**α-N-Acetylgalactosaminidase from Infant-associated Bifidobacteria Belonging to Novel Glycoside Hydrolase Family 129 Is Implicated in Alternative Mucin Degradation Pathway**

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**Background:** The degradation pathway of the intestinal mucin by bifidobacteria is poorly understood.

**Results:** A novel α-N-acetylgalactosaminidase, NagBb, was identified from *Bifidobacterium bifidum* JCM 1254.

**Conclusion:** NagBb might be involved in intracellular degradation of Tn antigen (GalNAc1-Ser/Thr).

**Significance:** NagBb represents a novel glycoside hydrolase family 129 in the CAZy database.

Bifidobacteria inhabit the lower intestine of mammals including humans where the mucin gel layer forms a space for commensal bacteria. We previously identified that infant-associated bifidobacteria possess an extracellular membrane-bound endo-α-N-acetylgalactosaminidase (EngBF) that may be involved in degradation and assimilation of mucin-type oligosaccharides. However, EngBF is highly specific for core-1-type O-glycan (Galβ1–3GalNAcα1-Ser/Thr), also called T antigen, which is mainly attached onto gastroduodenal mucins. By contrast, core-3-type O-glycans (GlcNAcβ1–3GalNAcα1-Ser/Thr) are predominantly found on the mucins in the intestines. Here, we identified a novel α-N-acetylgalactosaminidase (NagBb) from *Bifidobacterium bifidum* JCM 1254 that hydrolyzes the Tn antigen (GalNAcα1-Ser/Thr). Sialyl and galactosyl core-3 (Galβ1–3GalNAcβ1–3( Neu5Acα2–6)GalNAcα1-Ser/Thr), a major tetrasaccharide structure on MUC2 mucin primarily secreted from goblet cells in human sigmoid colon, can be serially hydrolyzed into Tn antigen by previously identified bifidobacterial extracellular glycosidases such as α-sialidase (SiaBb2), lacto-N-biosidase (LnbB), β-galactosidase (BbgIII), and β-N-acetylgalactosaminidases (BbhI and BbhII). Because NagBb is an intracellular enzyme without an N-terminal secretion signal sequence, it is likely involved in intracellular degradation and assimilation of Tn antigen-containing polypeptides, which might be incorporated through unknown transporters. Thus, bifidobacteria possess two distinct pathways for assimilation of O-glycans on gastroduodenal and intestinal mucins. NagBb homologs are conserved in infant-associated bifidobacteria, suggesting a significant role for their adaptation within the infant gut, and they were found to form a new glycoside hydrolase family 129.

Bifidobacteria naturally occur in human intestines and are predominant in those of newborn infants. They give various beneficial effects to the host such as stimulation of the immune response, prevention of the growth of pathogenic enterobacteria, and suppression of inflammatory and allergic responses; therefore, they are recognized as probiotics (1). Because they mainly reside in the lower intestines where the sugars are highly limited, they possess various glycosidases to hydrolyze indigestible oligosaccharides and glycoconjugates. Endo-α-N-acetylgalactosaminidase (EC 3.2.1.97) acts on the O-glycosidic linkage of GalNAcα1-O-Ser/Thr in the core structures of mucin-type oligosaccharides to release oligosaccharides from glycoproteins. Several pathogenic and non-pathogenic bacteria possess enzymes with this type of activity (2–5). Previously, we identified the gene *engBF* encoding endo-α-N-acetylgalactosaminidase from *Bifidobacterium longum* subsp. *longum* JCM 1217 and subsequently found that the homologous genes are conserved in various infant-associated bifidobacteria (6). The EngBF and its homologs in bacteria were classified into a new glycoside hydrolase (GH) family, 101, in the CAZY database (7). EngBF showed relatively strict specificity toward the core-1-type O-glycan (Galβ1–3GalNAcα1-Ser/Thr) compared with the other GH101 enzymes (8–10). The β1,3-linked Gal in the core-1 structure is known to be highly resistant to common β-galactosidases from general intestinal bacteria. However,
bifidobacteria can release the core-1 disaccharide from mucin glycoproteins through the action of the extracellular membrane-bound EngBF, incorporate it into the cytosol through a specific ATP-binding cassette-type transporter on the plasma membrane (11–13), and finally phosphorylate it by a specific phosphorylase in the cytosol (14). These findings suggest that bifidobacteria selectively grow in the intestines by assimilating mucin-type oligosaccharides utilizing a unique degradation pathway. In fact, the isomeric disaccharide Galβ1–3GlcNAc, a building unit of the type-1 chain in human milk oligosaccharides, which can be recognized by the same transporter and phosphorylase, specifically enhanced the growth of bifidobacteria in vitro (15).

