HAYATI
Journal of Biosciences

Vol. 33 No. 6, November 2026 1594-1604
DOI:10.4308/hjb.33.6.1594-1604
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Phenotypic Characterization and Genomic Profiling of Prebiotic Utilization in Human-Derived Indonesian *Bifidobacterium* Isolates

Figo[1], Patricia Celine[2], Hans Victor[1], Juandy Jo[1,3], Melanie Cornelia[4], Tjie Jan Tan[1], Marcelia Sugata[1*]

[1]*Department of Biology, Faculty of Health Sciences, Universitas Pelita Harapan, Tangerang 15810, Indonesia*
[2]*Faculty of Health, Medicine and Behavioural Sciences, The University of Queensland, Brisbane, Queensland, Australia*
[3]*Mochtar Riady Institute for Nanotechnology, Universitas Pelita Harapan, Tangerang 15810, Indonesia*
[4]*Department of Food Technology, Faculty of Science and Technology, Universitas Pelita Harapan, Tangerang 15811, Indonesia*

ARTICLE INFO

Article history:
Received June 26, 2026
Received in revised form July 22, 2026
Accepted September 14, 2026
Available Online October 29, 2026

KEYWORDS:
Bifidobacterium animalis,
Bifidobacterium breve,
fermentation activity,
gene prediction,
growth

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ABSTRACT

Bifidobacterium species are recognized for their beneficial roles in gut health, while prebiotics serve as selective substrates that support the growth of beneficial microorganisms. This study evaluated the responses of human-derived Indonesian *Bifidobacterium* isolates to different prebiotic substrates and explored their genomic potential for carbohydrate utilization. Four strains—*B. animalis* BR2-5, *B. breve* BS2-PS1, BS2-PS2, and BS2-PB3—were assessed using fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), xylo-oligosaccharide (XOS), inulin, maltodextrin, and L-arabinose. *In vitro* fermentation assays were conducted to evaluate growth responses and pH changes, while genome annotation was performed to identify putative genes related to carbohydrate utilization. *B. animalis* BR2-5 exhibited growth in all tested substrates, whereas *B. breve* strains grew primarily in maltodextrin, FOS, GOS, and inulin. GOS supported the highest growth response across all strains. Genome annotation revealed putative carbohydrate-active enzyme genes potentially associated with carbohydrate utilization pathways. These findings provide baseline information for the development of locally sourced synbiotic formulations and future investigations of Indonesian *Bifidobacterium* isolates.

1. Introduction

The use of natural microbial resources to promote human health has gained increasing attention as part of preventive nutrition and microbiome-based healthcare strategies. Among these resources, probiotics and prebiotics are widely recognized for their ability to modulate the intestinal microbiota and support host well-being. Probiotics are defined as live microorganisms that confer health benefits on the host when administered in adequate amounts, whereas prebiotics are selectively utilized substrates that promote the growth and activity of beneficial microorganisms in the gastrointestinal tract (Hill *et al.* 2014; Gibson *et al.* 2017). The complementary relationship between probiotics and prebiotics has stimulated growing interest in the development of synbiotic products that combine both components to enhance their functional effects.

Members of the genus *Bifidobacterium* are among the most extensively studied probiotic bacteria due to their important role in the human gut microbiota and their association with various health benefits. These Gram-positive, anaerobic bacteria are commonly detected in the gastrointestinal tract of healthy infants and adults and contribute to the maintenance of gut homeostasis

*Corresponding Author
E-mail Address: marcelia.sugata@lecturer.uph.edu

through carbohydrate fermentation, production of short-chain fatty acids, and modulation of immune responses (O'Callaghan and van Sinderen 2016; Abdelhamid *et al.* 2019). Previous studies have demonstrated that specific *Bifidobacterium* strains may alleviate gastrointestinal disorders, improve intestinal function, and contribute to host immune regulation (O'Callaghan and van Sinderen 2016; Abdelhamid *et al.* 2019). More recently, certain bifidobacterial species have also been reported to exhibit anticancer and anti-inflammatory activities, further highlighting their potential as functional microorganisms for human health applications (Faghfoori *et al.* 2021).

