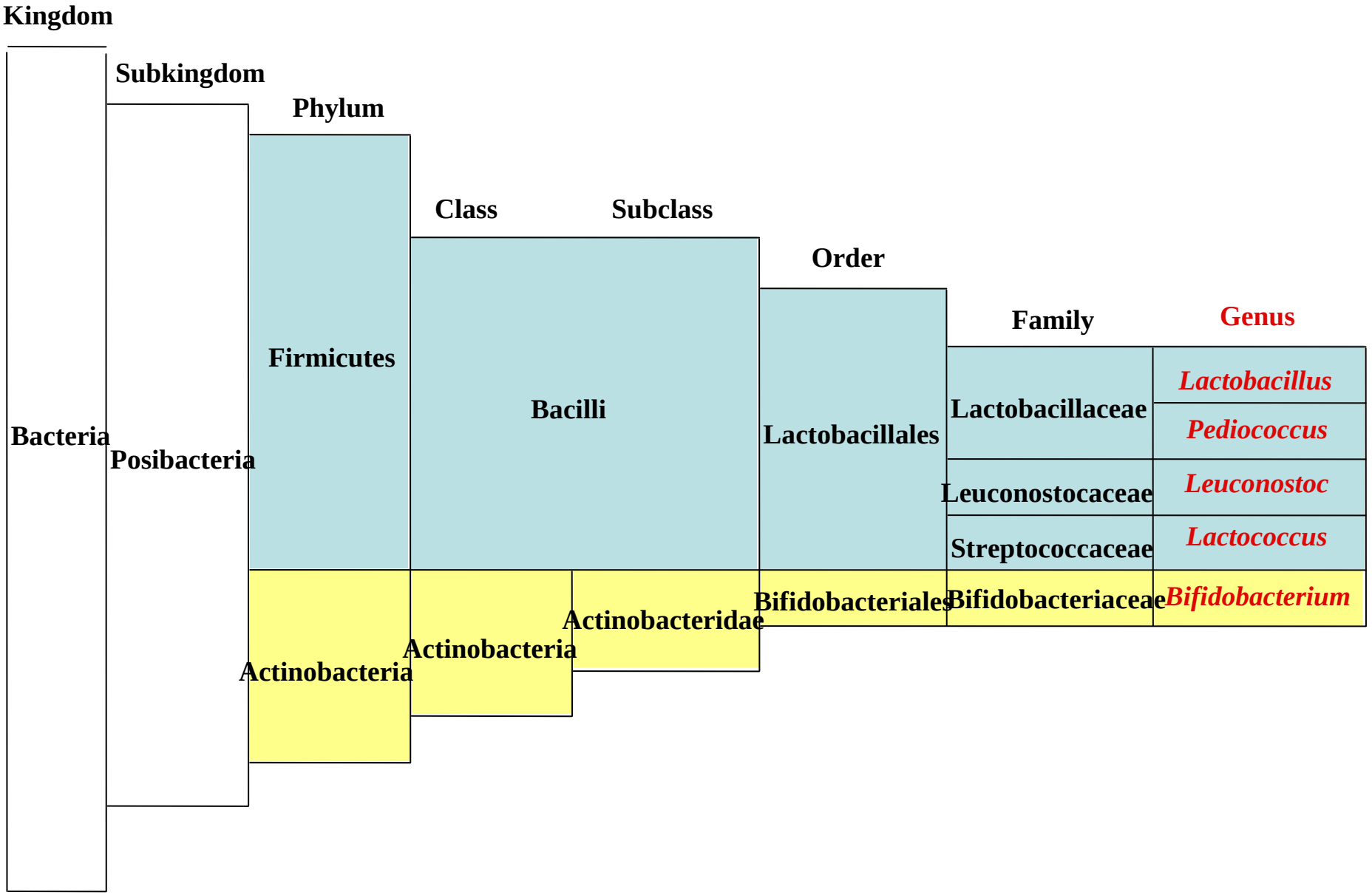
Kenji YAMAMOTO

Graduate School of Biostudies, Kyoto University



Bifidobacterium longum

Classification of Major Lactic Acid Bacteria



History of Bifidobacteria

1899 Tissier

Tissier found and isolated a new bacterium from infant's feces, and named the bacteria as '*Bacillus bifidus communis*'.
This is the finding of bifidobacteria.

1924 Orla-Jensen

Orla-Jensen proposed to establish new genus '*Bifidobacterium*', but the proposal was not accepted.

1933~1934 Weiss and Rettger

They regarded bifidobacteria as a species of Lactobacilli and classified into genus '*Lactobacillus'.*

1958~ Ochi and Mitsuoka et al, Rogosa et al, Sharpe, Reuter, Werner

Bifidobacteria were excluded from genus '*Lactobacillus'* and genus '*Bifidobacterium*' was newly established.

Various Bifidobacteria were Isolated from Various Sources

Human Origin

B. adolescentis	(adult faeces)
B. angulatum	(adult faeces)
B. bifidum	(child and adult faeces, vagina)
B. breve	(child faeces, vagina)
B. catenulatum	(child and adult faeces, vagina)
B. denticolens	(buccal cavity, adult faeces)
B. dentium	(buccal cavity, adult faeces)
B. gallicum	(adult faeces)
B. infantis	(child faeces, vagina)
B. inopinatum	(buccal cavity)
B. longum	(child and adult faeces, vagina)
B. pseudocatenulatum	(child faeces)

Aminal Origin

B. animalis	(rat, chicken, rabbit and calf faeces)
B. asteroides	(bees)
B. boum	(rumen, piglet faeces)
B. choerium	(piglet faeces)
B. coryneforme	(bees)
B. cuniculi	(rabbit faeces)
B. gallinarum	(chicken faeces)
B. indicum	(bees)
B. magnum	(rabbit faeces)
B. merycicum	(rumen)
B. pseudolongum pseudolongum	(piglet, chicken, calf and rat faeces, rumen)
B. pseudolongum globosum	(piglet, chicken, calf and rat faeces, rumen)
B. pullorum	(chicken faeces)
B. ruminantium	(rumen)
B. saeculare	(rabbit faeces)
B. suis	(piglet faeces)
B. thermophilum	(piglet, chicken and calf faeces, rumen)

Environmental and Food Origins

B. lactis	(fermented milk)
B. minimum	(sewage)
B. subtile	(sewage)
B. thermacidophilum	(anaerobic digester)

Most of Bifidobacteria are found in human and animal sources

Bifidobacteria Decrease with Aging of Human

Infant (breastfed)	Adolescent	Elderly

	Infant (breastfed)	Adolescent	Elderly
Ratio in flora	**90~%**		**~6%**
Major species	*B. infantis* *B. breve* *B. longum*	→	*B. adolescentis* *B. longum* *B. catenulatum*

Ratio of Bifidobacteria in human flora is decreased
and their species are changed with aging

Bifidobacteria are Approved as Probiotics

CIAA ERNA ehpm european botanicalforum

Brussels, 20 July 2007

LIST OF REFERENCES Part 1: Vitamins, Minerals, Protein, Carbohydrates, Fats, Fibre, Probiotics, Foods, Beverages and Diets

The number of LAB listed in 'CIAA EU Nutrition and Health Claims Regulation'

Lactobacilli	Number
L. acidophilus	15
L. bulgaricus	2
L. casei	9
L. coryniformis	2
L. fermentum	1
L. gasseri	4
L. helveticus	4
L. jonsonii	5
L. paracasei	5
L. plantarum	3
L. reuteri	3
L. rhamnosus	8
Total	61

Bifidobacteria	Number
B. adolescentis	2
B. animalis	18
B. bifidum	5
B. breve	3
B. infantis	2
B. longum	4
Total	34

Potential effects

- **Intestinal health**
- **Digestive health**
- **Natural defense**
- **Immune defense**
- **Skin health**

Cited from;
http://www.efosz.hu/letoltes/FCP28307E%20tudref1.pdf

In addtion to Lactobacilli, Bifidobacteria are also approved as major probiotic bacteria.

Bifidobacteria are Applied as Food Additives and Dietary Supplements

Representative food supplemented with Bifidobacteria

↓

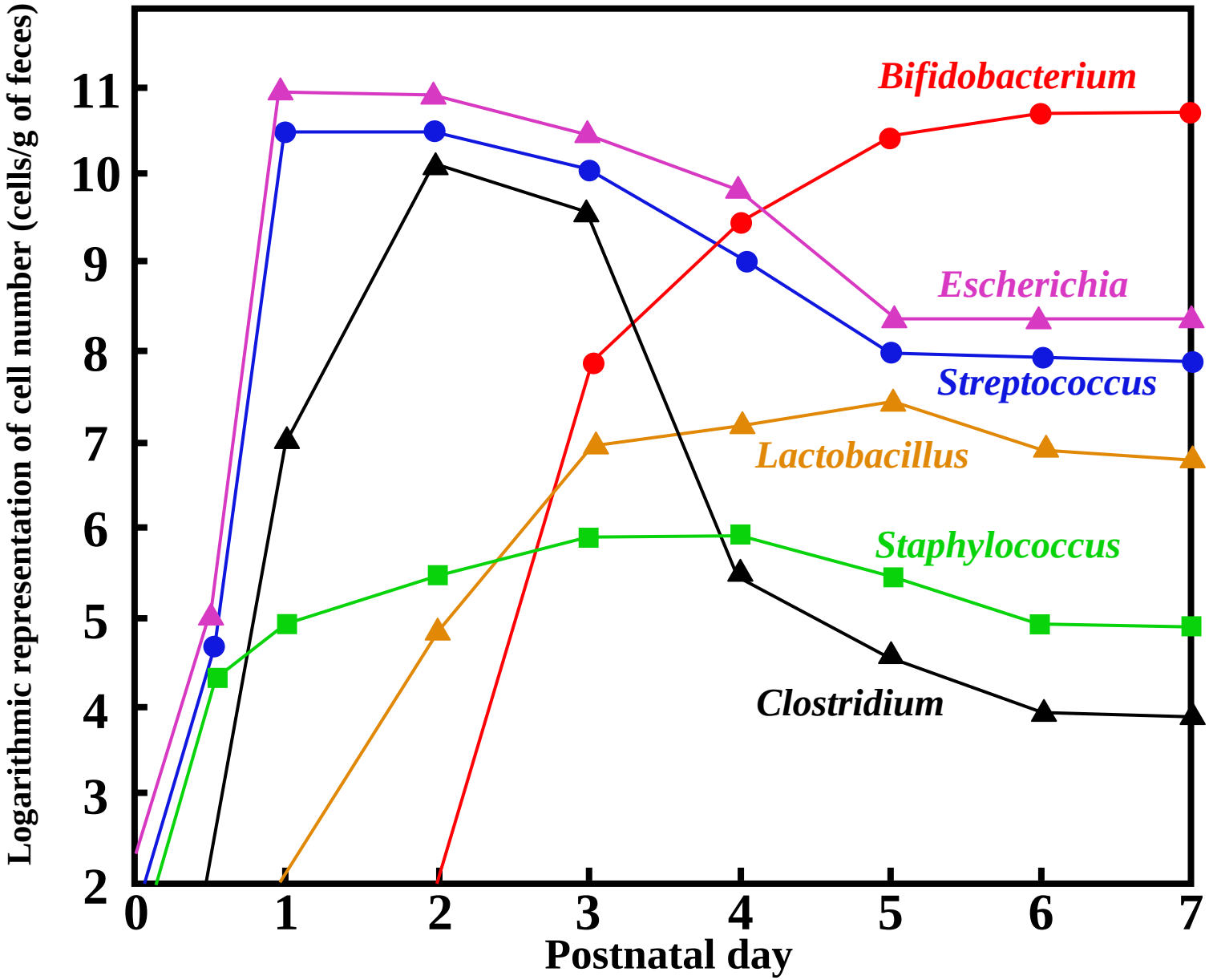
Yogurt

Cited from; 'Morinaga Milk Industry Co., Ltd.' (http://www.morinagamilk.co.jp/index.html) and 'DANONE JAPAN Co., Ltd.' (http://www.danone.co.jp/index.html)

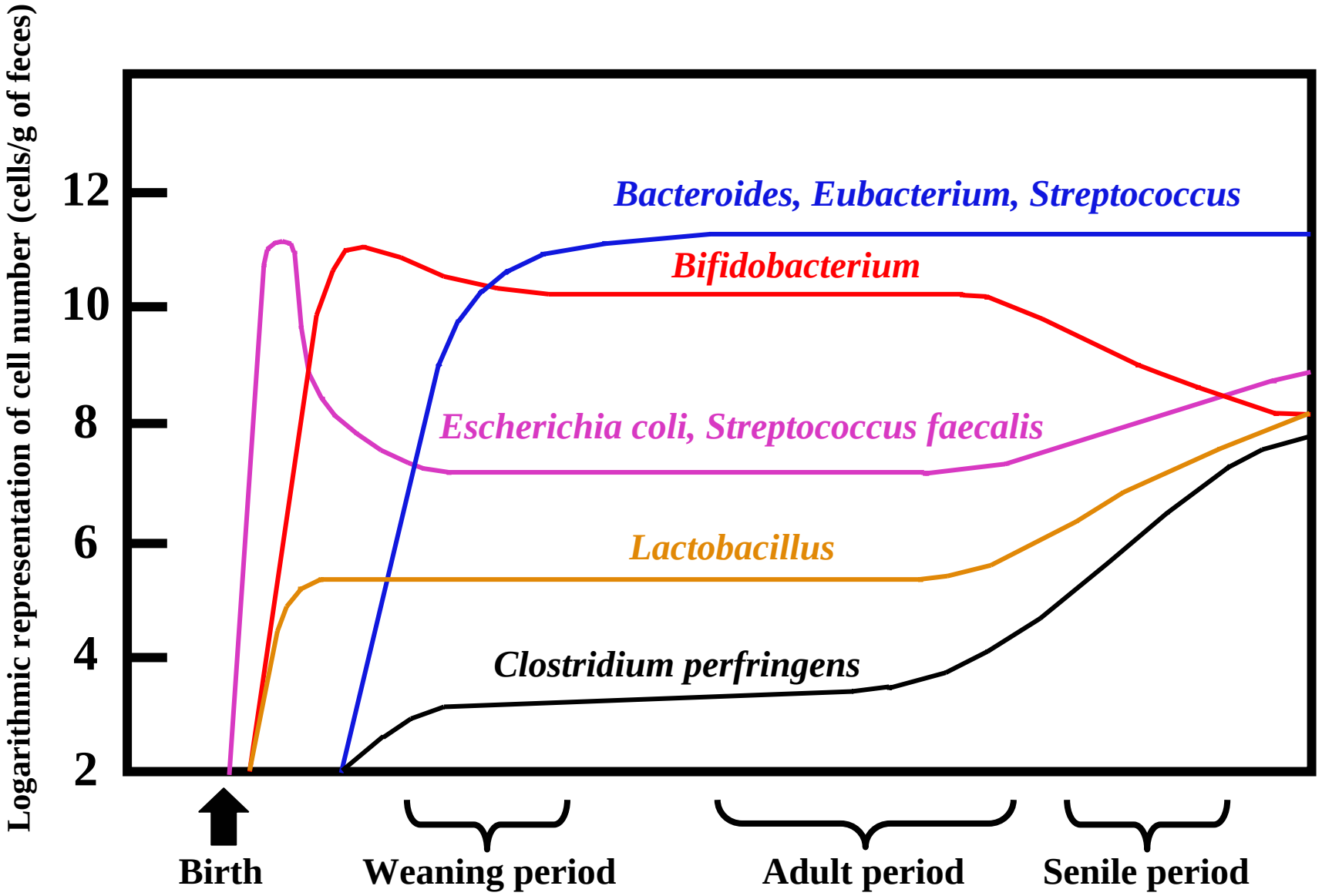
Bifidobacteria are ingested as dietary supplement