Mucins are secreted from specific mucin-producing epithelial cells in mucosal tissues and form a gel layer to protect the epithelia from pathogenic bacteria and viruses, digestive enzymes, and acidic digestive juice. In the gastrointestinal tract, the stomach and duodenum mainly secrete MUC5AC and MUC6, whereas the small and large intestines secrete MUC2, MUC3, and MUC4. It has been reported that MUC2 is the major mucin secreted from goblet cells in human colon (16) and that a swollen layer of MUC2 supplies the space for commensal bacteria (17, 18). The structures of O-glycans on mucin are quite different depending on the source of mucosal tissues. Gastric and duodenal mucins generally contain the core-1 and the core-2 (Galβ1–3(GlcNAcβ1–6)GalNAcα1–Ser/Thr) structures. Recent studies revealed that MUC2 in the sigmoid colon mainly contains the core-3 structure (GlcNAcβ1–3GalNAcα1–Ser/Thr) (19). As mentioned above, EngBF acts on the core-1 structure with a 300 times higher rate than core-3; thus, we speculate that EngBF is involved in degradation of gastroduodenal mucins, which are released and transported from the stomach and duodenum.

To better understand the degradation and assimilation of the intestinal mucin by bifidobacteria, we searched the genome of Bifidobacterium bifidum JCM 1254 using the sequence of EngBF as a query. As a result, we found an uncharacterized gene encoding a putative intracellular protein showing very slight similarity to EngBF. Heterologous expression of this protein revealed that it showed glycosidase activity with substrate specificity distinct from EngBF. Interestingly, its putative homologs were conserved in infant-associated bifidobacteria, and they were found to form a novel GH family.

**EXPERIMENTAL PROCEDURES**

**Culture and Genome Sequence of B. bifidum JCM 1254—**B. bifidum JCM 1254 was cultured on Gifu Anaerobic Medium (GAM) broth (Nissui Pharmaceutical, Japan) for 16 h at 37 °C under anaerobic conditions using Anaeropack-Anaero (Mitsubishi Gas Chemical, Japan). Genomic DNA was extracted from bacterial cells using standard methods. Draft sequencing of the genome of B. bifidum JCM 1254 was performed using a Genome Sequencer 20 System (Roche Applied Science). The details will be reported elsewhere.

**Expression and Purification of Recombinant NagBb—**The full-length DNA fragment encoding the 634-amino acid polypeptide of NagBb was amplified by high fidelity PCR using PrimeSTAR HS DNA polymerase (Takara Bio, Japan), genomic DNA from B. bifidum JCM 1254 as a template, and the following primers: forward, 5′-ggatccgtagatgcaaccctgGtcc; and reverse, 5′-actcagatgagacgtcgtgct. The PCR product was digested with EcoRI and XhoI and ligated into the corresponding sites of pET23b (+) (Novagen, Germany) to express C-terminal His6-tagged protein. The Escherichia coli BL21(DE3)ΔlacZ strain that lacks β-galactosidase activity was transformed with the constructed plasmid and cultured in Luria-Bertani liquid medium containing 100 μg/ml ampicillin at 25 °C. When the optical density at 600 nm (A600) reached 0.5, isopropyl β-D-1-thiogalactopyranoside was added to the culture to a final concentration of 0.5 mM. After additional culture for 5 h, cells were harvested and lysed in BugBuster Protein Extraction Reagent (Novagen) to obtain cell-free extract. His6-tagged NagBb was purified by a HisTrap HP column (1 ml; GE Healthcare) followed by Superdex 200 10/300 GL (GE Healthcare) gel filtration in line with the ÄKTA Explorer (GE Healthcare). Active fractions were collected, concentrated, and desalted using an Amicon Ultracel-10K (EMD Millipore, Germany). Protein concentrations were determined by the BCA protein assay reagent (Thermo Scientific-Pierce) using bovine serum albumin as a standard.