The growth and functional performance of Bifidobacteria are strongly influenced by the availability of suitable carbohydrate substrates. Various prebiotics, including fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), and inulin, have been shown to selectively stimulate bifidobacterial growth. However, the ability to utilize these substrates differs considerably among species and even among strains within the same species (Gibson *et al.* 2017; González-Herrera *et al.* 2020). Such variation is primarily associated with differences in carbohydrate transport systems and carbohydrate-active enzymes encoded within individual genomes. Consequently, understanding strain-specific prebiotic utilization is essential for selecting effective probiotic candidates and designing targeted synbiotic formulations (McLaughlin *et al.* 2015; Wang *et al.* 2020).

Advances in whole-genome sequencing have provided valuable opportunities to investigate the genetic basis of prebiotic utilization in probiotic bacteria. Genomic analysis enables the identification of genes encoding carbohydrate transporters, glycoside hydrolases, and other enzymes involved in oligosaccharide metabolism, thereby complementing phenotypic assessments of bacterial growth on specific substrates. For example, Marbun *et al.* (2026) combined genomic and phenotypic analyses to investigate galacto-oligosaccharide (GOS) and fructo-oligosaccharide (FOS) utilization by *Bifidobacterium breve* BS2-PS1. The study identified genes involved in oligosaccharide transport and degradation and demonstrated that the strain exhibited corresponding growth responses in GOS- and FOS-supplemented media. These findings highlight the value of integrating genomic profiling with experimental validation to distinguish strain-specific carbohydrate utilization phenotypes. Integrating *in vitro* fermentation assays with genomic analyses can therefore provide a more comprehensive understanding of probiotic functionality and substrate preference.

Despite extensive studies on commercially available *Bifidobacterium* strains, information regarding the prebiotic utilization capabilities of indigenous isolates from Indonesia remains limited. Human-derived bacterial strains originating from different geographical regions may possess unique metabolic characteristics due to variations in host diet, lifestyle, and microbial ecology. For example, a recent comparative genomic study of human gut bifidobacteria revealed substantial heterogeneity in carbohydrate utilization capabilities among bifidobacterial populations, reflecting differences in the distribution of carbohydrate-metabolism genes and adaptation to diverse ecological niches within the human gut (Arzamasov *et al.* 2025). These findings suggest human-derived isolates from different geographical and ecological backgrounds may harbor unique functional traits that are not fully represented by commercially available probiotic strains. Therefore, investigating locally isolated strains may contribute to the discovery of probiotic candidates with distinctive functional properties and potential applications in regionally relevant synbiotic products.

In the present study, four human-derived *Bifidobacterium* strains isolated in Indonesia were investigated, namely *Bifidobacterium animalis* BR2-5 obtained from infant feces and *Bifidobacterium breve* BS2-PS1, BS2-PS2, and BS2-PB3 isolated from human breast milk (Dosan *et al.* 2024). The objective of this study was to evaluate the ability of these strains to utilize six commercially available prebiotics, namely FOS, GOS, XOS, inulin, maltodextrin, and L-arabinose, through fermentation-based growth assays. In addition, whole-genome analyses were performed to identify genes potentially involved in carbohydrate metabolism and prebiotic utilization. The findings of this study are expected to improve understanding of strain-specific prebiotic utilization among Indonesian *Bifidobacterium* isolates and support the development of scientifically informed synbiotic applications.

2. Materials and Methods

2.1. Materials

Four human-derived *Bifidobacterium* strains were used in this study, namely *Bifidobacterium animalis* BR2-5 isolated from infant feces and *Bifidobacterium breve* BS2-PS1, BS2-PS2, and BS2-PB3 isolated from human breast milk. The strains and their whole-genome sequences were obtained from the Biology Study Program, Faculty of Health Sciences, Universitas Pelita Harapan. Stock cultures were maintained on Trypticase-

Phytone-Yeast extract (TPY) agar and routinely propagated in TPY broth under anaerobic conditions at 37°C using Thermo Scientific™ AnaeroGen sachets prior to experimental use. The prebiotic substrates evaluated in this study were fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), inulin, maltodextrin, and L-arabinose.

