Research Article

Comparative Genomics Revealed Genetic Diversity and Species/Strain-Level Differences in Carbohydrate Metabolism of Three Probiotic Bifidobacterial Species

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Strains of *Bifidobacterium longum*, *Bifidobacterium breve*, and *Bifidobacterium animalis* are widely used as probiotics in the food industry. Although numerous studies have revealed the properties and functionality of these strains, it is uncertain whether these characteristics are species common or strain specific. To address this issue, we performed a comparative genomic analysis of 49 strains belonging to these three bifidobacterial species to describe their genetic diversity and to evaluate species-level differences. There were 166 common clusters between strains of *B. breve* and *B. longum*, whereas there were nine common clusters between strains of *B. animalis* and *B. breve*. Further analysis focused on carbohydrate metabolism revealed the existence of certain strain-dependent genes, such as those encoding enzymes for host glycan utilisation or certain membrane transporters, and many genes commonly distributed at the species level, as was previously reported in studies with limited strains. As *B. longum* and *B. breve* are human-residential bifidobacteria (HRB), whereas *B. animalis* is a non-HRB species, several of the differences in these species' gene distributions might be the result of their adaptations to the nutrient environment. This information may aid both in selecting probiotic candidates and in understanding their potential function as probiotics.

1. Background

The genus *Bifidobacterium* has been classified into 48 taxa, including 39 species and nine subspecies [1], three of which (*Bifidobacterium longum*, *Bifidobacterium breve*, and *Bifidobacterium animalis*) are commonly applied as bifidobacterial probiotics in the food industry. Many studies have been performed on the functionality of probiotic bifidobacteria in the host, and the properties and functionality of probiotics have generally been thought to be strain dependent [2, 3]. However, previous reports have indicated that certain characteristics, such as the ability to generate vitamins [4], organic acids [5], and antimicrobial components [6, 7] as well as tolerance to stress [8, 9], are species dependent, although there is a degree of variation among strains. In several cases, it

is unclear whether certain characteristics are species common or strain specific.

Recent work has provided genomic information for all bifidobacterial species/subspecies [1, 10]. However, because the number of available genomic sequences from each species was limited in these studies, it is difficult to distinguish whether a number of unique genes are species or strain specific.

To further elucidate bifidobacterial genetic diversity and to evaluate species-level differences, we performed comparative genomic analyses of 49 strains belonging to three bifidobacterial species, or *B. longum*, *B. breve*, and *B. animalis*, which are commonly applied as probiotics in the food industry. The use of a large set of genome sequences based on a relatively large number of strains of each species allowed the identification of pan-genome structures and the elucidation of differences in core genome structures among these species. In the present report, given that bifidobacteria have been reported to utilise many unique metabolic pathways for sugar fermentation [11–19], additional analysis was focused on the carbohydrate transport/metabolism of each species/strain.

2. Materials and Methods

2.1. Bacterial Strains and Cultivation Conditions. The bacterial strains used in this study and general information about these strains are listed in Table 1. The bifidobacterial strains were obtained from stock cultures maintained in the Morinaga Culture Collection (MCC; Morinaga Milk Industry Co., Ltd., Zama, Japan) and the American Type Culture Collection (ATCC, VA). Each strain was cultivated in Difco Lactobacilli MRS (Becton Dickinson, NJ) supplemented with 0.05% L-cysteine-HCl (Kanto Chemical, Tokyo, Japan) at 37°C for 16 h under anaerobic conditions before DNA extraction. The microorganisms were collected by centrifugation, washed once with sterile saline, resuspended in an equivalent volume of sterile saline, and used as seed cultures for fermentation studies.

2.2. Assimilation of Human Milk Oligosaccharides. Modified MRS was prepared by removing glucose from the original components and was supplemented with LNT (Dextra Laboratories Ltd., Reading, UK) at a final concentration of 2%. An aliquot of seed culture from each bifidobacterial strain was then inoculated into $200 \,\mu$ L of the modified MRS, with a final concentration of 1%. Growth of each bifidobacterial strain was measured by absorbance at OD600 after cultivation under anaerobic conditions at 37°C for 24 h and 48 h. Experiments were performed in triplicate.

2.3. Genome Sequencing and Bioinformatics Analyses. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. The preparation of genomic libraries was performed with 1 ng of genomic DNA using the Nextera XT DNA Sample Preparation Kit (Illumina Inc., CA) according to the manufacturer's instructions. After PCR amplification and cleanup, the fragment size distribution of the tagmented DNA was analysed using an Agilent 2100 Bioanalyzer and the High Sensitivity DNA Analysis Kit (Agilent Technologies, Santa Clara, CA). The libraries were sequenced using a MiSeq Personal Sequencing System and the MiSeq Reagent Kit v2 (500 cycles) (Illumina Inc.). Quality trimming and de novo assembly of the raw paired-end reads were performed using the CLC Genomics Workbench (v 6.0) software package (CLC bio, Aarhus, Denmark) with default settings, except for contig length (minimum contig length = 2,000 bp). Seventeen genomes that were sequenced *de novo* in this study were automatically annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) 2.0 and were manually checked as part of the process of genome submission to GenBank. Our sequencing efforts resulted in multiple contigs (Table 1). The datasets for the genome annotations for the other 32 strains published previously were retrieved from the FTP server of NCBI [20].

2.4. Pan-Genome Analysis. For all of the 49 bifidobacterial strains included in this study, a pan-genome calculation was performed using the PGAP [21]. The ORF content of each genome was organised into functional gene clusters using the gene family (GF) method. Under the GF method, the total protein sequences of each strain were mixed together, with each gene being marked as a strain identifier. BLASTALL searches were performed among the mixed protein sequences, and the filtered BLAST results were clustered using the Markov Cluster algorithm [22], which has been widely used in other studies on prokaryotic genomes and in programs designed to search for orthologues among multiple strains. For each gene pair in a given cluster, the global match region was no less than 50% of the longer gene protein sequence, and the identity was also no less than 50%. The minimum score value and E-value applied in BLAST were 50 and 1e-8. Pan-genome profile analysis, genetic variation analysis of functional genes, and function enrichment analysis of gene clusters were then performed. KEGG Orthology (KO) numbers were assigned by the KEGG Automatic Annotation Server (KAAS) using the bidirectional best-hit method [23].