**Preparation of Cell-free Extract of B. bifidum JCM 1254—**B. bifidum JCM 1254 was precultured for 20 h at 37 °C under anaerobic conditions using Anaeropack-Anaero in 10 ml of basal medium (16 g/liter nutrient broth “Eiken” (Eiken Chemical, Japan), 5 g/liter yeast extract, 3 g/liter dipotassium hydrogen phosphate, 1 g/liter polyoxyethylene sorbitan monooleate (Tween 80), 10 g/liter sodium L-asorbate, and 0.5 g/liter L-cysteine hydrochloride) supplemented with 10 g/liter Glc. A suitable amount of precultured cells were inoculated in 10 ml of basal medium supplemented with 2.5 g/liter concentration of each sugar (Glc, GlcNAc, GalNAc, Galβ1–3GlcNAc (20), or Galβ1–3GalNAc (21)) to make its A600 0.050. After cultivation for 24 h under the same condition as for the preculture, cells were harvested and suspended in BugBuster Master Mix solution (Novagen) supplemented with 0.1 mg/ml N-acetylglucosaminidase SG (Seikagaku Biobusiness, Japan), and the suspension was incubated for 16 h at 20 °C to lyse the cells. The cell-free extract was obtained by centrifugation (20,000 × g for 10 min).

**Enzyme Assay for NagBb—**The NagBb assay was carried out using para-nitrophenyl (pNP)-glycosides in 50 mM sodium acetate buffer (pH 5.0) at 37 °C, and the released para-nitrophenol was quantified by measuring the absorbance at 405 nm. pNP-di- or trisaccharides with various core structures of mucin-type oligosaccharides were chemically synthesized (22); the other pNP-glycosides including GalNAcα1–pNP were from Sigma-Aldrich. 4,6-Dimethoxy-1,3,5-triazin-2-yl α-N-acetylgalactosaminide (GalNAcα1–DMT) was synthesized by stirring 1-(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (Tokyo Chemical Industry, Japan), 2,6-lutidine (Nacalai Tesque, Japan), and GalNAc in water at room temperature as described (23, 24) and then purified by silica gel 60 (0.063–0.200 mm) column chromatography (1 × 20 cm) with ethyl acetate/methanol (7:1, v/v) as an eluent. Hydrolyses of substrates were then monitored by silica gel TLC (Merck 5553, Germany) with 1-butanol/acidic acid/water (2:1:1, v/v/v) as a developing solvent, and the released sugars were visualized using
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TABLE 1

| Sugars used for cultivation | Cell growth (A_{600})a | para-nitrophenol releasing activityb |
|-----------------------------|------------------------|-------------------------------------|
| Glc                         | 0.522 ± 0.172          | 0.434 ± 0.088                       |
| GlcNAc                      | 0.854 ± 0.079          | 0.332 ± 0.018                       |
| GalNAc                      | 0.517 ± 0.054          | 0.974 ± 0.032                       |
| Galβ1–3GlcNAc               | 0.787 ± 0.069          | 0.438 ± 0.066                       |
| Galβ1–3GalNAc               | 0.742 ± 0.070          | 0.832 ± 0.034                       |

a Data represent mean ± S.D. of cell lysates obtained by three independent cultivations.

b The cells corresponding to 1 ml of A_{600} = 1.0 culture were lysed in 50 μl of lysis solution.