Cited from; 'MORISHITA JINTAN Co., Ltd.' (http://www.jintan.co.jp/) and 'SUNTORY LIMITED' (http://www.suntory.co.jp/)

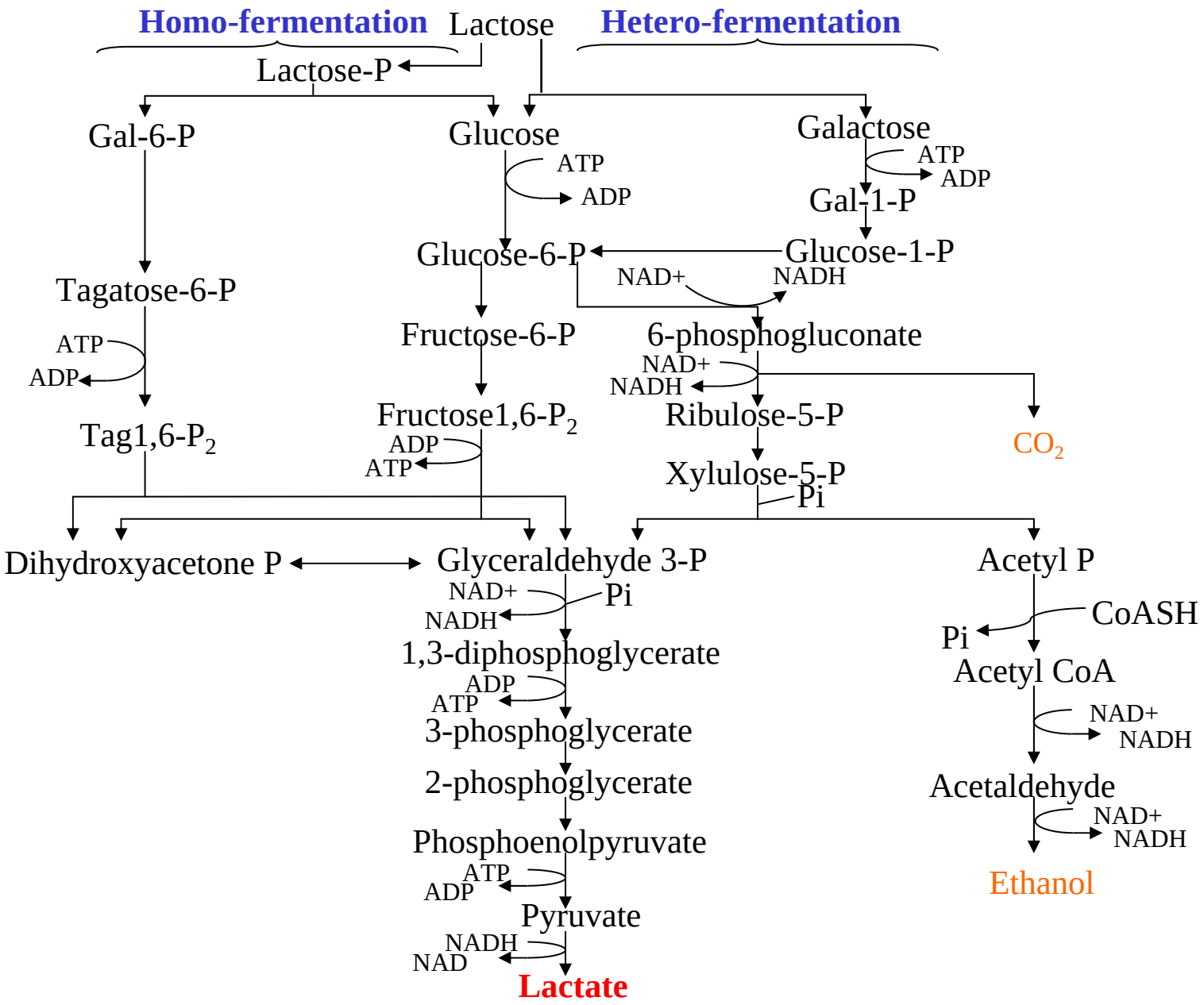
Alteration of Microbial Phase in Newborn Infant's Feces



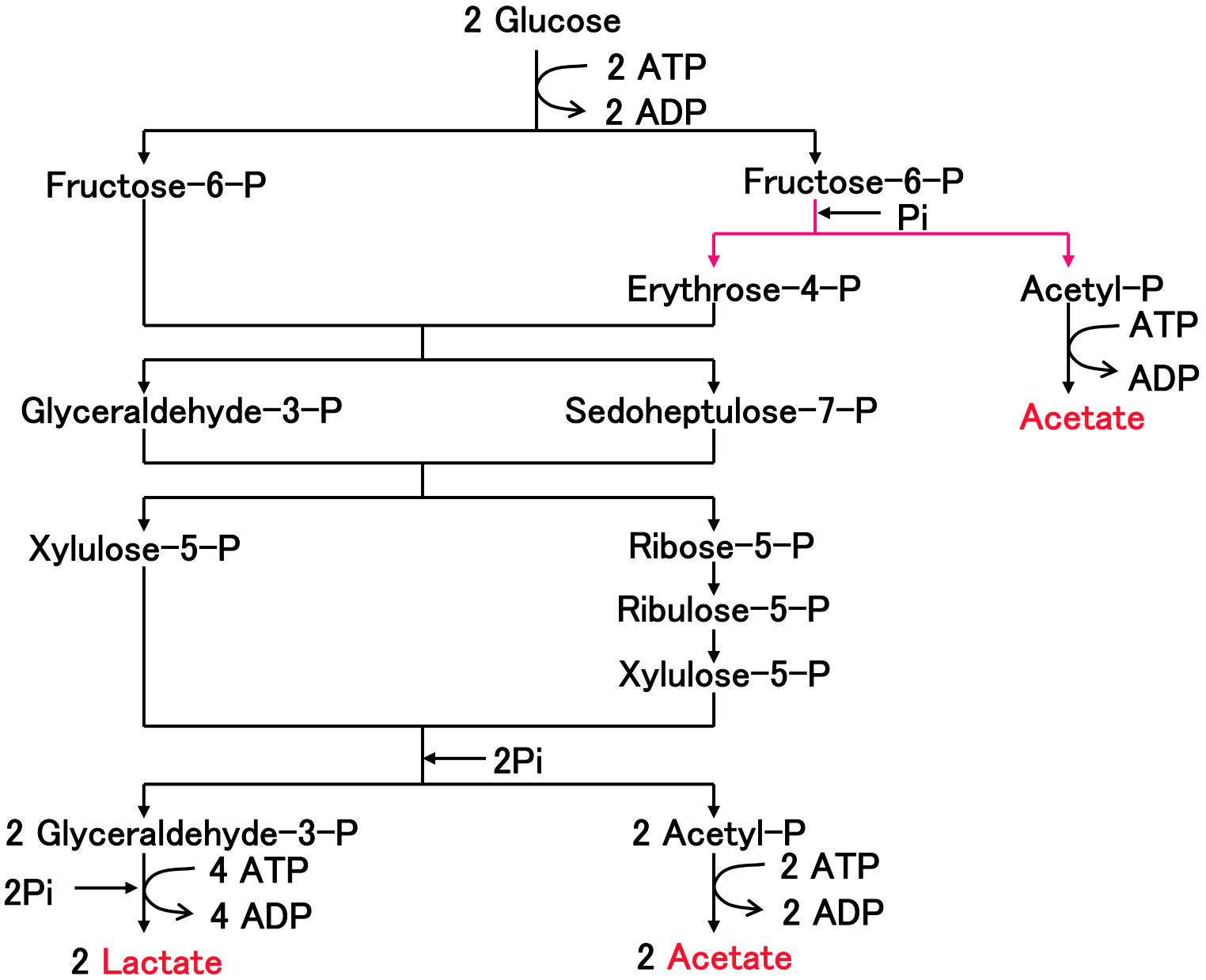
Alteration of Intestinal Microflora in Human Lifetime



Metabolic Pathway of Sugar in Lactic Acid Bacteria

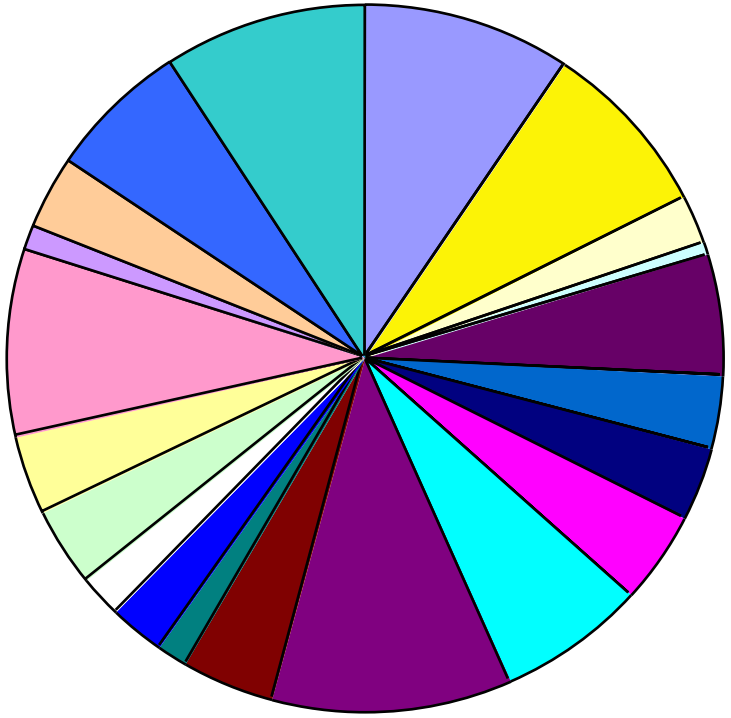


Metabolic Pathway of Sugar in Bifidobacteria



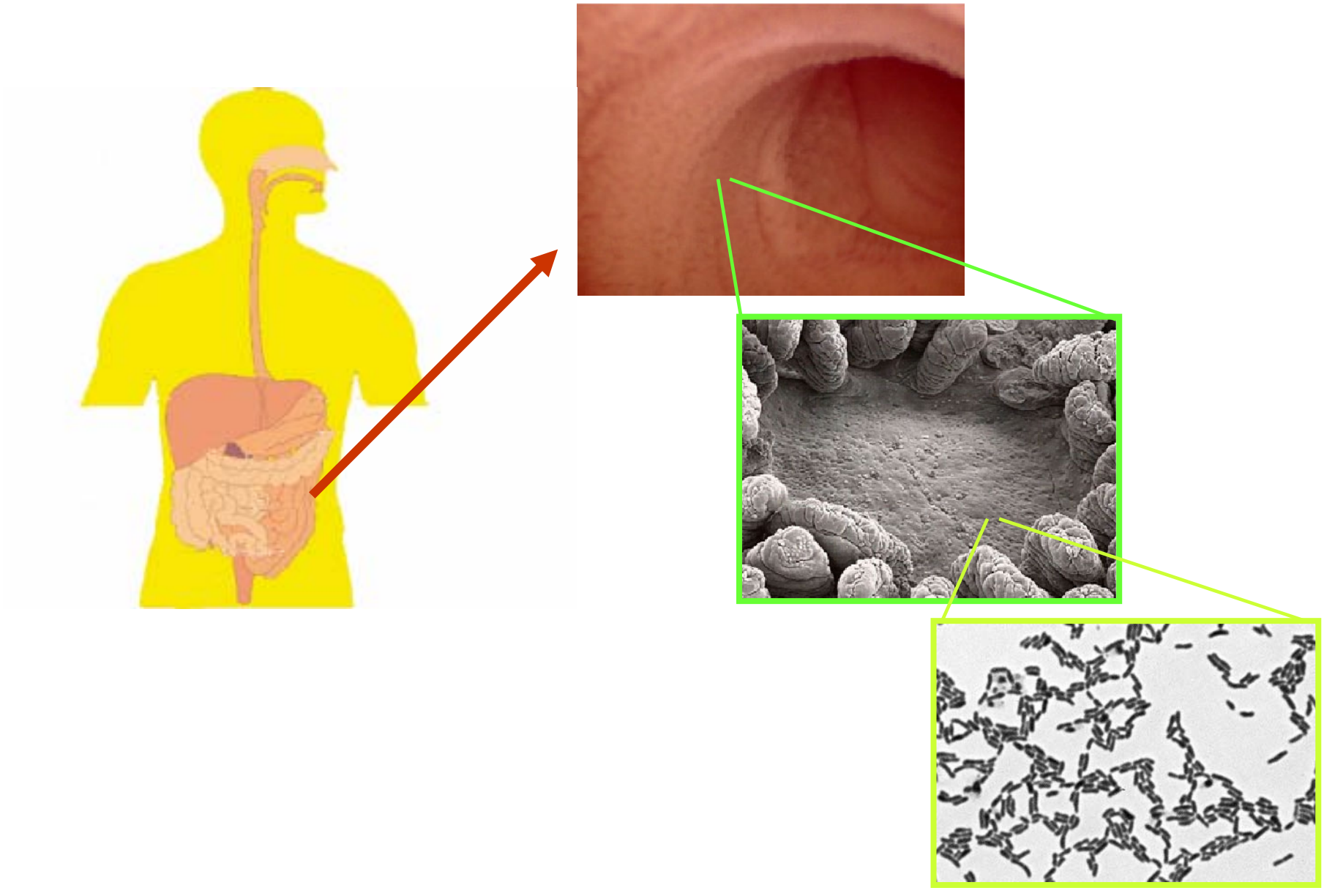
Analysis of Genomic DNA from *Bifidobacterium bifidum* JCM1254