2.5. *Phylogenetic Trees.* Pan-genome-based phylogenetic trees were generated according to a gene distance matrix, which was calculated based on genes that were absent or present in each strain [21], using UPGMA algorithms in PHYLIP. The sequences of 16S rRNA genes were aligned using the ClustalW alignment tool [24] with default parameters, and phylogenetic trees were constructed using UPGMA algorithms in MEGA6.06 [25]. Bootstrap values were calculated using 500 bootstrap replicates. The supertree was built using FigTree v1.4.0.

2.6. Nucleotide Sequence Accession Numbers. The de novo sequence and annotation data reported herein have been deposited in GenBank under the accession numbers AVQA00000000-AVQE00000000 and AWFK00000000-AWFV00000000.

3. Results and Discussion

3.1. Phylogenetic Trees for Each Bifidobacterial Species. Based on the pan-genome profiles and 16S rRNA gene sequences, phylogenetic trees were constructed for each species, using *Gardnerella vaginalis* ATCC 14018 as an outgroup species. Each phylogenetic tree was divided into two clusters. However, the classification of subspecies of certain strains based on the genotypes of the strains was contradictory to that previously defined based on phenotypes (Table 1, Supplementary files 1–3 in Supplementary Material available online at http://dx.doi.org/10.1155/2015/567809). *B. animalis* subsp. *animalis* ATCC 27536 and ATCC 27674 fell into the clade with the type strain of *B. animalis* subsp. *lactis*, whereas B. *longum* subsp. *longum* JDM 301 and *B. longum* subsp. *infantis* 157F fell into the clades with the type strains of *B. longum*

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Shecies	Genotyl	oe based on	Strain	Status	Chromosome	Plasmid Conti	o Lenoth (khn)	CDSs	Source	GenBank accession
cheere	16SrRNA	Whole genes					(Jan) manar a	8	221200	number
B. longum ssp. infantis	infantis	infantis	ATCC 15697	Complete	1		2,833	2,416	Human infant faeces	CP001095
B. longum ssp. longum	infantis	infantis	JDM301	Complete	1		2,478	1,958	Human vagina	CP002010
B. longum ssp. longum	longum	longum	BBMN68	Complete	1		2,266	1,804	Human elderly faeces	CP002286
B. longum ssp. infantis	longum	longum	157F	Complete	1	2	2,409	1,999	Human infant faeces	AP010890, AP010891,
, , ,)) -		- - (,					and AP010892
B. longum ssp. longum	longum	longum	JCM 1217	Complete	I		2,385	1,924	Human intant taeces	AP010888
B. longum ssp. longum	longum	longum	KACC 91563	Complete	1	2	2,396	1,985	Human infant faeces	CP002794, CP002795, and CP002796
B. longum	longum	longum	NCC2705	Complete	1	1	2,260	1,728	Human infant faeces	AE014295 and AF540971
										CP000605,
B. longum	longum	longum	DJO10A	Complete	1	2	2,390	2,000	Human adult faeces	AF538868, and A E538860
B. longum ssp. longum	longum	longum	F8	Complete	1		2,385	1,681	Human feces	FP929034
B. longum ssp. longum	longum	longum	ATCC 55813	Unfinished		140	2,373	2,109	Human infant faeces	ACH100000000
B. longum ssp. longum	longum	longum	CCUG 52486	Unfinished		55	2,453	2,240	Human elderly faeces	ABQQ00000000 ABQQ00000000000000000000000
B. longum ssp. longum	longum	longum	1-6B	Unfinished		171	2,686	2,425	Human children feces	AJTF00000000
B. longum ssp. longum	longum	longum	2-2B	Unfinished		141	2,625	2,412	Human children feces	AJTJ00000000
B. longum ssp. longum	longum	longum	35B	Unfinished		131	2,514	2,260	Human infant faeces	AJTI00000000
B. longum ssp. longum	longum	longum	44B	Unfinished		62	2,559	2,262	Human infant faeces	AJTM00000000
B. breve	breve	breve	ACS-071-V-Sch8b	Complete	1		2,327	1,826	Human vagina	CP002743
B. breve	breve	breve	UCC2003	Complete	1		2,423	1,852	Human infant faeces	CP000303
B. breve	breve	breve	DSM 20213	Unfinished		117	2,298	2,263	Human infant faeces	ACCG00000000
B. breve	breve	breve	CECT 7623	Unfinished		34	2,314	1,725	Human milk	AFVV00000000
B. breve	breve	breve	MCC0121	Unfinished		27	2,436	2,079	Human infant faeces	AVQA00000000
B. breve	breve	breve	MCC0305	Unfinished		22	2,287	1,927	Human adult faeces	AWFR00000000
B. breve	breve	breve	MCC0476	Unfinished		15	2,234	1,851	Human adult faeces	AVQB00000000
B. breve	breve	breve	MCC1094	Unfinished		23	2,327	1,944	Human infant faeces	AWFS00000000
B. breve	breve	breve	MCC1114	Unfinished		33	2,487	2,136	Human infant faeces	AVQC00000000
B. breve	breve	breve	MCC1128	Unfinished		25	2,480	2,143	Human infant faeces	AVQD00000000
B. breve	breve	breve	MCC1340	Unfinished		23	2,373	1,986	Human infant faeces	AWFT00000000
B. breve	breve	breve	MCC1454	Unfinished		14	2,457	2,111	Human infant faeces	AWFU00000000
B. breve	breve	breve	MCC1604	Unfinished		15	2,206	1,830	Human elderly faeces	AVQE00000000
B. breve	breve	breve	MCC1605	Unfinished		37	2,324	1,976	Human elderly faeces	AWFV00000000
B. animalis ssp. animalis	animalis	animalis	ATCC 25527	Complete	1		1,933	1,538	Rat feces	CP002567
B. animalis ssp. animalis	animalis	animalis	ATCC 27672	Unfinished		9	1,990	1,532	Rat feces	AWFQ00000000
B. animalis ssp. animalis	animalis	animalis	MCC0483	Unfinished		27	2,176	1,750	Rat feces	AWFK00000000
B. animalis ssp. animalis	animalis	animalis	MCC0499	Unfinished		11	2,134	1,721	Rat feces	AWFN00000000
B. animalis ssp. animalis	animalis	animalis	MCC1489	Unfinished		14	1,910	1,474	Guinea pig feces	AWFO00000000

TABLE 1: General genome features of Bifidobacterium species included in this study.