The activity of NagBb was assayed using pNP-monosaccharides as substrates revealed that the recombinant protein exhibited exo-α-N-acetylgalactosaminidase activity toward GalNAcα1-pNP (specific activity, 456 milliunits/mg of protein) but did not show any other exoglycosidase activities for the following substrates: GalNAcβ1-pNP, GlcNAcα1-pNP, GlcNAcβ1-pNP, Galα1-pNP, Galβ1-pNP, Glcα1-pNP, Glcβ1-pNP, Fucα1-pNP, Fucβ1-pNP, Manα1-pNP, Manβ1-pNP, Araα1-pNP, Araβ1-pNP, Xylα1-pNP, and Xylβ1-pNP. Also the enzyme did not hydrolyze the following pNP-disaccharides substrates: Galα1–4Glcβ1-pNP, Glcβ1–4Glcβ1-pNP, GlcNAcβ1–4GlcNAcβ1-pNP, Galβ1–3GlcNAcβ1-pNP, and Galβ1–3GalNAcβ1-pNP. However, the enzyme weakly hydrolyzed Galβ1–3GalNAcα1-pNP, a standard substrate for EngBF (specific activity, 17.1 milliunits/mg of protein). These results suggest that this enzyme is a novel α-N-acetylgalactosaminidase that shows a major exo activity with a minor endo activity. Thus, we named this gene nagBb (DDBJ accession number AB636148).

To confirm the expression of NagBb in the bifidobacterial cells, we incubated GalNAcα1-pNP with the cell-free extracts of B. bifidum JCM 1254 cultured in the presence of several types of sugars. The activities were detected in all extracts; however, the activities were ~2-fold higher in the lysates from the cells cultured in the presence of GalNAc or Galβ1–3GalNAc than in the absence of GalNAc (Table 1). Because EngBF (8) and GH36 α-galactosidase MelA/Aga2 (27, 28) never act on GalNAcα1-pNP, this result suggests that the nagBb gene is constitutively expressed and that its expression is enhanced in the presence of GalNAc. No activity was detected in the culture supernatants and the non-disrupted cell suspensions, indicating that NagBb is expressed intracellularly as predicted by its amino acid sequence.

Substrate Specificity of NagBb—To characterize the detailed substrate specificity of NagBb, we incubated the recombinant enzyme expressed in E. coli with various natural substrates containing α-linked GalNAc and analyzed the reaction mixtures by TLC (Fig. 1). NagBb completely hydrolyzed GalNAcα1-Ser, the minimum structure of the Tn antigen. The enzyme acted very weakly on GalNAcα1-UDP and GalNAcα1–3Galβ1–4Glc but not at all on Neu5Acα2–6GalNAcα1–Ser (sialyl Tn antigen) and GalNAcα1–3(Fucα1–2)Gal (blood group A trisaccharide). The K_m and k_cat values for GalNAcα1-Ser were estimated to be 2.2 mM and 47.6 s⁻¹, respectively, but those for other substrates could not be determined accurately due to high K_m values and low solubility. Therefore, only k_cat/K_m values were estimated (Table 2).

Next, we investigated endo-type activities of NagBb using synthetic pNP-substrates with various core structures of mucin-type O-glycans. Hydrolyses were monitored by measuring released para-nitrophenol (Table 2) and also by detecting released oligosaccharides (Fig. 1). As mentioned above, NagBb hydrolyzed core-1 structure (Galβ1–3GalNAcα1-pNP) 27 times slower than GalNAcα1-pNP. In addition, NagBb very slowly hydrolyzed core-3 (GlcNAcβ1–3GalNAcα1-pNP) and core-8 (Galα1–3GalNAcα1-pNP) type structures but not other core structures. Core-7 (GalNAcα1–6GalNAcα1-pNP) may be sequentially hydrolyzed from the non-reducing termini...
nus by exo-α-N-acetylgalactosaminidase activity because the disaccharide was never detected. From these results, we concluded that the natural substrate for NagBb occurring in the intestines is essentially the Tn antigen.