Total number of contigue bp ; 2,218,867
Total number of presumed ORF ; 1,769

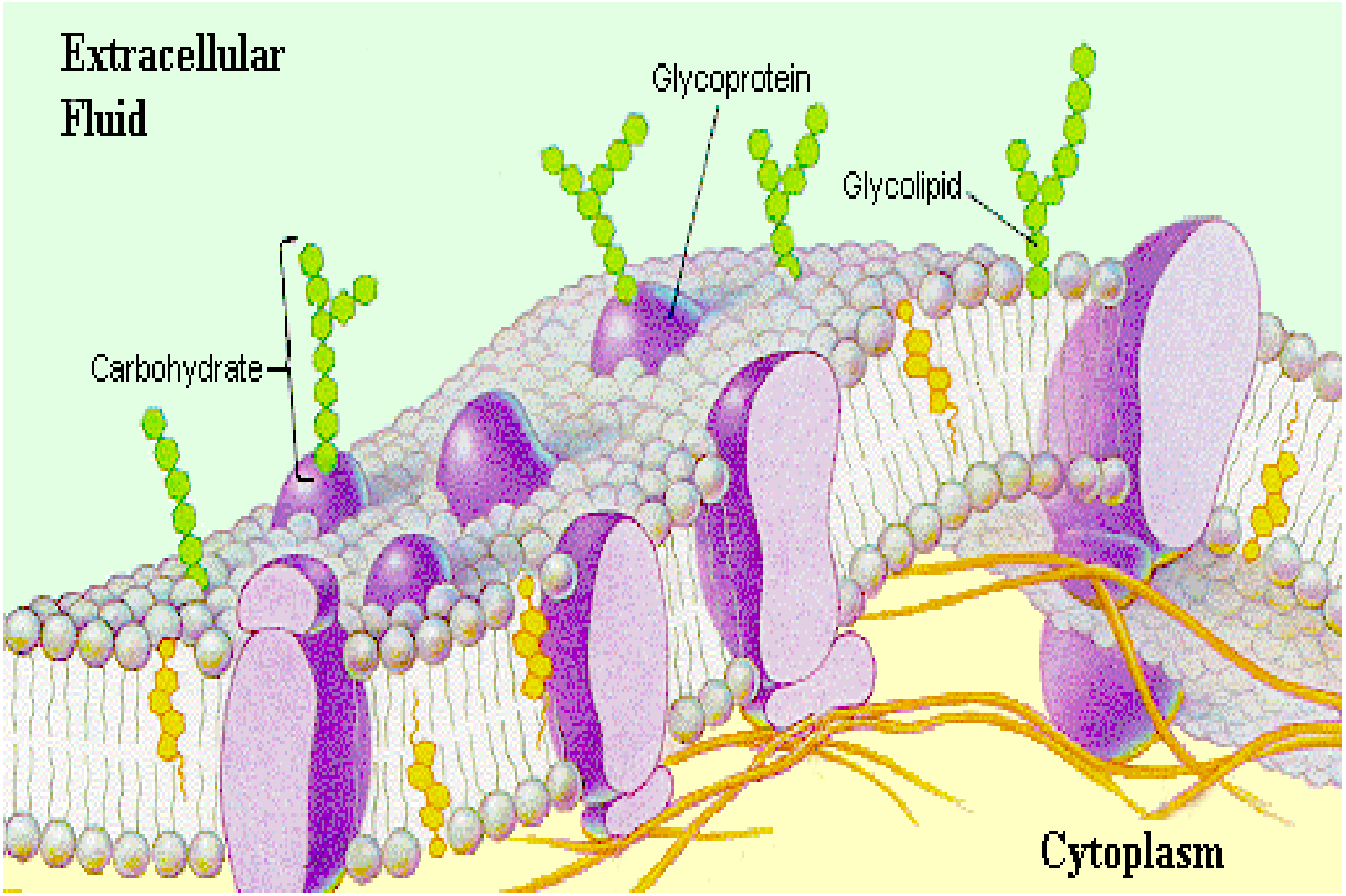


Clusters of Orthologous Groups (COG) classification	Ratio (%)
Amino acid transport and metabolism	9.3
Carbohydrate transport and metabolism	8.3
Cell cycle control, cell division, chromosome partitioning	2.1
Cell motility	0.5
Cell wall/membrane/envelope biogenesis	5.6
Chromatin structure and dynamics	0.1
Coenzyme transport and metabolism	3.4
Cytoskeleton	0.0
Defense mechanisms	3.3
Energy production and conversion	4.2
Extracellular structures	0.0
Function unknown	6.6
General function prediction only	10.9
Inorganic ion transport and metabolism	4.1
Intracellular trafficking, secretion, and vesicular transport	1.6
Lipid transport and metabolism	2.3
Not annotated	2.0
Nuclear structure	0.0
Nucleotide transport and metabolism	3.7
Posttranslational modification, protein turnover, chaperones	3.5
RNA processing and modification	0.1
Replication, recombination and repair	8.5
Secondary metabolites biosynthesis, transport and catabolism	1.0
Signal transduction mechanisms	3.3
Transcription	6.4
Translation, ribosomal structure and biogenesis	9.2

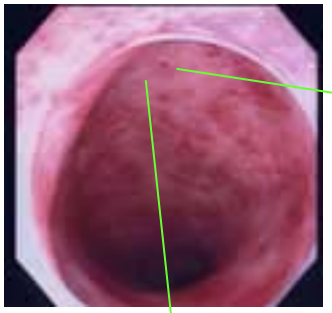
Relationship between Intestinal Tract and Befidobacteria

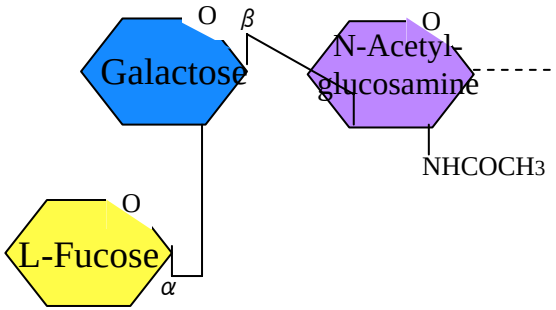


Schematic Model of Sugar Chains on Cell Surface Membrane

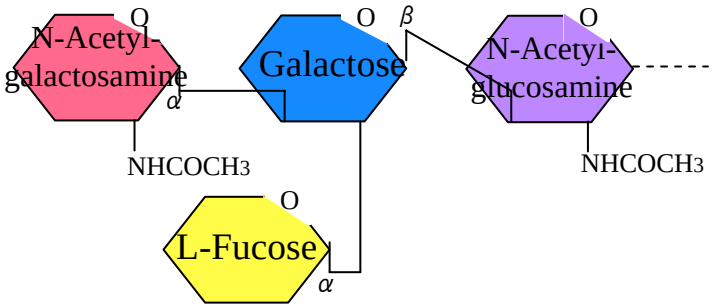


Structure of Determinant of ABO-type Blood Group Substances

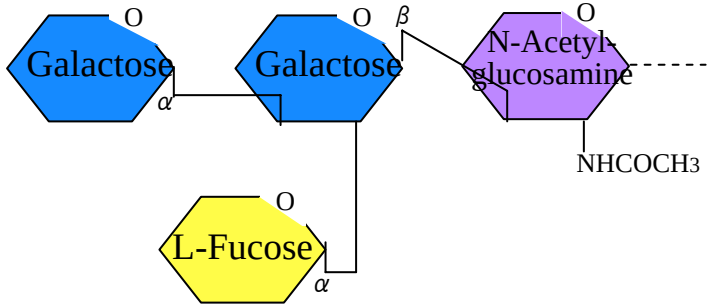




H(O)-type blood determinant

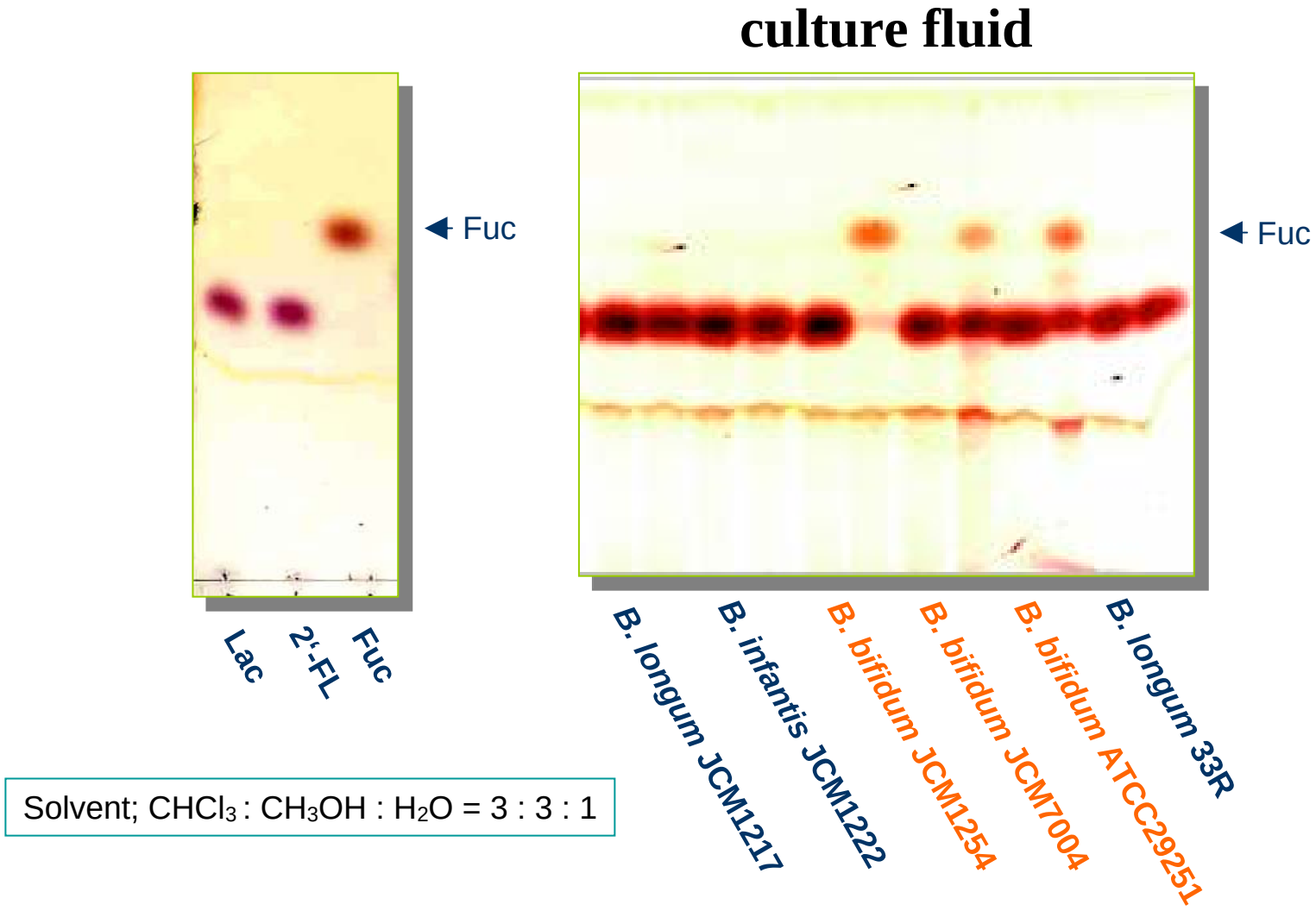


A-type blood determinant



B-type blood determinant

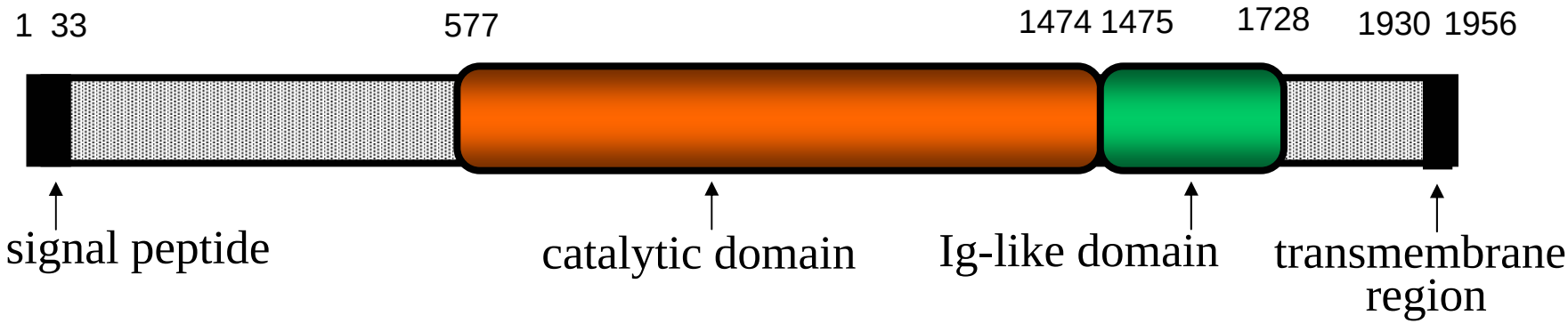
Distribution of 1,2-α-L-Fucosidase Activity in Bifidobacteria



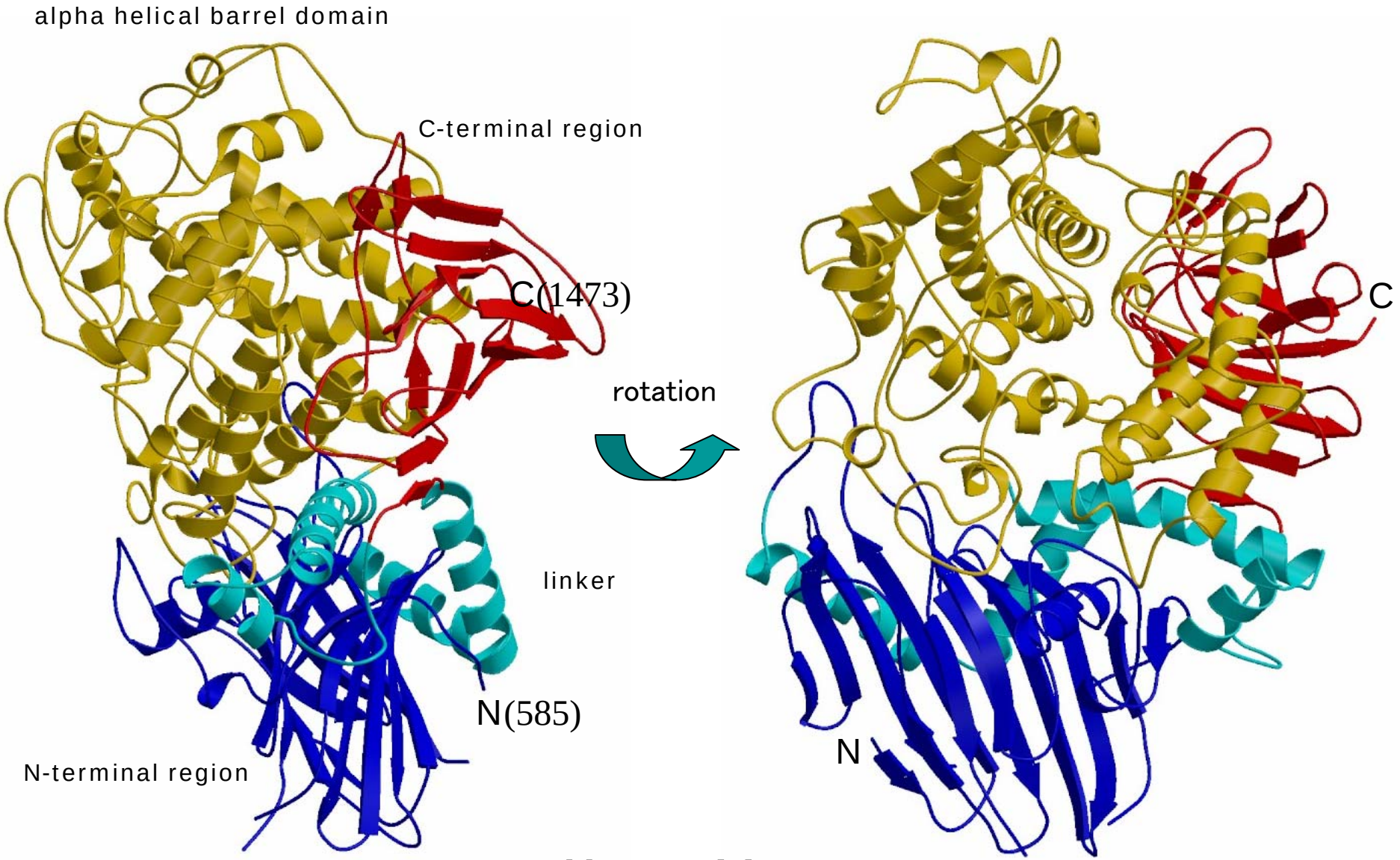
Solvent; $CHCl_3$: CH_3OH : H_2O = 3 : 3 : 1