					TABLE 1: Cont	inued.					
Species	Genoty 16SrRNA	/pe based on Whole genes	Strain	Status	Chromosome	Plasmid	Contig	Length (kbp)	CDSs	Source	GenBank accession number
B. animalis ssp. lactis	lactis	lactis	AD011	Complete	1			1,934	1,527	Human infant faeces	CP001213
B. animalis ssp. animalis	lactis	lactis	ATCC 27536	Unfinished			18	1,912	1,561	Chicken feces	AWFL00000000
B. animalis ssp. animalis	lactis	lactis	ATCC 27673	Unfinished			21	1,937	1,576	Sewage	AWFP00000000
B. animalis ssp. animalis	lactis	lactis	ATCC 27674	Unfinished			18	1,912	1,561	Rabbit feces	AWFM00000000
B. animalis ssp. lactis	lactis	lactis	B420	Complete	1	1		1,939	1,561	No information	CP003497
B. animalis ssp. lactis	lactis	lactis	BB-12	Complete	1			1,942	1,642	Yoghurt	CP001853
B. animalis ssp. lactis	lactis	lactis	Bi-07	Complete	1			1,939	1,597	No information	CP003498
B. animalis ssp. lactis	lactis	lactis	Bl-04	Complete	1			1,939	1,567	Human infant faeces	CP001515
B. animalis ssp. lactis	lactis	lactis	Bl12	Complete	1			1,938	1,518	Colonoscopic sample	CP004053
B. animalis ssp. lactis	lactis	lactis	BLCI	Complete	1			1,944	1,518	No information	CP003039
B. animalis ssp. lactis	lactis	lactis	BS01	Unfinished			7	1,932	1,572	No information	AHGW000000000000000000000000000000000000
B. animalis ssp. lactis	lactis	lactis	CNCM I-2494	Complete	1			1,943	1,660	No information	CP002915
B. animalis ssp. lactis	lactis	lactis	DSM 10140	Complete	1			1,938	1,565	Yoghurt	CP001606
B. animalis ssp. lactis	lactis	lactis	610NH	Unfinished			28	1,916	1,578	No information	ABOT00000000
B. animalis ssp. lactis	lactis	lactis	V9	Complete	1			1,944	1,572	Human infant faeces	CP001892

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FIGURE 1: Venn diagram of the homologous clusters shared among the core genes. The surfaces are approximately proportional to the number of genes.

subsp. *infantis* and B. *longum* subsp. *longum*, respectively. In this study, we adopted subspecies of these strains based on the phylogenetic trees for further analyses.

3.2. Core and Pan-Genome Structures. After clustering the functional genes for each species, respective totals of 5,471, 4,053, and 2,833 clusters and 966, 1,221, and 1,092 core clusters were obtained for B. longum, B. breve, and B. animalis (Supplementary file 4). These numbers are similar to those reported previously [11, 26, 27]. However, we identified a wider variety of genomes in B. longum compared with a previous report [11]. This result may suggest that B. longum has an open pan-genome; that is, B. longum may exhibit a robust ability to import new genes, allowing it to adapt to each ecological niche over its long history of evolution. In the current study, to reveal differences among species, a total of 90,442 genes from the 49 strains were jointly clustered. These genes were divided into 8,818 homologous clusters, and there were 584 common clusters among all of the bifidobacterial strains (Figure 1). Additional analysis revealed that 404 of these clusters were commonly clustered with G. vaginalis ATCC 14018 as an outgroup species. Therefore, the number of specific gene clusters among the three bifidobacterial species was estimated to be less than 180. There were 166 common clusters between strains of B. breve and B. longum, whereas there were nine common clusters between strains of B. animalis and B. longum and four common clusters between strains of B. animalis and B. breve (Figure 1).

3.3. Metabolism of Host Glycans. Bifidobacteria have been reported to employ many unique metabolic pathways for sugar fermentation to enable them to utilise diverse carbohydrates in the intestine that are not utilised by their hosts [11–19]. Figure 2 shows the distributions of genes involved in the metabolism of host glycans, such as human milk oligosaccharides (HMOs), mucin, and N-glycans.

3.3.1. HMOs. A 43 kb cluster in the genome of *B. longum* subsp. *infantis* ATCC 15697 has been reported to be one of the

most characteristic gene clusters for the utilisation of HMOs [28].

Seven homologous genes encoding extracellular solutebinding proteins predicted to bind oligosaccharides (SBP family 1) were found in only two strains of *B. longum* subsp. *infantis*, whereas homologues of the major facilitator superfamily (Blon_2331, 2332) were found in all strains except for *B. animalis* subsp. *lactis* AD011. Two of four gene homologues encoding glycoside hydrolases (alpha-galactosidase and beta-N-acetylhexosaminidase) were found in strains of *B. breve* as well as *B. longum* subsp. *infantis*. Fucosidase (Blon_2336) and sialidase (Blon_2348) homologues were found only in *B. longum* subsp. *infantis*. However, certain strains of *B. breve* possessed other gene clusters related to fucose and sialic acid incorporation (Figure 2).

Regarding the core structure of HMOs, three of the four predominant HMOs (lacto-N-fucopentaose I (LNFP I), lacto-N-difucohexaose I (LNDFH I), and lacto-N-tetraose (LNT)) exhibit the LNT structure. Therefore, the enzyme required for the utilisation of LNT is crucial in the utilisation of HMOs and the colonisation of the infant intestine. Certain strains belonging to B. bifidum, B. longum subsp. longum, B. longum subsp. infantis, and B. breve have been reported to act as LNT consumers [29, 30]. The last two species incorporate intact LNT via an unidentified transporter and then hydrolyse it intracellularly using LNT β -1,3-galactosidase (Blon_2016) [31]. In our study, using type strains, we confirmed the ability of *B. longum* subsp. longum, B. longum subsp. infantis, and B. breve, but not B. animalis subsp. animalis and B. animalis subsp. lactis, in the utilisation of LNT (Supplementary file 5). Unexpectedly, Blon_2016 homologues were also present in B. animalis strains. Based on phylogenetic analysis, Yoshida et al. [31] discovered that there is a close homologue of this gene (amino acid identity > 95%) that falls into a clade of strains of infant gut-related species (i.e., B. breve, B. longum subsp. infantis, and B. longum subsp. longum), whereas the gene in B. animalis was observed to be distant from this clade. These results imply the existence of different substrate specificity for LNT β -1,3galactosidase, although further investigations are necessary to examine this issue. B. bifidum and certain strains of B. longum subsp. longum produce a secretory enzyme, LNBase, that hydrolyses LNT into lacto-N-biose (LNB) and lactose [32, 33]. In the present study, homologous genes encoding LNBase (BLLJ_1506) and the chaperone for this enzyme (BLLJ_1505) were found in two strains of *B. longum* subsp. longum. The LNBase of B. longum subsp. longum has been reported to be able to hydrolyse the GlcNAc β 1-3Gal linkage in LNT, LNFP I, and sialyllacto-N-tetraose. These results imply the existence of different mechanisms for the utilisation of LNT decorated by fucose and sialic acid. B. bifidum might digest LNT after releasing its own fucosidase and sialidase, whereas B. longum subsp. longum lacks these enzymes but can directly degrade decorated LNT.