General Properties of NagBb—General enzyme properties of NagBb were determined using GalNAc1-pNP as a substrate. The enzyme was stable over the pH range of 3.0–11.0 and most active at pH 5.0 ± 0.5 (supplemental Fig. S3, A and B). The optimal temperature for activity was found to be 55 °C, and the enzyme was stable up to 45 °C (supplemental Fig. S3, C and D). These pH and thermal profiles of NagBb provide sufficient enzyme property for functioning in human intestines. The enzyme was stable over the pH range of 3.0–11.0 and most active at pH 5.0 ± 0.5 (supplemental Fig. S3, A and B). The optimal temperature for activity was found to be 55 °C, and the enzyme was stable up to 45 °C (supplemental Fig. S3, C and D). 

Catalytic Mechanism and Critical Residues of NagBb—To determine the stereochemical course of the hydrolysis catalyzed by NagBb, GalNAc1-pNP was incubated with NagBb, and the anomeric configuration of the released GalNAc was analyzed by normal-phase HPLC (Fig. 2). Generally, an α-anomeric sugar is eluted from a normal-phase column slightly faster than the corresponding β-anomer (30, 31). After a 2-min

![Figure 1. Substrate specificity of NagBb. Substrates (250 μM for pNP-glycosides; 3 mM for the others) were incubated with recombinant NagBb (14 ng/μl for pNP-glycosides; 50 ng/μl for the others) and analyzed by TLC. Gn and GNB, standard GalNAc and Galβ1–3GalNAc; Tn, GalNAcα1–Ser; sTn, Neu5Acα2–6GalNAcα1–Ser; GDP-Gn, GalNAcα1–UDP; A tri, GalNAcα1–3(Fucα1–2)Gal; Gn-Lac, GalNAcα1–3Galβ1–4Glc; Core-1–Core-8, pNP derivatives of each core glycan; Gn-pNP, GalNAcα1–pNP. Plus and minus, presence and absence of NagBb; asterisk, contaminant in the core-4-pNP sample.](image1)

**TABLE 2**

Kinetic parameters of NagBb from *B. bifidum* JCM 1254

| Substrate                     | Alternative name | Hydrolysis | para-nitrophenol releasing activity | K<sub>m</sub> | k<sub>cat</sub> | k<sub>cat</sub>/K<sub>m</sub> |
|-------------------------------|-----------------|------------|-----------------------------------|------------|-------------|-----------------|
| GalNAcα1-Ser                  | Tn antigen      | +          | 2.2 ± 0.11                        | 47.6 ± 1.66| 21.6        |
| Neu5Acα1-6GalNAcα1-Ser        | Sialyl Tn antigen| −          | ND                               | ND         | 0.03        |
| GalNAcα1–3Galβ1–4Glc          | +               | ND         | ND                               | ND         | 2.35        |
| GalNAcα1–3(Fucα1–2)Gal        | A trisaccharide | −          | ND                               | ND         | 0.02        |
| GalNAcα1-UDP                  |                | ND         | ND                               | ND         | 0.08        |
| GalNAcα1-pNP                  |                | 456 ± 5.5  | ND                               | ND         | 0.03        |
| GalNAcα1-DMT                  |                | 17.1 ± 0.79| ND                               | ND         | 0.08        |
| Galβ1–3(GalNAcβ1–6)GalNAcα1-pNP| Core-2        | 5.8 ± 0.40 | ND                               | ND         | 0.03        |
| GlcNAcβ1–3GalNAcα1-pNP        | Core-3          | −          | ND                               | ND         | 0.08        |
| GlcNAcβ1–3(GlcNAcβ1–6)GalNAcα1-pNP| Core-4        | −          | ND                               | ND         | 0.03        |
| GalNAcα1–3GalNAcα1-pNP        | Core-5          | −          | ND                               | ND         | 0.03        |
| GlcNAcβ1–6GalNAcα1-pNP        | Core-6          | −          | ND                               | ND         | 0.03        |
| GalNAcα1–6GalNAcα1-pNP        | Core-7          | +          | 0.6 ± 0.19                       | ND         | <0.01       |
| Galα1–3GalNAcα1-pNP           | Core-8          | +          | ND                               | ND         | <0.01       |

* Kinetic parameters were determined by quantifying the released GalNAc using an HPLC system. Data represent mean ± S.D. (n = 3).