Structure of 1,2-α-L-Fucosidase from *B.bifidum* JCM1254

AfcA (1959 amino acids) **Fuc α 1-2 Gal β 1-3GlcNAc** β 1-3Gal β 1-4Glc

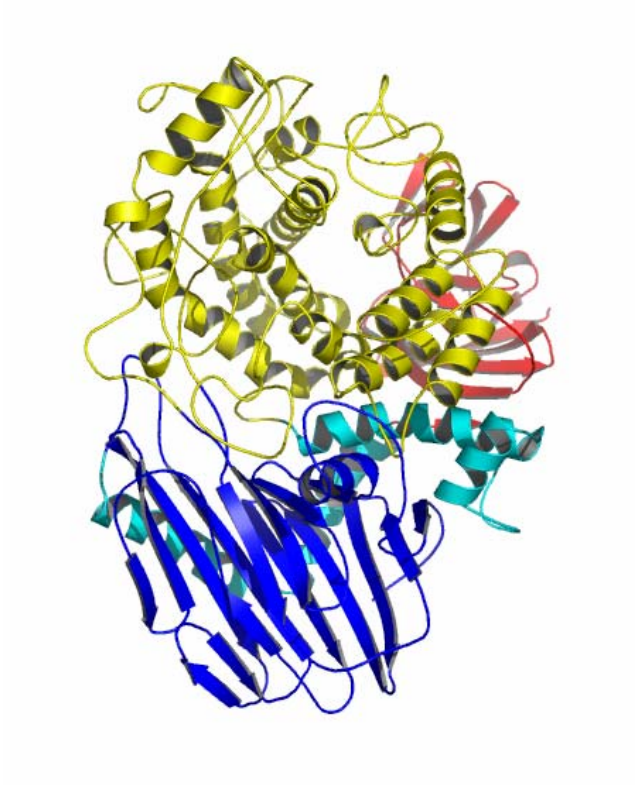


Overall Structure of Catalytic Domain of 1,2-α-L-Fucosidase

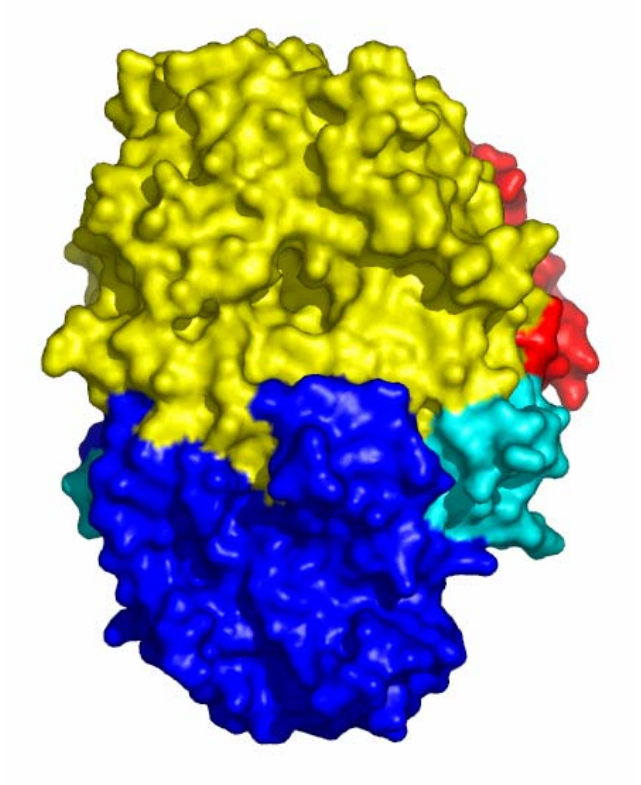


ribbon model

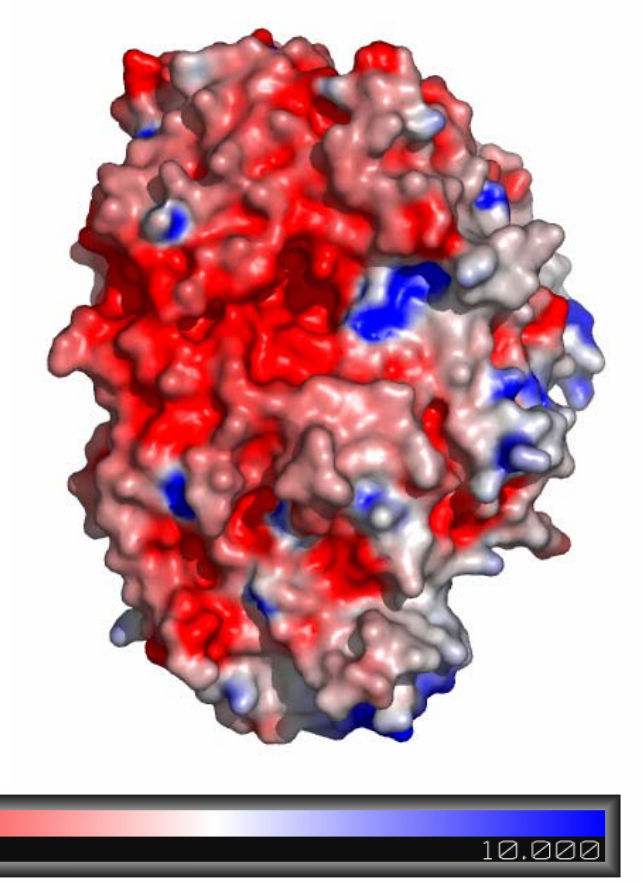
Overall Structure of Catalytic Domain of 1,2-α-L-Fucosidase



ribbon model



molecular surface



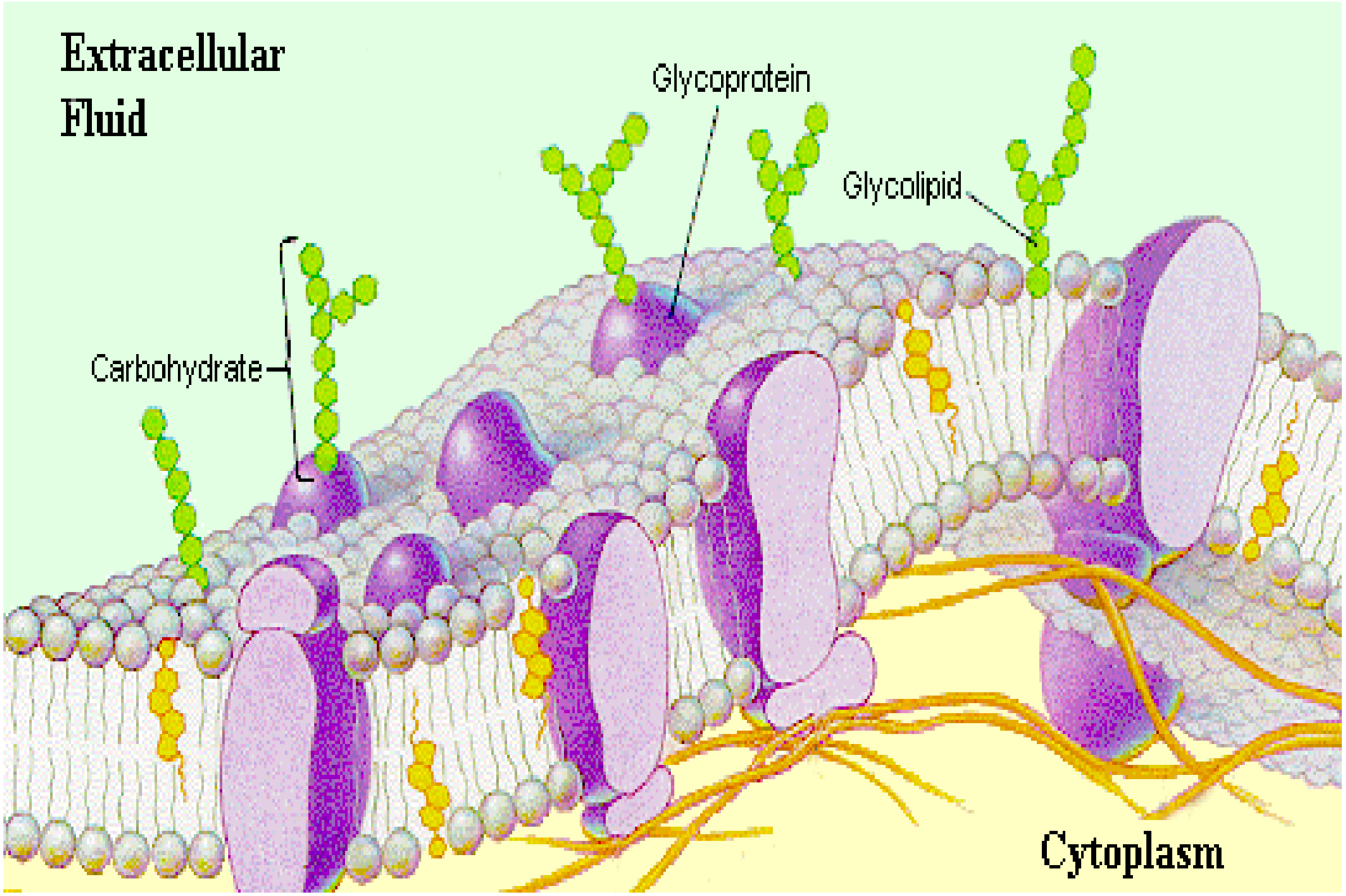
electrostatic potential map

Substrate Specificity of *B.bifidum* 1,2-α-L-Fucosidase

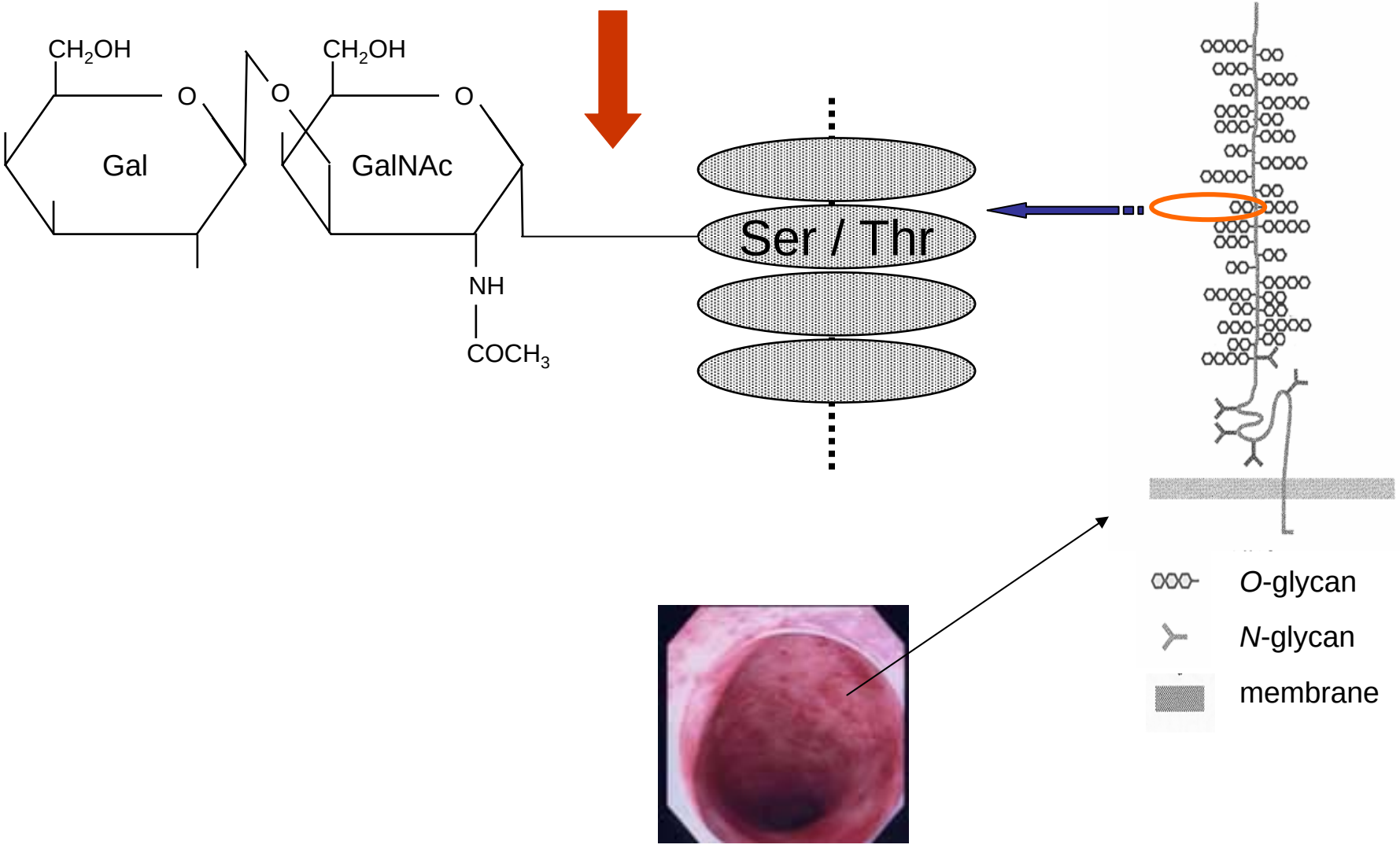
Substrate	Structure	Activity (%)
2'-FL	Galβ1→4Glc ↑2 Fucα1	100
H(II)	Galβ1→4GlcNAc ↑2 Fucα1	74
H	Galβ1 ↑2 Fucα1	27
A	GalNAcα1→3Galβ1 ↑2 Fucα1	5.9
B	Galα1→3Galβ1 ↑2 Fucα1	trace
LNF P-I	Galβ1→3GlcNAcβ1→3Galβ1→4Glc ↑2 Fucα1	61
3-FL	Galβ1→4Glc ↑3 Fucα1	trace
LNF P-V	Galβ1→3GlcNAcβ1→3Galβ1→4Glc ↑3 Fucα1	trace
LNF P-II	Galβ1→3GlcNAcβ1→3Galβ1→4Glc ↑4 Fucα1	ND
FDA C	Fucα1 ↓6 GlcNAcβ1→4GlcNAc	ND
*p*NP-Fuc	Fucα1→*p*NP Fucβ1→*p*NP	ND

LNFP; lacto-*N*-fucopentaose FDAC; fucosyldiacetylchitobiose 'Trace' indicates less than 1% activity.