Subsequently, the liberated LNB is imported into the cells via the galacto-N-biose (GNB)/LNB transporter for further degradation [34]. The disaccharide is then phosphorolysed by a GNB/LNB phosphorylase to produce Gal-1-P and GlcNAc

	LON	INF	BRE	ANI	LAC	
	e	ch 33		22	10 m = = = =	
	B B B B B B B S S 81 581 0 A 0 A	217 2156 705 697 697 697 705 705 705 705 705 705 705 705 705 70	212 1121 1121 114 114 114 128 128 128 128 128 128 128 128 128 128		1 753 767 767 1 1 1 1 1 1 1 1 0 9 9	
	11.6 351.2 3	MBC CIS OC A		:22222	D01 222222222222222222222222222222222222	
	D CCI B ATC	N X X Q Q N N N N	MC M MC M MC M MC	THE WORK	B B B B B B B B B B B B B B B B B B B	
Description		- Sector				Reference
43 kbp HMO cluster						Blon 2331 [28]
Galactoside symporter	and the second second second	and the second				Blon_2332 [28]
Integrase						Blon_2333 [28]
Beta-galactosidase	and the second					Blon_2334 [28]
Alpha-galactosidase Alpha-L-fucosidase						Blon 2336 [28]
RbsD or FucU transport						Blon_2337 [28]
Dihydrodipicolinate synthase						Blon_2338 [28]
Short chain dehydrogenase Mandelate racemase						Blon_2339 [28] Blon_2340 [28]
Hypothetical protein						Blon_2341 [28]
Sugar ABC transporter permease						Blon_2342 [28]
Sugar ABC transporter permease						Blon_2343 [28]
Sugar ABC transporter permease						Blon 2345 [28]
Sugar ABC transporter permease						Blon_2346 [28]
Family 1 extracellular solute-binding protein						Blon_2347 [24, 28]
Exo-aipna-siaiidase N-Acetylneuraminate lyase						Blon 2349 [28]
Family 1 extracellular solute-binding protein				1		Blon_2350 [24, 28]
Family 1 extracellular solute-binding protein						Blon_2351 [24, 28]
Family 1 extracellular solute-binding protein						Blon 2353 [28]
Family 1 extracellular solute-binding protein						Blon_2354 [24, 28]
Beta-N-acetylhexosaminidase						Blon_2355 [28]
Haloacid dehalogenase domain-containing protein hydrolase						Blon_2356 [28]
Family 1 extracellular solute-binding protein						Blon_2357 [28]
Beta-lactamase				T		Blon_2358 [28]
Binding-protein-dependent transport system						Blon_2359 [28]
Binding-protein-dependent transport system						Plan 2260 [20]
Inner membrane protein						Bion_2360 [28]
Sugars ABC transporter ATP-binding protein						Blon_2361 [28]
Stalidase cluster LacI-type transcriptional regulator						Blon 0641 [28]
Transcriptional regulator, GntR family			a a a a a a a a a a			Blon_0642 [28]
UDP-N-acetylglucosamine diphosphorylase						Blon_0643 [28]
ROK family transcriptional regulator						Bion_0644 [28]
NanE						Blon_0645 [28]
Sialidase A						Blon_0646 [28]
Oligopeptide-binding protein oppA						Blon_0647 [28] Blon_0648 [28]
fusion between oligopeptide transport permease						Blon_0640 [20]
oppC and ATP-binding protein oppD						BIOII_0649 [28]
Oligopeptide transport ATP-binding protein						Blon_0650 [28] Blon_0651 [28]
Fucosidase cluster						Dioii_0001 [20]
Two-component response regulator						Blon_0243 [28]
Two-component sensor protein						Blon_0244 [28]
MFS transporter MFS transporter permease						Blon 0247 [28]
Alpha-L-fucosidase						Blon_0248 [28]
LacI-type transcriptional regulator						Blon_0423 [28]
Alpha-L-fucosidase						Blon 0426 [28]
Permease of ABC transporter for sugars						Blon_0341 [25]
Sugar ABC transporter permease						Blon_0342 [25]
ABC transporter substrate-binding protein						Bion_0343 [25]
domain-containing protein						Blon_0344 [25]
MFS transporter permease						Blon_0345 [25]
α-fucosidase						Blon_0346 [25, 26]
LNT β-1,3-galactosidase	· · · · · · · · · · ·				a a a a a a a a a a a a a a a a	Blon_2016 [31]
Chaperon for lacto-N-biosidase						BLLJ_1505 [33]
Lacto-N-DIOSIGASE GNB/LNB cluster				I	1	DLLJ_1506[33]
Phosphotransferase family			· · · · · · · · <u>· · · · · · · · · · · </u>			Blon_2171 [34]
Lacto-N-biose phorylase						Blon_2172 [34]
Permease protein of ABC transporter system for sugars						Blon_2173 [34]
Solute-binding protein of ABC transporter						Blon 2174 [34]
system for sugars						DION_2174 [54]
Galactose-1-phosphate unidylyltransferase Permease protein of ABC transporter system for						Bion_21/5 [34]
sugars						Blon_2176 [34]
UDP-glucose 4-epimerase						Blon_2177 [34]
GluNAc/GalNAc related gene	1					Blop 0881 [24 25]
N-Acetylglucosamine-6-phosphate deacetvlase						Blon_0882 [24, 25]
Beta-hexosaminidase A						Blon_0732 [27]
Glycosyl hydrolase						Blon_2468 [42] Blon_2470 [42]
PTS system, N-acetylglucosamine-specific IIBC						DIOII_24/0 [42]
component						Blon_2471 [42]
Phosphoenolpyruvate-protein phosphotransferase						Blon_0178 [42]
Phosphocarrier protein HPr Endo-beta-N-acetylolucosaminidase	-					BION_0177 [42] BLD 0197 [42]
Endo-alpha-N-acetylgalactosaminidase						BLD_1258 [39]
α -N-acetylgalactosaminidase						NagBb [38]
Endo-beta-N-acetylglucosaminidase						-
α -mannose related gene				I	1	
Alpha-mannosidase						Blon_0868 [42]
Alpha-mannosidase				1		Blon_0869 [42]
source-omaing protein for manno-	L			L	1	

FIGURE 2: Distribution of genes involved in the metabolism of host glycans, such as human milk oligosaccharides, mucin, and N-glycans. The abbreviations used for species/subspecies are as follows: INF, *B. longum* subsp. *infantis*; LON, *B. longum* subsp. *longum*; BRE, *B. breve*; ANI, *B. animalis* subsp. *animalis*; LAC, *B. animalis* subsp. *lactis*.