* Not determined.

* Sequentially hydrolyzed from non-reducing terminus by exo activity.

![Figure 2. Stereochemical analysis of hydrolysis catalyzed by NagBb. GalNAcα1-pNP (2 mM) was incubated with NagBb in 20 mM sodium acetate buffer (pH 5.0) at 37 °C for the indicated time period. The reaction mixtures were immediately analyzed by normal-phase HPLC.](image2)
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enzyme reaction, almost exclusively GalNAccαOH was detected (α/β ratio, 94:6). Then the ratio of the α- and β-anomers of GalNAc gradually changed to reach an equilibrium (α/β ratio, 55:45) due to the slow mutarotation of GalNAccαOH. This data confirmed that NagBb catalyzes the hydrolysis reaction via the retaining mechanism.

We previously identified the catalytic residues of EngBF by docking analysis of its three-dimensional structure with Galβ1–3GalNAc (29). Alignment of NagBb and EngBF revealed that several critical residues are aligned; i.e. Asp-435 in NagBb corresponds to the catalytic nucleophile Asp-789 in EngBF, and Asp-330 in NagBb corresponds to “fixer” Asp-682, the third essential residue in EngBF (supplemental Fig. S1). Asp-682 in EngBF is an important residue forming hydrogen bonds with O4 and O6 of GalNAc and O6 of Gal. However, the catalytic acid/base residue corresponding to Glu-822 in EngBF revealed that several critical residues are aligned:

Transglycosylation Reaction—Some of the GH101 enzymes including EngBF catalyze a transglycosylation reaction in which the released Galβ1–3GalNAc is transferred to the hydroxyl group of a suitable acceptor via the same anomeric linkage because they are retaining glycosidases (5, 34, 35). To test whether NagBb catalyzes a transglycosylation reaction, we incubated 3 mM GalNAcc1-pNP and 35 ng/μl NagBb in the presence of 100 mM Ser as an acceptor in 50 mM sodium acetate buffer (pH 5.0) at 37 °C. The reaction mixture was analyzed by the Prominence amino acid analyzer equipped with a post-column fluorescence labeling system using o-phthalaldehyde and a Shim-pack Amino-Na column. A, heat-inactivated enzyme; B, active enzyme; C, standard GalNAcc1-pSer.

Phylogenetic Analysis of NagBb and Its Homologs—Database searches revealed that NagBb homologs are present in the genome of several Bifidobacterium species such as another strain of B. bifidum, PRL2010 (36); B. longum subsp. longum NCC2705 (37); B. longum subsp. infantis ATCC 15697 (38); and Bifidobacterium breve DSM20213, which are frequently found species in the intestines of newborn infants. These homologs are similar in size (627–634 amino acids), and all are predicted to be intracellular soluble enzymes. Three species, B. longum subsp. longum, B. longum subsp. infantis, and B. breve, possess highly similar homologs (96–99% identities in amino acid sequences), and NagBb showed 76–78% identities with these homologs. Possible NagBb homologs were found in several other enterobacteria such as Clostridium scindens and Eubacterium biiforme (57 and 44% identities, respectively) but not in common species such as Bacteroides fragilis, Bacteroides thetaiotaomicron, and Clostridium perfringens. The predicted extracellular lipoproteins were also found as distant relatives in Victivallis vadensis and Candidatus Solibacter utitsatus (33 and 25% identities, respectively). The phylogenetic tree of these close homologs and related proteins including conserved domains of GH101 enzymes was constructed using the neighbor-joining method (Fig. 4). Although NagBb homologs and GH101 enzymes may share a common ancestral protein, these
groups can be clearly divided. Thus, we propose that NagBb and its homologs should be assigned to a new family GH 129.