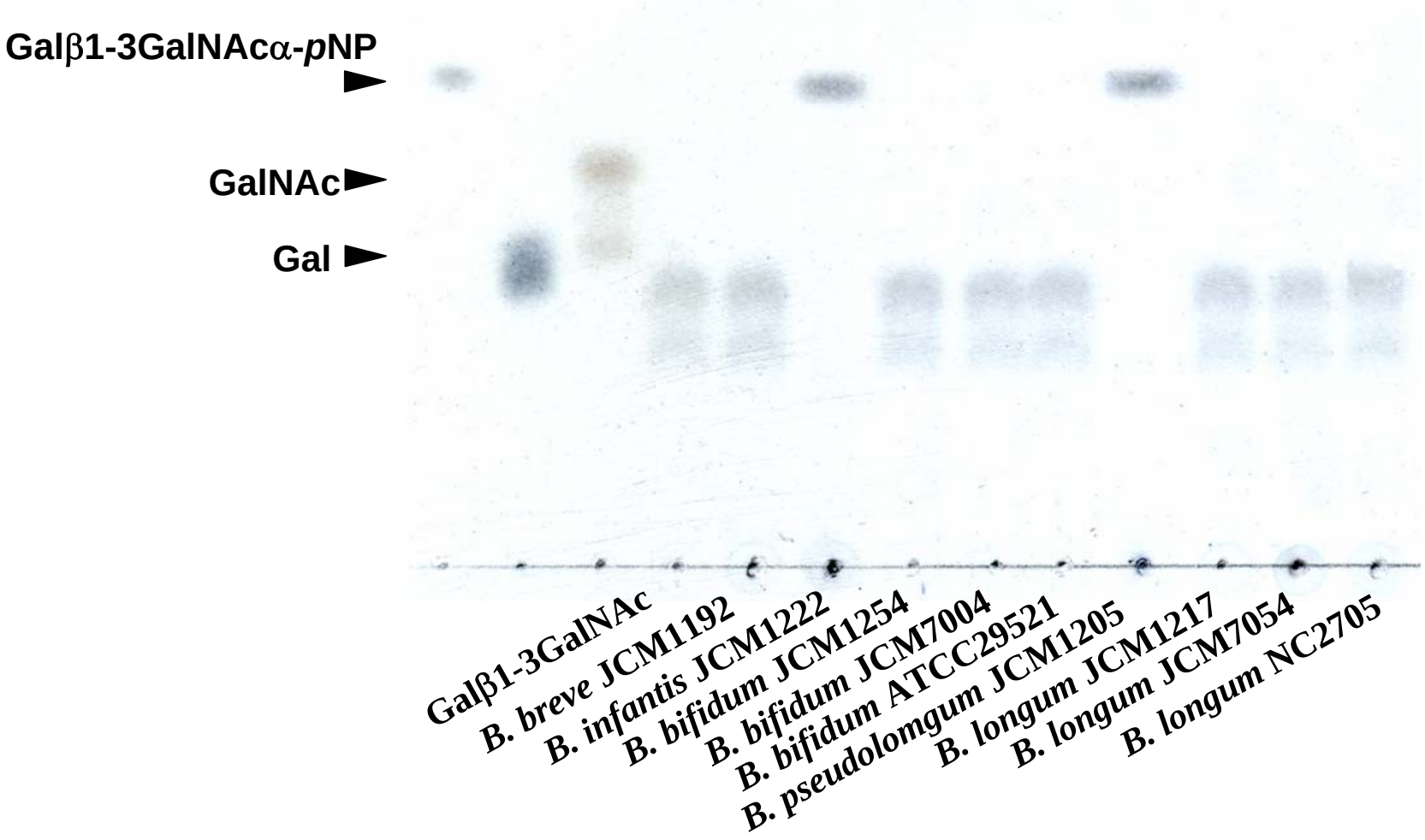
Schematic Model of Sugar Chains on Cell Surface Membrane



Endo-α-*N*-acetylgalactosaminidase

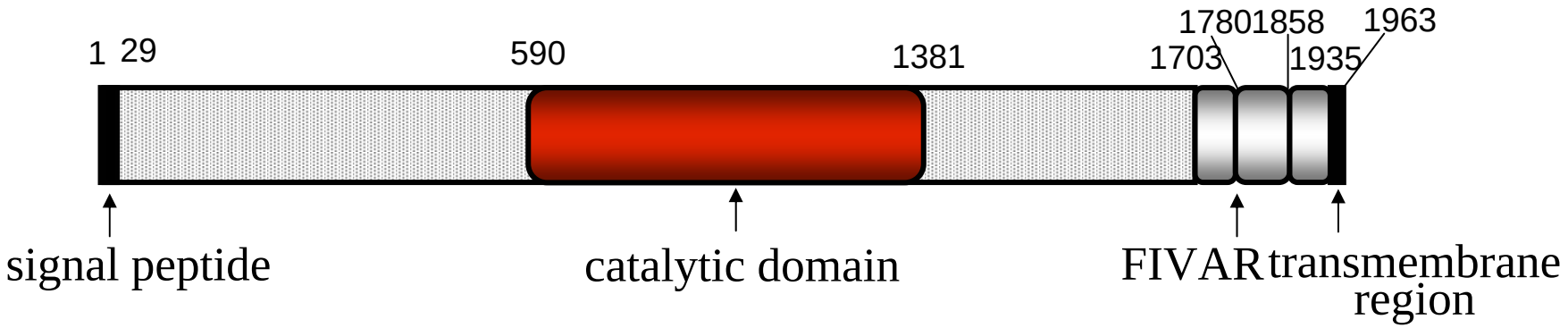


Endo-α-*N*-acetylgalactosaminidase Activity of Various Bifidobacteria

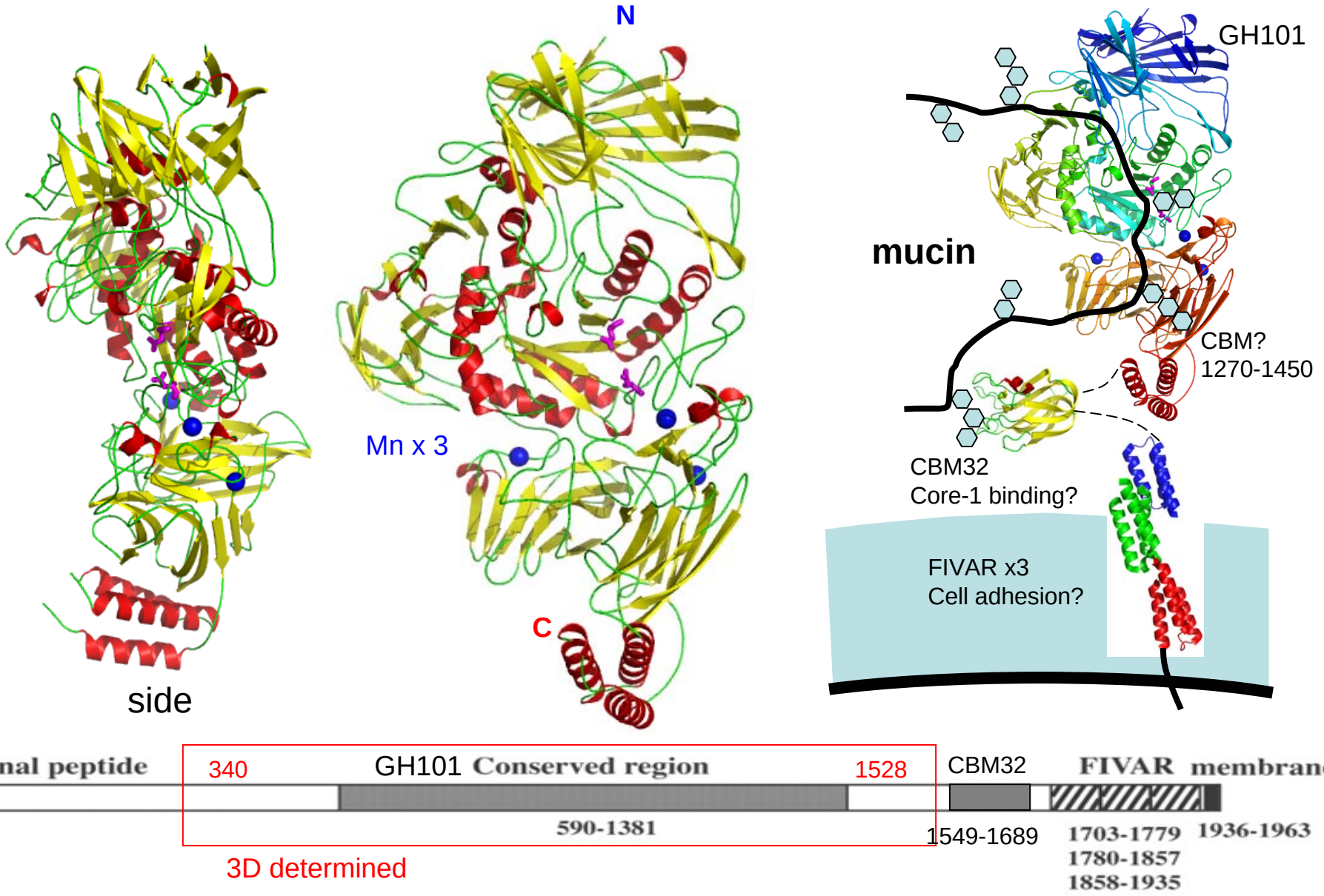


Structure of Endo-α-*N*-acetylgalactosaminidase from *B.longum* JCM1217

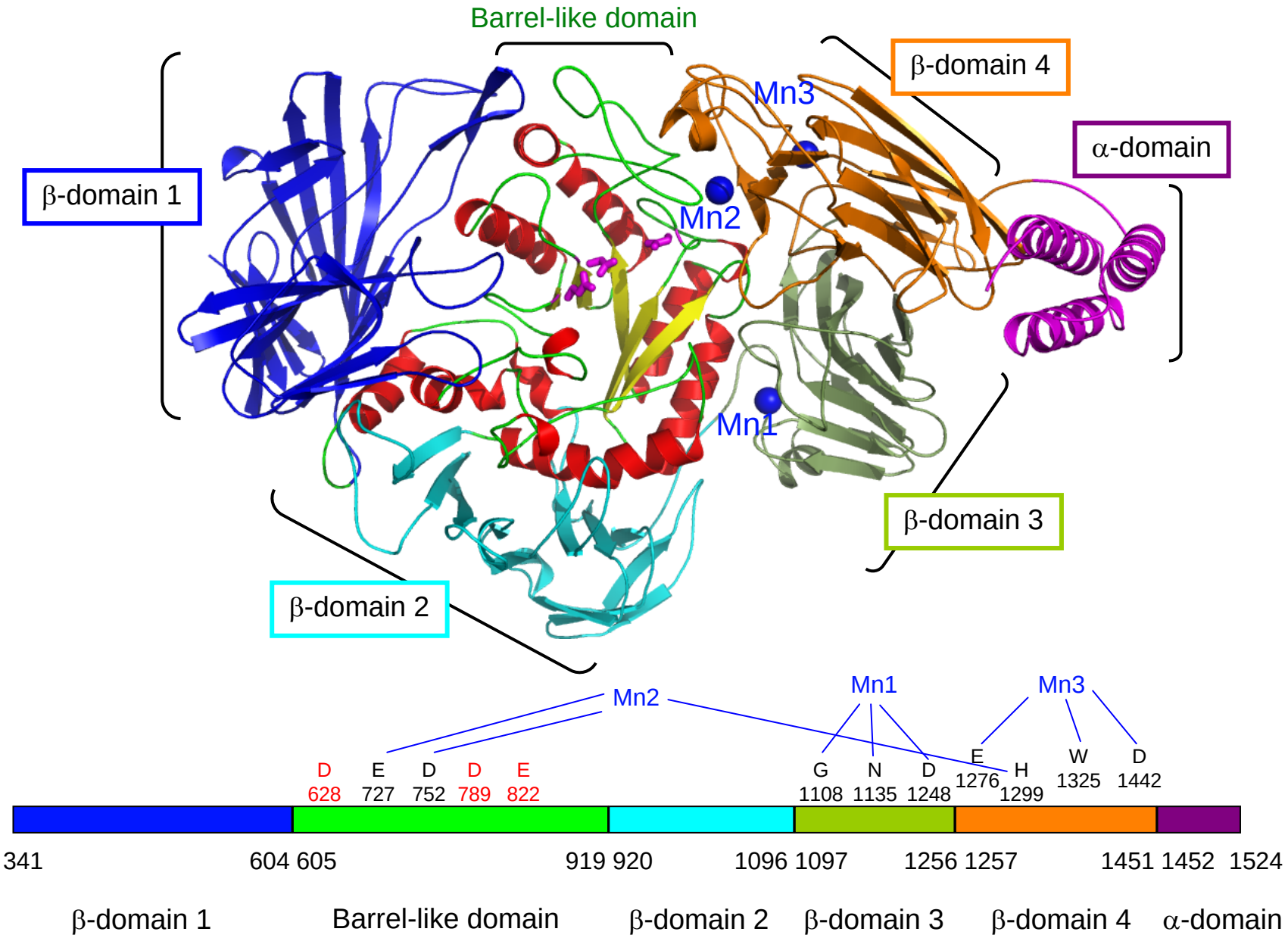
EngBF (1966 Amino Acids) **Gal β 1-3GalNAc α** -O-Thr/Ser