FIGURE 3: Distribution of genes involved in the metabolism of plant-derived sugars predicted by the CAZy database. The abbreviations used for species/subspecies are as follows: INF, *B. longum* subsp. *infantis*; LON, *B. longum* subsp. *longum*; BRE, *B. breve*; ANI, *B. animalis* subsp. *animalis*; LAC, *B. animalis* subsp. *lactis*.

and is further metabolised [34]. In the current study, a sevengene operon involved in this pathway was observed in nearly all strains of *B. longum* and *B. breve* and in three strains of *B. animalis* subsp. *animalis*. This distribution is in complete accord with results regarding *in vitro* LNB utilisation [35]. A previous report revealing a strong ability to grow in human milk also supports these claims [36].

3.3.2. Mucin and N-Glycans. Another substrate for this metabolic pathway is GNB, which is a core structure of gastrointestinal mucin. Mucins are extensively O-glycosylated proteins and are thought to serve as a potential carbon source for gut microbiota. Studies have revealed that the main core structures of gastric/duodenal and intestinal mucins are core-1, 2-type and core-3-type O-glycans, respectively [37, 38]. A homologous gene encoding α -N-acetylgalactosaminidase, acting on core-3-type O-glycan (NagBb) [39], was observed in 2 strains of B. longum subsp. infantis, 11 strains of B. longum subsp. longum, and 13 strains of B. breve in the present study. In contrast, a gene encoding endo- α -N-acetylgalactosaminidase, which is predicted to release GNB-containing glycans from core-1-type O-glycan mucin (BLD_1258) [40], was observed only in B. longum subsp. longum. In addition, the terminal ends of glycoconjugates in the suckling gut have been reported to predominantly consist of sialic acid, whereas in the adult, they predominantly consist of fucose [41, 42]. In our study, nearly all strains of B. longum subsp. infantis and B. breve contained a gene encoding sialidase, which might be useful for this species to colonise the infant intestine.

Most strains of *B. longum* and *B. breve*, but not *B. animalis* strains, were found to possess genes involved in the pathway for the utilisation of N-acetylglucosamine (GlcNAc) and GalNAc, which are the major constituents of host glycans. Furthermore, gene homologues encoding endo-beta-N-acetylglucosaminidase (BLD_0197) [43], which releases complex N-glycans from human milk glycoproteins, and alpha-mannosidase (Blon_0868, 0869) were found in certain strains of these two species. These enzymes involved in the degradation of host glycans might play a role in the utilisation of intrinsic carbohydrate sources.

Bifidobacteria are generally residents of the intestines of animals, including warm-blooded mammals and social insects, and several bifidobacterial species are typical inhabitants of the human gut (designated human-residential bifidobacteria or HRB). All strains of *B. longum* and *B. breve*, which are typical HRB, possess certain genes related to the pathway for the utilisation of LNT/LNB, which are core structures of type I oligosaccharides that are specific to human breast milk (Figure 2). Among HRB strains in our study, nearly all possessed gene operons involved in the GNB/LNB pathway, whereas genes upstream of HMO utilisation, such as fucosidase, sialidase, and LNBase, were species/strain dependent. These results suggest that each HRB strain might evolve to assimilate HMOs, for which hundreds of types of structures have been reported [44]. In contrast, B. animalis is a non-HRB species; although certain strains of B. animalis subsp. lactis have also been isolated from humans, they have been found in faecal samples but rarely in colon mucosal samples [27, 45]. In addition, previous reports have shown that *B. animalis* is a strictly monophyletic group, and the evolutionary distance between B. animalis and species of HRB was shown to be relatively far based on 16S rRNA multigene alignments and comparative genomics [11, 13, 27, 46]. Our results indicate that there are fewer homologues involved in the degradation of host glycan by *B*. animalis (Figure 2). These results suggest that the observed differences in the gene distribution might be the result of the adaptation of these strains to their residential environments.

3.4. Carbohydrate-Active Enzymes for Plant-Derived Sugars. Regarding extrinsic carbohydrates, a variety of resistant fibres, which can be dietary compounds, are delivered to the colon, where bifidobacteria reside. Based on CAZy classification, all of the strains possessed a large number of homologous genes encoding GH 13 family members (Figure 3), which has previously been reported as a characteristic feature of bifidobacterial genomes [47, 48].