**DISCUSSION**

Mucins are secreted from mucosal tissues and protect epithelia from various outer environmental agents including bacteria. In the intestines, goblet cells secrete mucins that are mainly the MUC2 type. When MUC2 is secreted, it folds tightly and forms a dense inner gel layer, which functions as a barrier against intestinal bacteria (17). By contrast, MUC2 in the tight layer is converted into an expanded outer layer due to proteolytic cleavage; this layer is the habitat of commensal flora (18). Recently, O-glycan structures of MUC2 from the sigmoid colon of normal patients were determined (19). The structures were mainly based on the core-3-containing tetrasaccharide (Galβ1–3GalNAcβ1–3(Neu5Acα2–6)GalNAca1-Ser/Thr) and were further elongated by addition of GlcNAc, Gal, sulfated Gal, Neu5Ac, and Fuc. We previously identified several extracellular membrane-bound glycosidases from *B. bifidum* JCM 1254 that are involved in degradation of human milk oligosaccharides. These glycosidases may also function in degradation of O-glycans on MUC2 (Fig. 5). Namely, two α-L-fucosidases, GH95 AfcA specific for the α1,2-linkage (39, 40) and GH29 AfcB specific for the α1,3- and α1,4-linkages (41), can remove Fuc at the non-reducing termini except for any that are α1,6-linked. GH33 α-sialidase SiaBb2 can remove both α2,3- and α2,6-linked sialic acids (42). The type-1 chain (Galβ1–3GlcNAcβ1–3(Neu5Acα2–6)GalNAca1-Ser/Thr) is likely eliminated by GH20 lacto-N-biosidase LnbB (43), and the released lacto-N-biose (Galβ1–3GlcNAc) is incorporated into the cytosol via a Galβ1–3GalNAc/Galβ1–3GlcNAc transporter (11, 12). The type-2 chain (Galβ1–4GlcNAcβ1–3(Neu5Acα2–6)GalNAca1-Ser/Thr) is sequentially degraded by GH2 β-galactosidase BbgIII acting on N-acetyllactosamine (Galβ1–4GlcNAc) and GH20 β-N-acetylhexosaminidases BbhI and BbhII specific for GlcNAcβ1–3Galβ1–R (44). Through cooperation of these glycosidases, which were shown to be expressed constitutively in...
the normal condition, the Tn antigen could be uncovered on MUC2. Before or after the generation of the Tn structure, core proteins may be cleaved by some kind of protease and then incorporated into the cytosol of bifidobacteria and degraded by NagBb. The transporter responsible for Tn antigen-containing peptides is currently unknown. In the vicinity of the nagBb locus, there are putative ATP-binding cassette transporter genes, one of which may be a candidate for the transporter of Tn antigen-containing peptides. NagBb homologs are conserved in infant-associated bifidobacteria, i.e. B. longum subsp. longum, B. longum subsp. infantis, and B. breve. Thus, this novel degradation pathway for core-3 O-glycan is likely important for growth and adaptation of bifidobacteria in the gut of newborn infants. Because the NagBb homolog has never been found in pathogenic and opportunistic infectious bacteria, this novel pathway could be a promising target for the development of a highly selective bifidogenic factor, a so-called “prebiotic.” GalNAcα1-3Ser or α-GalNAc-containing glycosides might be better prebiotics, and the transglycosylation activity of NagBb may be exploited for their productions.

Exo-α-N-acetylgalectosaminidases (α-N-acetylgalectosaminidases; EC 3.2.1.49) have been found in GH27, GH36, and GH109 families. GH27 contains eukaryotic lysosomal GH109 families. GH27 contains eukaryotic lysosomal

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