Crystal Structure of Endo-α-*N*-acetylgalactosaminidase



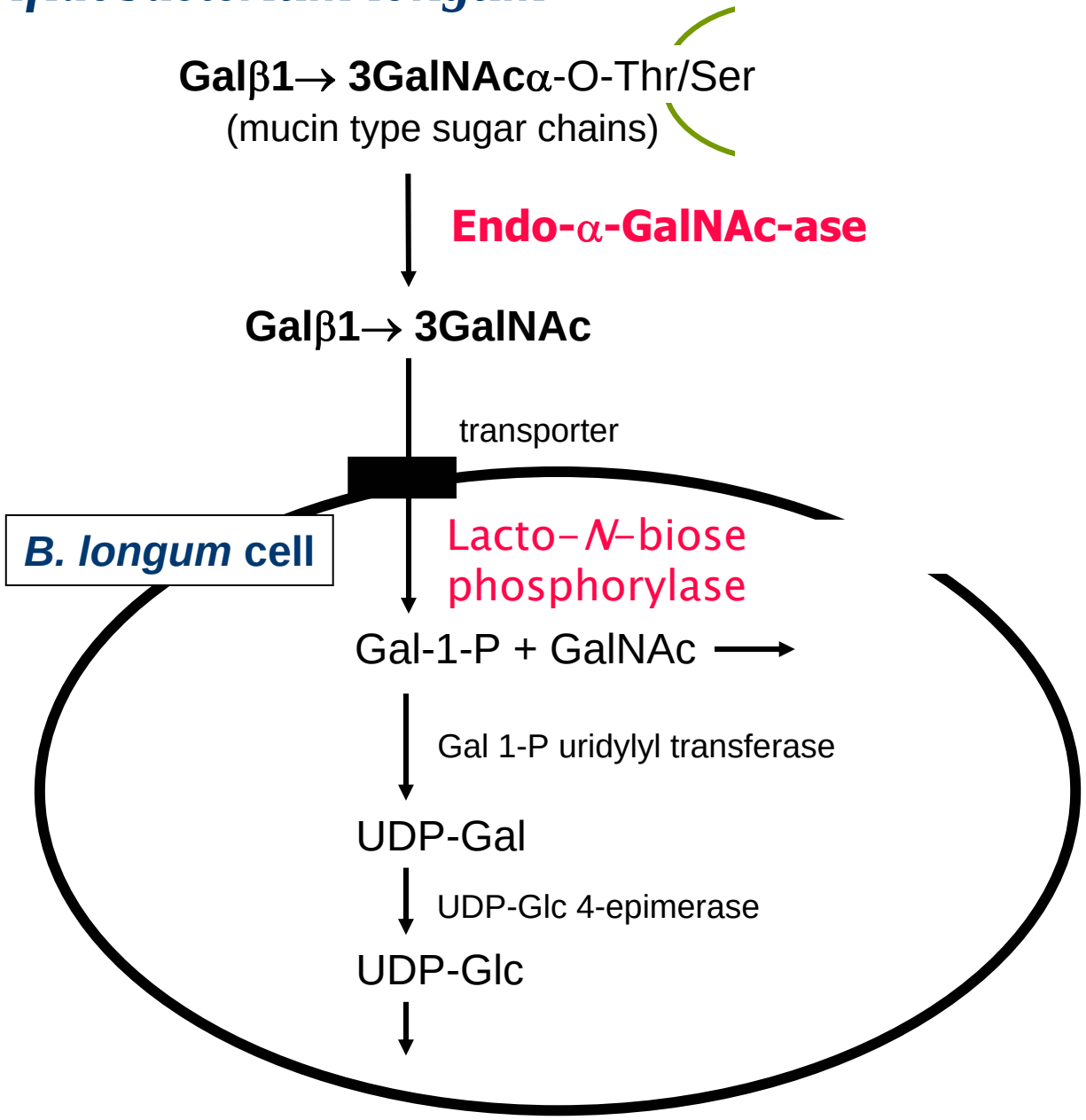
Structure of Endo-α-*N*-acetylgalactosaminidase



Substrate Specificity of *B.longum* Endo-α-*N*-acetylgalactosaminidase

Substrate	Type	Activity
GalNAc α -*p*NP	Tn antigen	−
Gal β 1-3GalNAc α -*p*NP	Core 1 (T antigen)	+++
Gal β 1-3(GlcNAc β 1-6)GalNAc α -*p*NP	Core 2	−
GlcNAc β 1-3GalNAc α -*p*NP	Core 3	+
GlcNAc β 1-3(GlcNAc β 1-6)GalNAc α -*p*NP	Core 4	−
GalNAc α 1-3GalNAc α -*p*NP	Core 5	−
GlcNAc β 1-6GalNAc α -*p*NP	Core 6	−
GalNAc α 1-6GalNAc α -*p*NP	Core 7	−
Gal α 1-3GalNAc α -*p*NP	Core 8	−
Glc β 1-3GalNAc α -*p*NP		++
Glc β 1-6GalNAc α -*p*NP		−
GalNAc β 1-3GalNAc α -*p*NP		++
Gal β 1-6GlcNAc α -*p*NP		−
Gal α 1-6GalNAc α -*p*NP		−
Gal β 1-3GlcNAc α -*p*NP		±
Gal β 1-3GalNAc-SPh		±

Putative Metabolic Pathway of Mucin Sugar Chain on *Bifidobacterium longum*



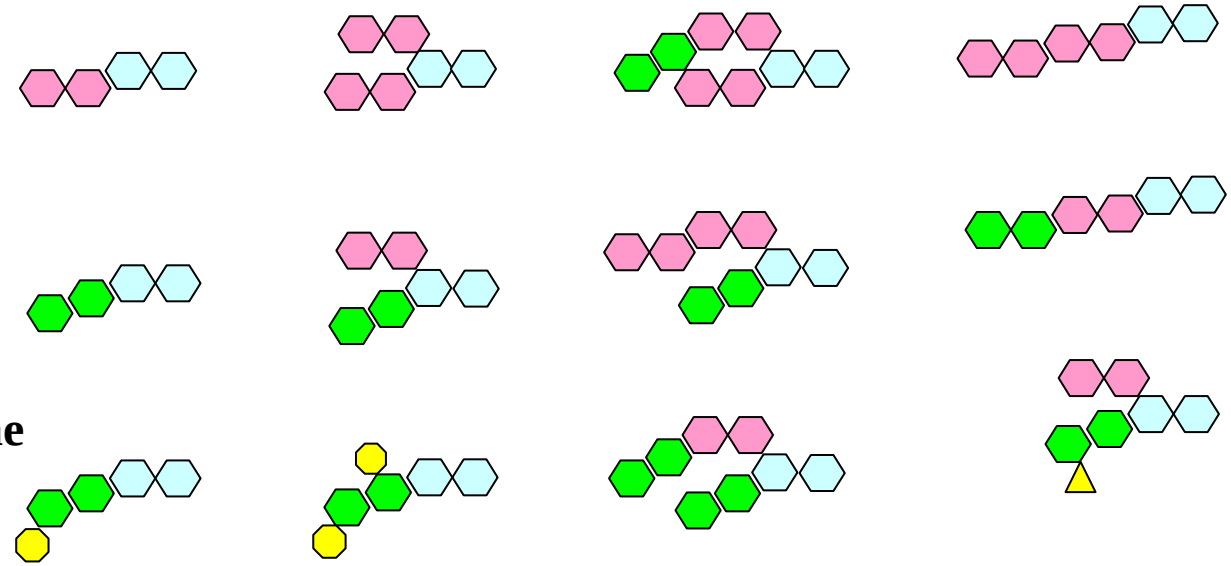
Model Structure of Human Milk Oligosaccharides

Disaccharide composition

Lactose

Lacto-*N*-biose

N-Acetyllactosamine

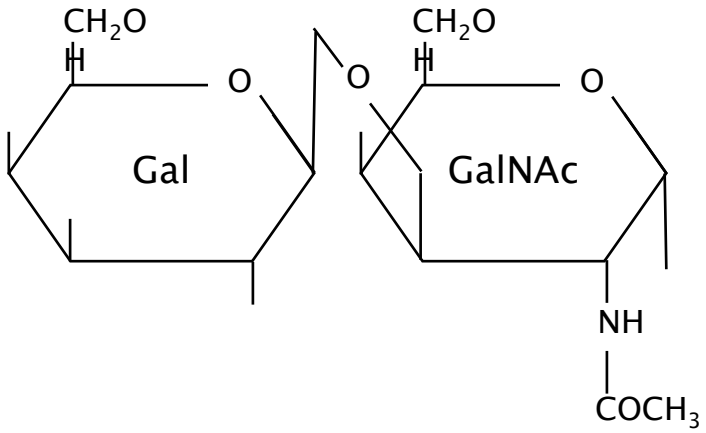


Modified sugar

Sialic acid

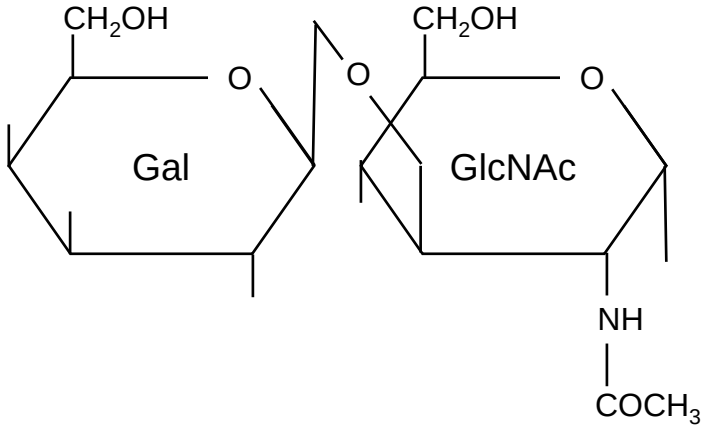
L-Fucose

Similarity of Core Structure of Mucin Sugar Chain with Core Structure of Human Milk Oligosaccharides



Core structure of sugar chain of Mucin glycoprotein

Galβ1-3GalNAc



Core structure of human milk oligosaccharides

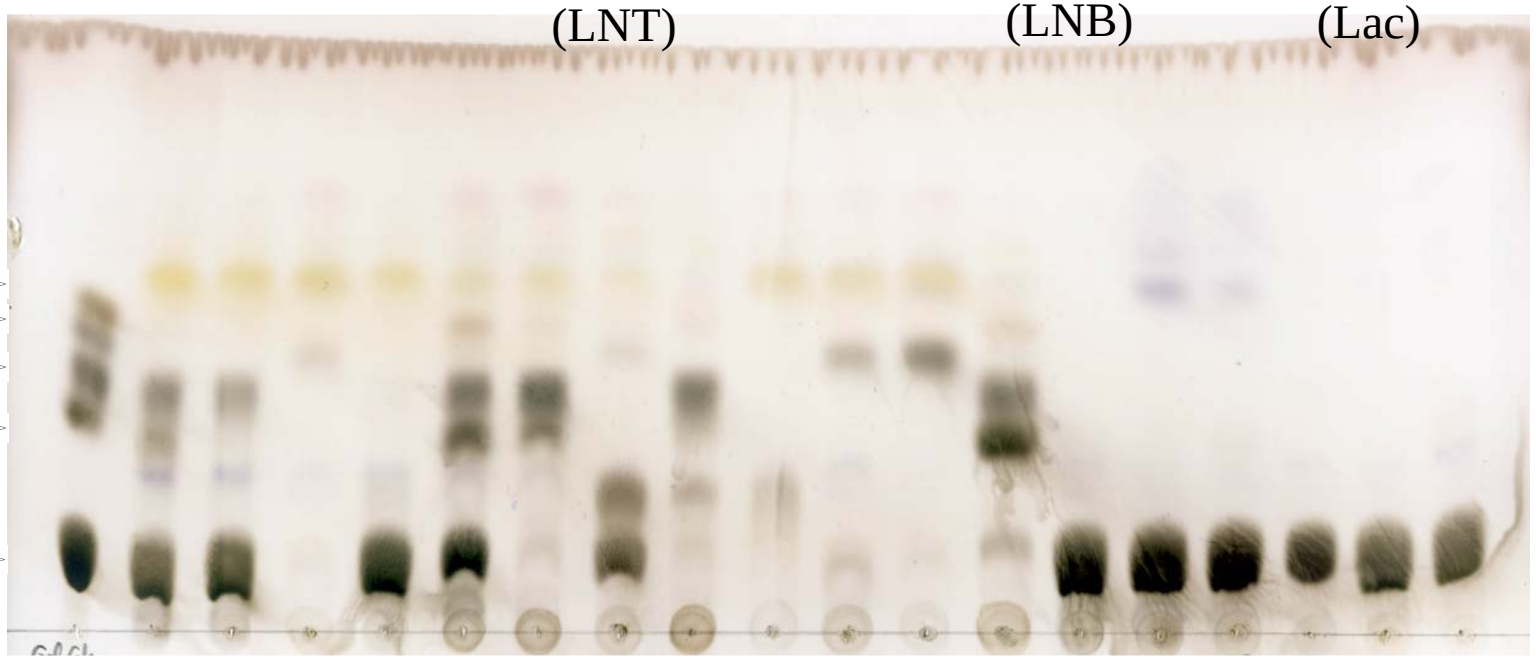
Galβ1-3GlcNAc

Lacto-*N*-biose

Screening of Lacto-*N*-biosidase Activity in Cell Suspension of Various Bifidobacteria and Other Lactic Acid Bacteria

Galβ1-3GlcNAcβ1-3Galβ1-4Glc → Galβ1-3GlcNAc + Galβ1-4Glc

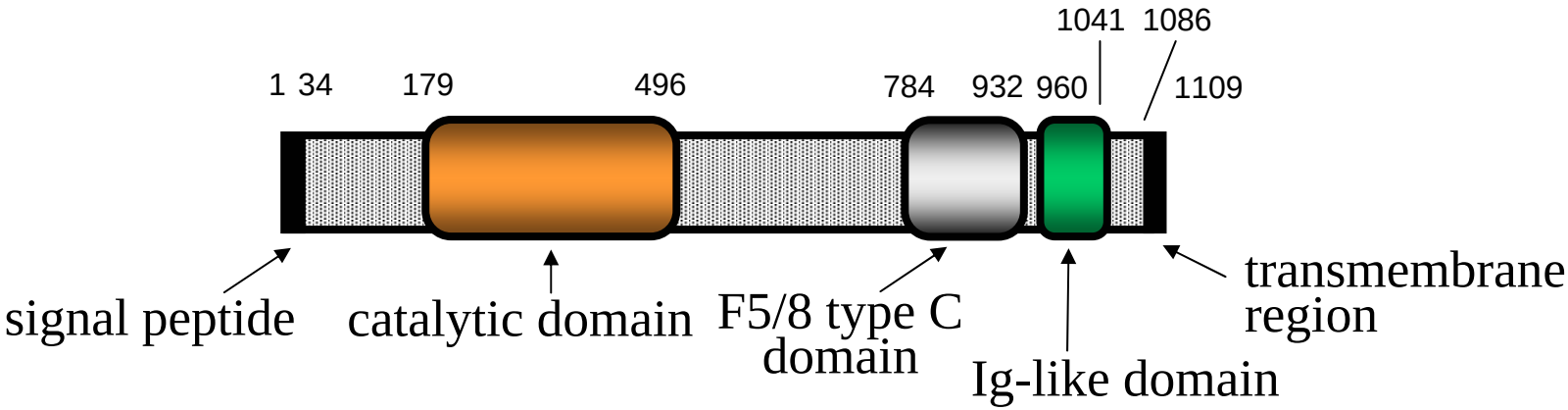
(LNT) (LNB) (Lac)



B. breve *clb*
B. breve 203
B. breve JCM 119
B. pseudolongum JCM 1205
B. longum 33R
B. longum JCM 1217
B. longum NCC 2705
B. longum JCM 7054
B. infantis 1222
B. bifidum 7004
B. bifidum JCM 1254
B. bifidum ATCC 29251
L. lactis JCM 1158
Lb. jonsonii JCM 8794
Lb. gasseri JCM 1130
Lb. rhamnosus ATCC 7469
Lb. casei sp.
Lb. GG