	LON	INF	BRE	Al	NI	LAC
	68 28 158 148 148 148 148 155 100 100 100 11217 11217 11217	C2705 C15697 M301	071_V_Sch8b BCT7263 SM20213 SM20213 SM20213 CC_0121 CC_0121 CC_0120 CC_114 CC_114 CC_1128 CC_1340 CC_1340 CC_1340 CC_1454	CC_1604 CC_1605 JCC2003 C25527 C25527	C_0499 C_1489 C_1489	0011 2.27536 2.27673 2.27674 4.20 2.12 4.20 1.07 1.12 1.1
KO assignment	ATO 1 4 3 3 2 2 1 1 2 1 2 1 2 2 2 2 2 2 2 2 2 2		N M M M M M M M M M M M M M M M M M M M	ATCO N	N N N	ATCO ATCO ATCO ATCO B B B B B B B B B B B B B B B B CNCI B B C C C C C C C C C C C C C C C C C
ABC transporters, prokaryotic type K02050 ABC.SN.P; NitT/TauT family transport system permease protein K02049 ABC.SN A: NitT/TauT family transport system ATP-binding protein						
K15553 ssuA; sulfonate transport system substrate-binding protein K15554 ssuC; sulfonate transport system permease protein		1		111111		· · · · · · · · · · · · · · · · ·
K15555 ssuB; sulfonate transport system ATP-binding protein [EC:3.6.3] K02055 ABC.SPS; putative spermidine/putrescine transport system substrate-binding protein K02053 ABC.SPP: putative spermidine/putrescine transport system permease protein						
K02054 ABC:SPI: putative spermidine/putrescine transport system permease protein K02052 ABC:SPI: putative spermidine/putrescine transport system ATP-binding protein				1111		
K05845 opuC; osmoprotectant transport system substrate-binding protein K05847 opuA; osmoprotectant transport system ATP-binding protein K10108 malE: maltose/maltodextrin transport system substrate-binding protein						
K10112 msmX, msmK, malK, sugC, ggtA, msiX; multiple sugar transport system ATP-binding protein K15770 cycB, ganO; arabinogalactan oligomer/maltooligosaccharide transport system substrate-binding protein						
K15771 ganP; arabinogalactan oligomer/maltooligosaccharide transport system permease protein K15772 ganQ; arabinogalactan oligomer/maltooligosaccharide transport system permease protein K10112 msmX. msmK. malK. supC. getA. msiK: multiple super transport system ATP-binding protein						
K10117 mmit; raffinose/stachyose/melibiose transport system usbrate-binding protein K10118 msmF; raffinose/stachyose/melibiose transport system usbrate-binding protein		1				
K10119 msmG; raffinose/stachyose/melibiose transport system permease protein K10112 msmX, msmK, malK, sugC, ggtA, msiK; multiple sugar transport system ATP-binding protein K10233 adE setC: alpha-chlocoide transport system permease protein		-				
K10112 msmX, msmX, msmK, malK, sugC, ggtA, msiK; multiple sugar transport system ATP-binding protein K17317 gtsC, glcG; glucose/mannose transport system permease protein	· · · · · · · · · · · · ·					
K10112 msmX, msmK, malK, sugC, ggtA, msiK; multiple sugar transport system ATP-binding protein K10236 thuE; trehalose/maltose transport system substrate-binding protein K10200 APC/SIN Cost Nuclear Studies and State State State States and States and States States States and States St		1				
K10201 ABC.NGC.P. N-acetylglucosamine transport system permease protein K10201 ABC.NGC.P. N-acetylglucosamine transport system permease protein		: : :				
K10242 cebG; cellobiose transport system permease protein K10112 msmX, msmK, malK, sugC, ggtA, msiK; multiple sugar transport system ATP-binding protein						
K1/331 dasc; r, N uacertyrcnitooise transport system permease protein K10112 msmX, msmK, malK, sugC, ggtA, msiK; multiple sugar transport system ATP-binding protein K17245 chiF: putative chibolose transport system permease protein						
K10188 lacE, araN; lactose/L-arabinose transport system substrate-binding protein K10189 lacF, araP; lactose/L-arabinose transport system permease protein		: :				
K17318 K17318, JiplA; putative aldouronate transport system substrate-binding protein K17319 JplB; putative aldouronate transport system permease protein K17320 JplC: putative aldouronate transport system permease protein						
KU726 POC polarity automotive transport system protein substrate-binding protein K10547 ABC.GGUS, chvE; putative multiple sugar transport system substrate-binding protein K10547 ABC.GGU.P, gguB; putative multiple sugar transport system permease protein				11111		
K10548 ABC.GGU.A, ggu.A; putative multiple sugar transport system ATP-binding protein K10555 lsrB; AI-2 transport system substrate-binding protein		1				
K1039 rbss; ribose transport system substrate-binding protein K10439 rbss; ribose transport system substrate-binding protein K10440 rbsC; ribose transport system permease protein		: :		11111		
K10441 rbsA; ribose transport system ATP-binding protein [EC:3.6.3.17] K17238 inoF; inositol-phosphate transport system permease protein		1		1111		
K10112 msmX, msmK, maiX, sug/, ggtA, msix; muitiple sugar transport system A1P-binding protein K05813 ugpB; sn-glycerol 3-phosphate transport system substrate-binding protein K05814 ugpA; sn-glycerol 3-phosphate transport system permease protein						
K05815 ugpE; sn-glycerol 3-phosphate transport system permease protein K10112 msmX, msmK, malK, sugC, ggtA, msiK; multiple sugar transport system ATP-binding protein						
K1/234 araN; arabinosaccharide transport system substrate-binding protein K17236 araQ; arabinosaccharide transport system permease protein K10112 msN, msnK, malK, sueC, getA, msiK; multiple suear transport system ATP-binding protein						
K02027 ABC.MS.S; multiple sugar transport system substrate-binding protein K02025 ABC.MS.P; multiple sugar transport system permease protein		• •				
K02026 ABC.MS.P.I; multiple sugar transport system permease protein K10112 msmX, msmK, malK, sugC, ggtA, msiK; multiple sugar transport system ATP-binding protein K02058 ABC SS: science sugar transport extens substrate.binding protein						
K02057 AEC.SS.P; simple sugar transport system permase protein K02056 AEC.SS.P; simple sugar transport system Permase protein K02056 ABC.SS.A; simple sugar transport system ATP-binding protein [EC.3.6.3.17]		: :				
K02069 ABC.X2.P; putative ABC transport system permease protein K02068 ABC.X2.A; putative ABC transport system ATP-binding protein K02040 net; hosenbot transport system pathetate binding motein						
K02037 pstS; phosphate transport system permease protein K02038 pstA; phosphate transport system permease protein		: :				
K02036 pstB; phosphate transport system ATP-binding protein [EC:3.6.3.27] K02044 phnD; phosphonate transport system substrate-binding protein						
K02042 phine; phosphonate transport system ATP-binding protein [EC:3.6.3.28] K02041 phnC; phosphonate transport system ATP-binding protein [EC:3.6.3.28] K10005 gluB; glutamate transport system substrate-binding protein						
K10006 gluC; glutamate transport system permease protein K10007 gluD; glutamate transport system permease protein						
K10008 guA; guitamate transport system A1P-binding protein K02424 fliY; cystine transport system substrate-binding protein K10009 ABC_CYSTP: cystine transport system permease protein						
K10010 ABC.CYST.A; cystine transport system ATP-binding protein [EC:3.6.3] K16958 tcyL; L-cystine transport system permease protein		: :			: : :	
K01999 JIVK; branched-chain amino-acid transport system substrate-binding protein K01997 JiVH; branched-chain amino-acid transport system permease protein K01998 JIVM: branched-chain amino-acid transport system permease protein						
K01995 livG; branched-chain amino-acid transport system ATP-binding protein K01996 livF; branched-chain amino-acid transport system ATP-binding protein				1111		
K11959 urtR; urea transport system substrate-omdung protein K11960 urtB; urea transport system permease protein K11961 urtC; urea transport system permease protein						
K11962 urtD; urea transport system ATP-binding protein K11963 urtE; urea transport system ATP-binding protein						
K020/3 mett; D-methionine transport system substrate-binding protein K02072 mett; D-methionine transport system permease protein K02071 mett; D-methionine transport system ATP-binding protein						
K16961 yxeM; putative amino-acid transport system substrate-binding protein K16962 yxeN; putative amino-acid transport system permease protein		11			:::	
K02030 ABC.PA.S; polar amino-acid transport system substrate-binding protein K02029 ABC.PA.P; polar amino-acid transport system permease protein K02028 ABC PA A: polar amino-acid transport system ATP-binding protein [EC:36321]						
K15580 oppA, mppA; oligopeptide transport system substrate-binding protein K15581 oppB; oligopeptide transport system permease protein		1				
K15582 oppC; oligopeptide transport system permease protein K10823 oppF; oligopeptide transport system ATP-binding protein K00305 ABC; PE S: pentide/nickel transport system substrate-binding protein				· · · · ·		
K02033 ABC.PE.P; peptide/nickel transport system permease protein K02034 ABC.PE.P1; peptide/nickel transport system permease protein						
K02031 ABC.PE.A; peptide/nickel transport system ATP-binding protein K02032 ABC.PE.A1; peptide/nickel transport system ATP-binding protein K02013 ABC.FEV.A1; iron complex transport system ATP-binding protein ICC3 6.3.34]						
K02006 chO; cobalt/nickel transport system ATP-binding protein K02006 chO; cobalt/nickel transport system ATP-binding protein		: :		11111	:::	
K03523 bioY; biotin transport system substrate-specific component K16783 bioN; biotin transport system permease protein						
K03523 bioY, biotin transport system substrate-specific component K03523 bioY, biotin transport system substrate-specific component K16923 qrT; energy-coupling factor transport system substrate-specific component						
K16925 ykoE: energy-coupling factor transport system substrate-specific component K16926 htsT: energy-coupling factor transport system substrate-specific component						
K10769 ecr1; energy-coupling factor transport system permease protein K16786 ecrA1; energy-coupling factor transport system ATP-binding protein [EC:3.6.3] K16787 ecrA2; energy-coupling factor transport system ATP-binding protein [EC:3.6.3]						
K02077 ABC.ZM.S; zinc/manganese transport system substrate-binding protein K02075 ABC.ZM.P; zinc/manganese transport system permease protein				· · · · · ·		
KU2UYA ABC.ZM.A; zinc/manganese transport system ATP-binding protein K09810 ABC.LPTA, lolD; lipoprotein-releasing system ATP-binding protein [EC:3.6.3] K09811 fix: cell division transport system permease protein						
K09812 ftsE; cell division transport system ATP-binding protein K02004 ABC.CD.P; putative ABC transport system permease protein	· · · · · · · · · · · · · ·		· · · · · · · · · · · · ·			· · · · · · · · · · · · · · · · · · ·
K02003 ABC.CD.A; putative ABC transport system ATP-binding protein K01992 ABC-2P; ABC-2 type transport system permease protein K01990 ABC-2 A: ABC-2 type transport system ATP-binding meriding						
K09013 sufC; Fe-S cluster assembly ATP-binding protein		1				

FIGURE 4: Continued.

				LON			INTE			20		_	AN				LAC		
				LOIN			nar		Di	u.			All				LAC		
		1_6B 2_2B 35B	44B 157F	2C55813 8MN68 UG5 2486	1010A F8 M1217	CO1563 CC2705	CC15697 DM301		MCC_0476 MCC_0476 MCC_1094	MCC_1114 MCC_1128	MCC_1340 MCC_1454 MCC_1604	MCC_1605 UCC2003	CC25527 C_27672 C_0483	C_0499 C_1489	D011 C_27536 C_27673 C_27673	8420 B_12	8.07 8.12	8501 SML_2494 SML_2494	610N
KO assiş	nment			TA BO	<u>a</u> <u>c</u>	XXX	ТР Н	AC .					A D M	M M	A D D TA			- ž	с <i>ж</i>
Major fa	cilitator superfamily (MFS)																		
K02100	araE; MFS transporter, SP family, arabinose: H+ symporter								 										
K08138	xylE; MFS transporter, SP family, xylose: H+ symporter																		
K08139	HXT; MFS transporter, SP family, sugar: H+ symporter																		
K06610	iolF; MFS transporter, SP family, inositol transporter																		
K02532	lacY; MFS transporter, OHS family, lactose permease											1.1							
K02429	fucP; MFS transporter, FHS family, L-fucose permease																		
K06901	pbuG; putative MFS transporter, AGZA family, xanthine/uracil permease																		
K03762	proP; MFS transporter, MHS family, proline/betaine transporter						1.1					1.1							
K08177	oxlT; MFS transporter, OFA family, oxalate/formate antiporter																		
K08151	tetA; MFS transporter, DHA1 family, tetracycline resistance protein																		
K08156	araJ; MFS transporter, DHA1 family, arabinose polymer transporter																		
K08166	mmr; MFS transporter, DHA2 family, methylenomycin A resistance protein											1.1							
K08167	smvA; MFS transporter, DHA2 family, methyl viologen resistance protein SmvA	1.1.1					1.1					1.1							
K08168	tetB; MFS transporter, DHA2 family, metal-tetracycline-proton antiporter						1.1					1.1							
K08217	mef; MFS transporter, DHA3 family, macrolide efflux protein						1.1					1.1		1.1					
K06902	UMF1; MFS transporter, UMF1 family											- 1							
K08369	ydjE; MFS transporter, putative metabolite: H+ symporter								 			1 1		1.1					
Phospho	otransferase system (PTS)								 			_							
K08483	PTS-ELPTSI, ptsi; pnospnotransterase system, enzyme I, Ptsi [EC:2.7.3.9]																		
K11189	PTS-HPK; phosphocarrier protein																		
K02///	PTS-GIC-EIIA, crr; PTS system, glucose-specific IIA component [EC:2.7.1.69]						. 1					1.1							
K02803	PTS Nag-EIIB, nage; PTS system, N-acetylglucosamine-specific IIB component [EC:2.7.1.69]						- 1					1.1		1.1					
K02804	PTS-Nag-EntC, hage; PTS system; N-acetylgucosamine-specific file component													11					
K02////	PTS-OR-FILA, CIT; PTS system, glucose-specific IA component [EC:27.1.09]													11					
K02750	PTS-Bgi-Fills, bgir; PTS system, beta-glucosides-specific file component [EC:2.7.1.09]													11					
K02/5/	PTS-bgt-Enc, bgt; FTS system, beta-glucosuce-spectric file component													11					
K02777	PTS-Glc-EIIA, Crt, PTS-system, glucose-specific IIA component [EC:27.1.69]																		
K02777	PTS-Glc-EIIA, Crt, PTS-system, glucose-specific IIA component [EC:27.1.69]																		
K02777	PTS-Glc-EIIA, Crt, PTS-system, glucose-specific IIA component [EC:27.1.69]																		
K02768	PTS-Eru-EIIA, etr. PTS system, glucose-specific IIA component [EC:2.7.1.09]																		
K02769	PTS-Fru-Fills (rule) PTS system fructose-specific IIB component [EC2.7.1.69]																		
K02770	PTS-Eru-EIIC, fruA: PTS system, fructose-specific IIC component																		
K11202	PTS-Fru2-EIIB: PTS system, fructose-specific IIB-like component [EC:2 7 1 69]																		
K02773	PTS-Gat-EIIA, gatA: PTS system, galactitol-specific IIA component [EC-2,7,1,69]																		
K02775	PTS-Gat-EIIC, gatC: PTS system, galactitol-specific IIC component																		
K02821	PTS-Ula-EIIA, ulaC, sgaA; PTS system, ascorbate-specific IIA component [EC:2,7,1.69]																		
K02822	PTS-Ula-EIIB, ulaB, sgaB; PTS system, ascorbate-specific IIB component [EC:2.7.1.69]																		
K03475	PTS-Ula-EIIC, ulaA, sgaT; PTS system, ascorbate-specific IIC component																		
K02806	PTS-Ntr-EIIA, ptsN; PTS system, nitrogen regulatory IIA component [EC:2.7.1.69]																		
Other tr	ansporters	•					· · · ·								•				
K06188	aqpZ; aquaporin Z								 			1.1							
K02440	GLPF; glycerol uptake facilitator protein											1.1							