Structure of Lacto-*N*-biosidase from *B.bifidum* JCM1254

LNBase (1112 Amino Acids) **Gal β 1-3GlcNAc** β 1-3Gal β 1-4Glc



Schematic Diagram of Structures of Two Sialidases

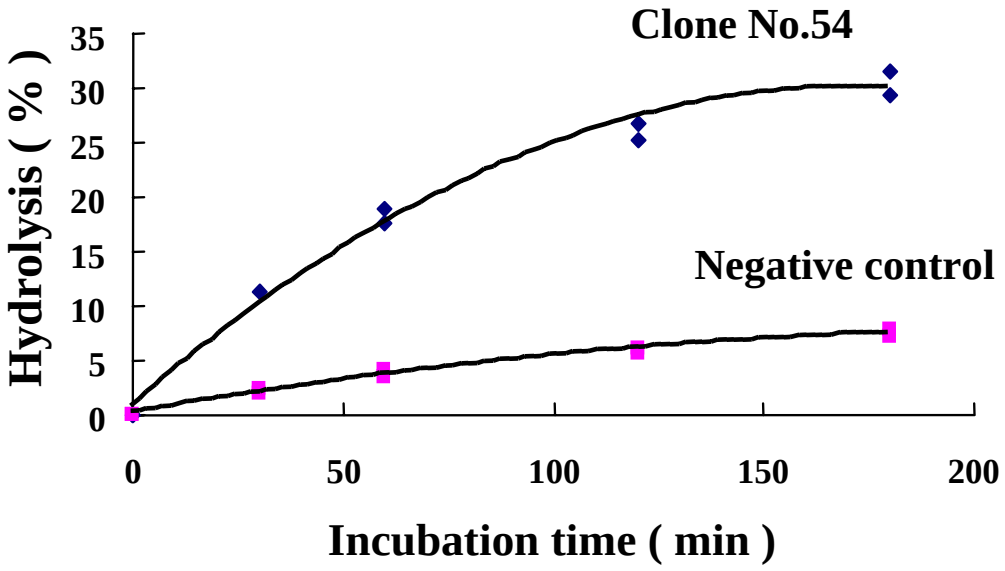
SiaBl

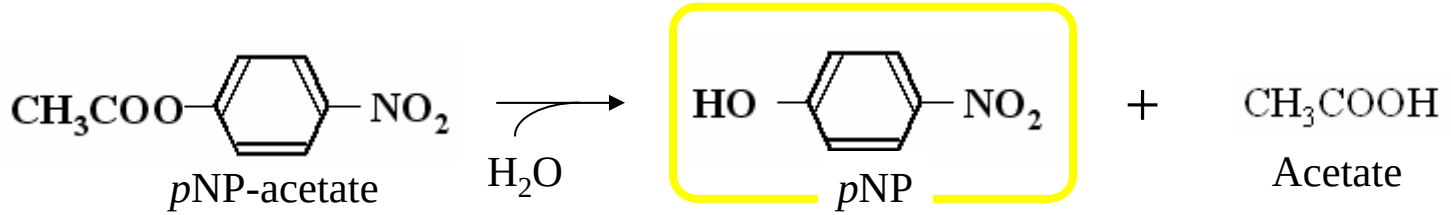
1795 AA

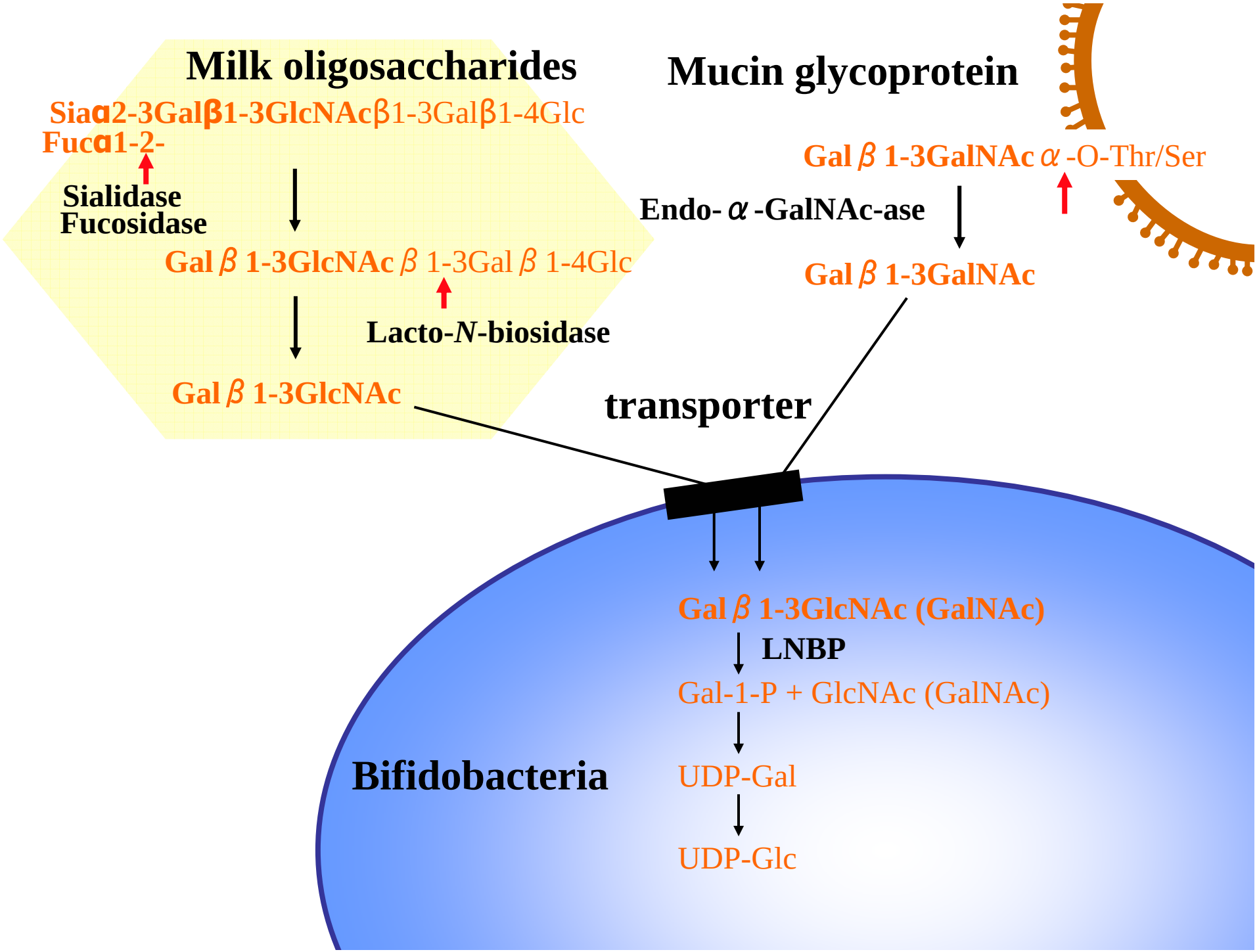
chor

SiaBl

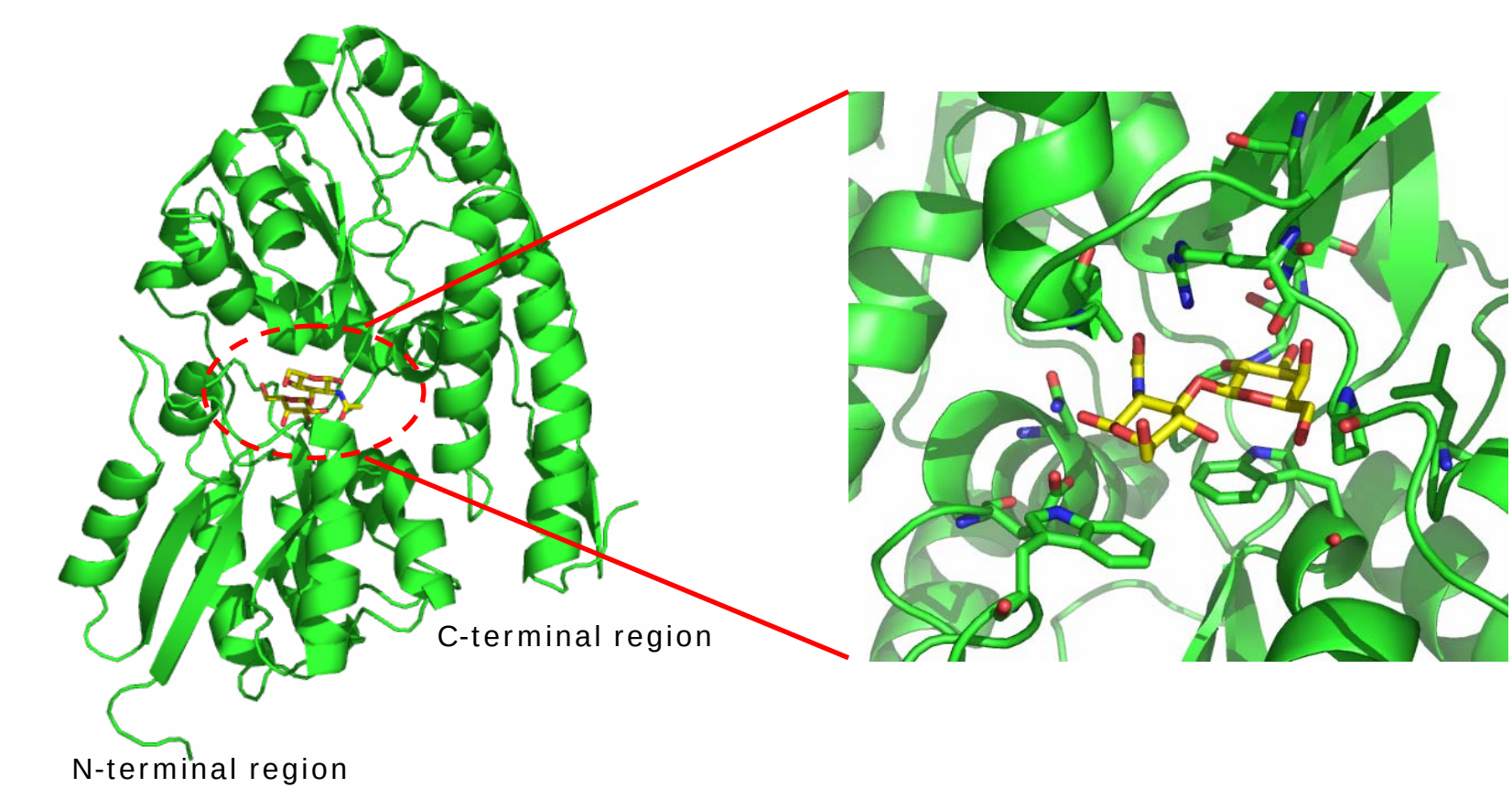
Esterase Activity of SiaBb1-Expressed Cell






Milk oligosaccharides
Siaα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc
Fucα1-2-
Sialidase
Fucosidase
Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc
Lacto-N-biosidase
Gal β 1-3GlcNAc
Mucin glycoprotein
Gal β 1-3GalNAc α -O-Thr/Ser
Endo- α -GalNAc-ase
Gal β 1-3GalNAc
transporter
Gal β 1-3GlcNAc (GalNAc)
LNBP
Gal-1-P + GlcNAc (GalNAc)
UDP-Gal
UDP-Glc
Bifidobacteria

Crystal Structure of Solute Binding Protein of ABC-Transporter from *B.longum* Specific for Lacto-*N*-biose

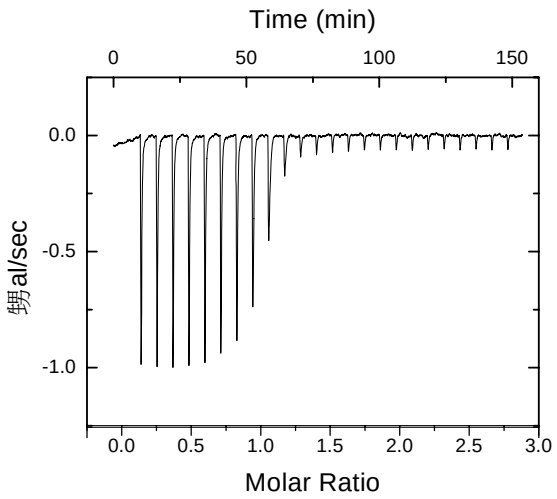


Solute binding protein of ABC transporter for sugars
1317 bp, 438 amino acids

Functional Analysis of Solute Binding Protein

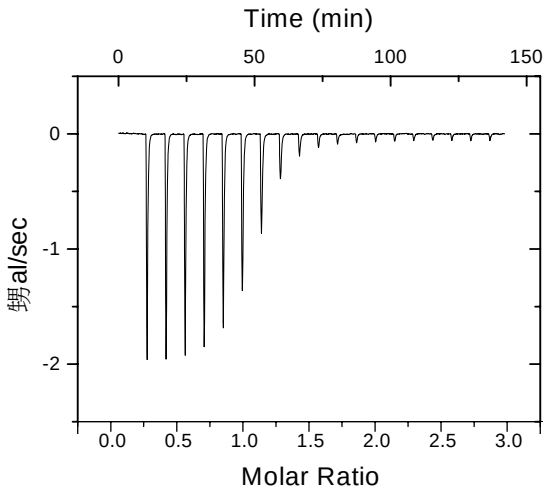
Isothermal titration calorimetry analysis

Gal（β1-3）GalNAc



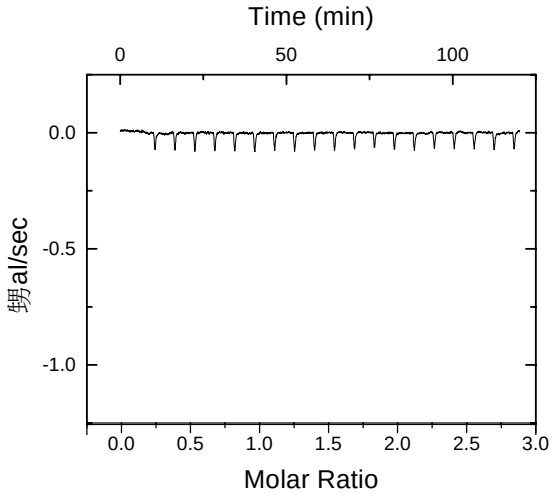
Protein; 5 μM
Ligand; 100 μM

Gal（β1-3）GlcNAc

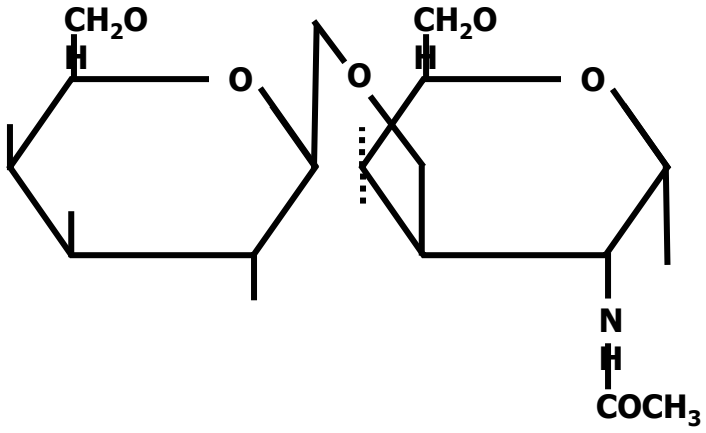


Protein; 10 μM
Ligand; 200 μM

Gal（β1-4）GlcNAc



Protein; 10 μM
Ligand; 200 μM



Galactose β1-3 *N*-Acetylaminosugar

Effect of Addition of Various Sugars on Growth of Bifidobacteria

Sugar	Structure	Relative growth
None		35
Non-prebiotic sugars		
Glucose	Glc	100
Lactose	Gal-β1,4-Glc	101
Prebiotic oligosaccharides		
Fructo-oligosaccharides	Glc-β1,2-Fru(-β2,1-Fru)n	68
Isomalto-oligosaccharides	Glc(-α1,6-Glc)n Glc-α1,6-Glc-α1,4-Glc	84
Gentio-oligosaccharides	Glc(-β1,6-Glc)n	73
Xylo-oligosaccharides	Xyl(-β1,4-Xyl)n	36
Raffinose	Gal-α1,6-Glc-β1,2-Fru	99
Lacto-*N* -biose	**Gal-β1,3-GlcNAc**	**85**

Effect of Addition of Lacto-*N*-biose to Culture Medium on Growth of Various Bifidobacteria

Bacteria (strain)	Growth (LNB / None) 24 h	48 h
Bifidobacteria		
B.adolescentis **(JCM1275)**	+	+
B.angulatum **(JCM7096)**	-	-
B.animalis lactis **(JCM10602)**	±	±
B.bifidum **(JCM1254)**	+	++
B.bifidum **(JCM1255)**	-	±
B.bifidum **(JCM7004)**	+	+
B.breve **(JCM1192)**	+	+
B.catenulatum **(JCM1194)**	+	+
B.gallicum **(JCM8224)**	±	±
B.longum **(JCM1217)**	++	++
B.longum **(JCM1222)**	+	++
B.longum **(JCM7054)**	++	++
B.pseudocatenulatum **(JCM1200)**	+	+
B.pseudolongum pseudolongum **(JCM1205)**	±	±
B.scardovii **(JCM12489)**	-	±

++	→	Highly effective	**(LNB/None = 2.00 ~ 2.99)**
+	→	Effective	**(LNB/None = 1.20 ~ 1.99)**
±	→	No effect	**(LNB/None = 0.80 ~ 1.19)**
-	→	Inhibit	**(LNB/None = ~ 0.79)**

Effect of Addition of Lacto-*N*-biose to Culture Medium on Growth of Various Intestinal Microorganisms

Bacteria (strain)	Growth (LNB / None) 24 h	48 h
Bacteroides		
B.ovatus **(JCM5824)**	+	+
B.thetaiotaomicron **(JCM5827)**	+	±
Clostridia		
C.celatum **(JCM1394)**		
C.hiranonis **(JCM10541)**	±	±
C.hylemonae **(JCM10539)**	+	+
C.spiroforme **(JCM1432)**		
C.scindens **(JCM6567)**	-	+
Enterococci		
E.pseudoavium **(JCM8732)**	±	±
E.raffinosus **(JCM8733)**	±	+
Eubacteria		
E.limosum **(JCM6421)**	±	±
E.cylindroides **(JCM10261)**		
Propionibacteria		
P.acnes **(JCM6425)**	±	+

++	→	Highly effective	**(LNB/None = 2.00 ~ 2.99)**
+	→	Effective	**(LNB/None = 1.20 ~ 1.99)**
±	→	No effect	**(LNB/None = 0.80 ~ 1.19)**
-	→	Inhibit	**(LNB/None = ~ 0.79)**

Acknowledgement

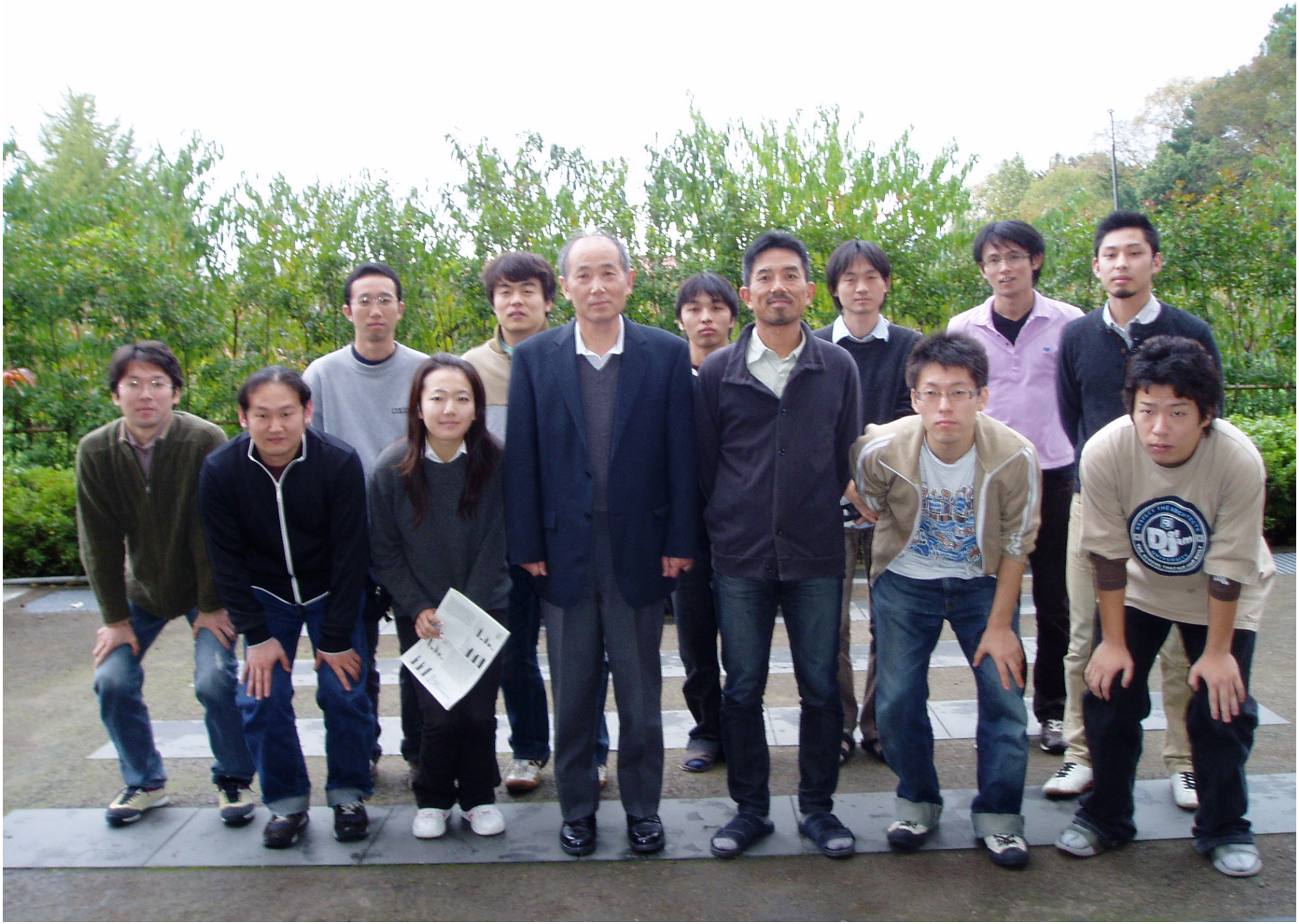
Kyoto University,
Graduate School of Biostudies
Dr.Y.Makimura
Ms.N.Nagamine
Ms.A.Sakuma
Ms.A.Tsuchiya

Shizuoka University,
Faculty of Agriculture
Prof.T.Usui
Dr.T.Murata

High Energy Accelerator Research Organization (KEK)
Dr.S.Wakatsuki
Dr.R.Katoh
Dr.M.Nagae

Ishikawa Prefectural University
Prof.H.Kumagai
Mr.J.Wada

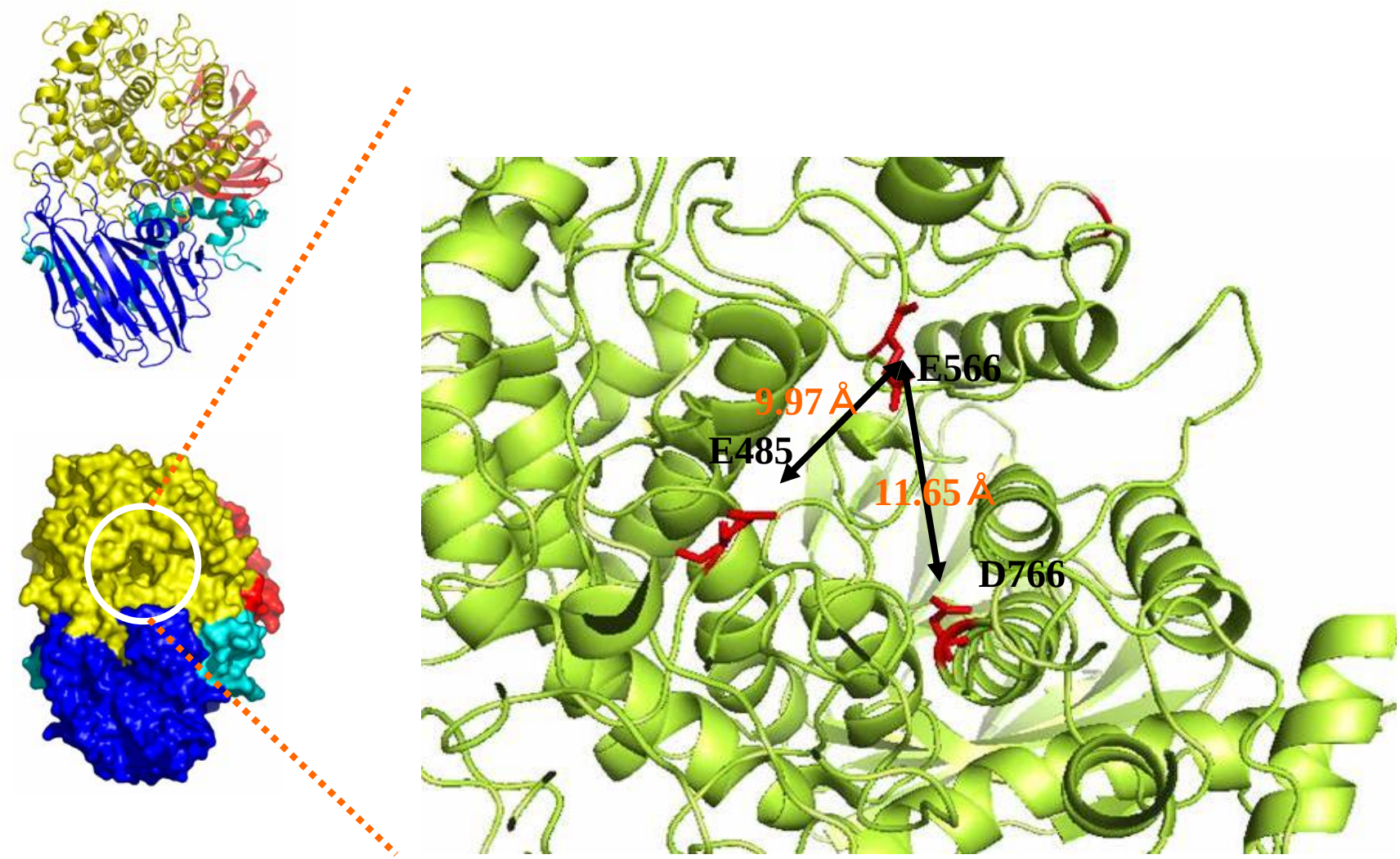
The University of Tokyo,
Department of Biotechnology
R.Suzuki
S.Fushinobu



Thank you for your attentions !

謝謝！

Candidate for Catalytic Residues of 1,2-α-L-Fucosidase



Binding Activity of *Lactobacillus casei* to Various Glycosphingolipids

Glycolipids	Structure	Relative Binding activity
Glucosylceramide	Glc β1-1'Cer	72
Galactosylceramide	Gal β1-1'Cer	46
Lactosylceramide	Gal β1-4Glc β1-1'Cer	89
CTH	Gal α1-4Gal β1-4Glc β1-1'Cer	100
Globoside	GalNAc β1-3Gal α1-4Gal β1-4Glc β1-1'Cer	0
Forssman hapten	GalNAc α1-3GalNAc β1-3Gal α1-4Gal β1-4Glc β1-1'Cer	0
GM3	NeuAc α2-3Gal β1-4Glc β1-1'Cer	0
GM2	GalNAc β1-4(NeuAc α2-3)Gal β1-4Glc β1-1'Cer	0
GM1	Gal β1-3GalNAc β1-4(NeuAc α2-3)Gal β1-4Glc β1-1'Cer	0
GD3	NeuAc α2-8NeuAc α2-3Gal β1-4Glc β1-1'Cer	0
GD1a	NeuAc α2-3Gal β1-3GalNAc β1-4(NeuAc α2-3)Gal β1-4Glc β1-1'Cer	0
GT1b	NeuAc α2-3Gal β1-3GalNAc β1-4Gal β1-4Glc β1-1'Cer 3 NeuAc α2-8NeuAc α2	0
GQ1b	NeuAc α2-8NeuAc α2-3Gal β1-3GalNAc β1-4Gal β1-4Glc β1-1'Cer 3 NeuAc α2-8NeuAc α2	0
GA1	Gal β1-3GalNAc β1-4Gal β1-4Glc β1-1'Cer	94
Lc4	Gal β1-4GlcNAc β1-3Gal β1-4Glc β1-1'Cer	35
SPG	NeuAc α2-3Gal β1-4GlcNAc β1-3Gal β1-4Glc β1-1'Cer	0
Sulfatide	HSO_3-3Gal β1-1'Cer	27

Gal, D-galactose; GalNAc, *N*-acetylgalactosamine; Glc, D-glucose; GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid; Cer, ceramide

Binding Activities of *Lactobacillus* to Various Glycosphingolipids

Glycosphingolipids	Structure	*Lb.casei*	*Lb.reuteri*	*Lb.johnsonii*
Glucosylceramide	Glcβ1-1'Cer	++	-	+
Galactosylceramide	Galβ1-1'Cer	+	+	nd
Lactosylceramide	Galβ1-4Glcβ1-1'Cer	++	+	nd
CTH	Galα1-4Galβ1-4Glcβ1-1'Cer	++	-	-
Isotriaose	Galα1-3Galβ1-4Glcβ1-1'Cer	nd	nd	+
Globoside	GalNAcβ1-3Gal α1-4Galβ1-4Glcβ1-1'Cer	-	-	-
Forssman hapten	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1'Cer	-	-	-
GM3	NeuAcα2-3Galβ1-4Glcβ1-1'Cer	-	-	-
GM2	GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1'Cer	-	-	nd
GM1	Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1'Cer	-	-	nd
GD3	NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ1-1'Cer	-	nd	nd
GD1a	NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1'Cer	-	-	nd
GT1b	NeuAcα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1'Cer 3 NeuAcα2-8NeuAcα2	-	nd	nd
GQ1b	NeuAcα2-8NeuAcα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1'Cer 3 NeuAcα2-8NeuAcα2	-	nd	nd
GA1	**Galβ1-3GalNAcβ1-**4Galβ1-4Glcβ1-1'Cer	++	++	++
Lc4	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1'Cer	+	nd	+
Lactotetraose	Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1'Cer	nd	nd	+
SPG	NeuAc α2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1'Cer	-	nd	-
Sulfatide	HSO3-3Galβ1-1'Cer	+	+	-

Gal, D-galactose; GalNAc, *N*-acetylgalactosamine; Glc, D-glucose; GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid; Cer, ceramide. nd : not done.

乳酸菌による抗体産生増強作用