FIGURE 4: Distribution of genes encoding membrane transporters for carbohydrate. The abbreviations used for species/subspecies are as follows: INF, *B. longum* subsp. *infantis*; LON, *B. longum* subsp. *longum*; BRE, *B. breve*; ANI, *B. animalis* subsp. *animalis*; LAC, *B. animalis* subsp. *lactis*.

These enzymes are typical enzymes for the degradation of alpha-glucopyranose units such as pullulan, starch, and amylopectin. In particular, B. longum subsp. longum was shown to be genetically well equipped for the fermentation of plant-derived sugars (Figure 3), which are assumed to be not introduced into the infant gut before weaning. A large number of GH 43 and 51 family members, which are enzymes responsible for the degradation of arabinose/xylose units such as arabinofuranoside and xylan, were found to be specific to B. longum subsp. longum, with strain specificity for several of these genes. Taken together with the distribution of genes for host glycan utilisation, such a wide range of genes for carbohydrate utilisation provides an advantage for colonisation of the human intestine by B. longum subsp. *longum*. This result might explain why only *B. longum* subsp. *longum* is a predominant species in both the infant and the adult intestines [49].

3.5. Membrane Transporters for Carbohydrate. KO assignment indicated several differences in the distribution of carbohydrate transporters at the subspecies level. The subspecies that possessed the greatest number of transporter homologues was *B. longum* subsp. *infantis* (83.5 \pm 2.1), including taxon-specific transporters for GlcNAc, phosphonate, and urea, whereas *B. animalis* subsp. *lactis* possessed the lowest number of these homologues (33.7 \pm 1.3). All three species can assimilate fructose; however, only species of *B. longum* exhibited a set of high-affinity fructose-specific ABC transporters (K02056, K02057, and K02058), which have been reported to be involved in efficiently converting fructose to acetate under certain gut conditions [5]. *B. longum* subsp. *longum*

lacked a homologous gene set for arabinogalactan transport. However, twelve strains of *B. longum* subsp. *longum* possessed a set of homologous genes encoding extracellular enzymes for the degradation of arabinogalactan (BLLJ_1840) [50]. *B. breve* also exhibited certain unique ABC transporters for lactose/Larabinose, ribose, and sn-glycerol 3-phosphate.

We found distinctive differences between the three species in terms of the distribution of genes related to the phosphotransferase system (PTS), which is known to be a multicomponent system specific for sugar uptake that operates in global carbon regulation in many bacteria [51]. Strains of *B. longum* and *B. breve* possessed three homologues, encoding phosphoenolpyruvate-protein kinase, phosphocarrier protein, and beta-glucoside-PTS, whereas none of the *B. animalis* strains exhibited these genes. Additionally, nearly all strains of *B. breve* possessed homologues of N-acetylglucosamine, glucose, fructose, and ascorbate, which are involved in the PTS (Figure 4).

4. Conclusions

Certain strains belonging to the three bifidobacterial species targeted in this study have been reported to exert numerous beneficial effects on human health as probiotics. Probiotic functionality is generally thought to be strain dependent. In fact, our analysis confirmed that certain genes, such as those encoding membrane transporters and enzymes for host glycan utilisation, are strain dependent. However, our data also demonstrated that there are common characteristics in each species that may be important in light of the species' health-promoting effects on their hosts. Additionally, differences in characteristics might be a result of adaptation to the nutrient environments of each species (such as HRB versus non-HRB). Our results support previous observations based on the investigation of certain type strains of bifidobacterial species and enable the qualification of several of these characteristics as species common or strain specific [33, 39, 50, 52]. Taken together with other characteristics, such as vitamin metabolism [4, 53], colonisation factors [54], and extracellular components [55, 56], we believe that these findings will help to predict the features of probiotic strains. However, further study is needed to evaluate other non-HRB and HRB bifidobacterial species to attain a better understanding of the characteristics of these bacteria as well as the mechanisms underlying their residence in the host intestine and their potential functions as probiotics.

Conflict of Interests

All authors are employees of Morinaga Milk Industry Co., Ltd., which has several probiotic products marketed worldwide.

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