2.2. Preparation of Prebiotic-Supplemented Media

Prebiotic solutions, FOS (Xian Virgin Biological Technology Co., Xian, China), GOS (Xian Virgin Biological Technology Co., Xian, China), XOS (Jiangsu Rongye Technology Co., Jiangsu, China), inulin (NOW Foods, Bloomingdale, IL, USA), maltodextrin (Qinhuangdao Lihua Starch Co., China), and L-arabinose (HiMedia Laboratories, Maharashtra, India), were prepared and sterilized using a 0.22 µm syringe filter.

TPY broth (1 L) consisted of 17.6 g of Tryptone Soya Broth (TSB) (HiMedia Laboratories, India), 5 g of peptone (HiMedia Laboratories, India), 5 g of glucose, 2.5 g of yeast extract (Condalab, Madrid, Spain), 1 mL of Tween-80 (Vivantis Technologies, Malaysia), 0.5 g of L-cysteine (NOW Foods, USA), and 2 g of K_2HPO_4 (Merck, Darmstadt, Germany).

For the fermentation activity assay, TPY broth without glucose was prepared at a two-fold (2×) concentration, and 4% prebiotic solutions were added at a ratio of 1:1 to obtain a final prebiotic concentration of 2%. TPY broth containing 2% glucose was used as the positive control. TPY agar was prepared by adding 1.5% bacteriological agar to the respective media.

2.3. Preparation of Bacterial Liquid Culture

Four strains of *Bifidobacterium* (*B. animalis* BR2-5, *B. breve* BS2-PS1, *B. breve* BS2-PS2, and *B. breve* BS2-PB3) were previously isolated and identified by Dosan *et al.* (2024). Each strain was inoculated onto TPY agar using a four-way streak method and incubated for 72 h. A single colony from each strain was subsequently transferred into 5 mL of TPY broth. All incubations were carried out under anaerobic conditions at 37°C, maintained using Thermo Scientific™ AnaeroGen sachets.

2.4. Fermentation Activity Assay

One milliliter of bacterial liquid culture from each strain, with an OD_{600} value between 0.8 and 1.0, was inoculated into 9 mL of TPY broth supplemented with 2% (w/v) of FOS, GOS, XOS, inulin, maltodextrin, or L-arabinose and incubated anaerobically at 37°C for 72 h. TPY broth containing 2% (w/v) glucose served as the positive control, whereas TPY broth without an added carbon source served as the negative control. Fermentation activity was assessed by changes in culture pH after growth in carbohydrate-supplemented media, as previously described for probiotic bacteria (Sugata *et al.* 2024). The pH of each medium was measured before inoculation (0 h) and after incubation (72 h) using a pH meter. pH reduction was calculated as the difference between the initial and final pH values.

2.5. Growth Performance Assay

The growth of *B. animalis* BR2-5, *B. breve* BS2-PS1, *B. breve* BS2-PS2, and *B. breve* BS2-PB3 in TPY broth supplemented with different prebiotics was evaluated by quantifying colony-forming units (CFU) after 72 h of incubation. The bacterial cultures were grown under the same treatment conditions described for the fermentation activity assay, including TPY broth supplemented with 2% (w/v) FOS, GOS, XOS, inulin, maltodextrin, or L-arabinose. TPY broth supplemented with 2% (w/v) glucose was used as the positive control, whereas TPY broth without an added carbon source was used as the negative control. Viable cell counts were determined using the CFU enumeration method, which is commonly used for growth assessment of probiotic bacteria in carbohydrate-supplemented media (Sugata *et al.* 2024). Following incubation, cultures were serially diluted in sterile saline solution and spread onto TPY agar plates. Plates were incubated anaerobically at 37°C for 72 h before colonies were enumerated. Cultures were plated and enumerated in triplicate. The viable cell counts (CFU/mL) obtained after 72 h of incubation were used to evaluate growth performance.

2.6. Statistical Analysis

All experiments were performed using three independent biological replicates. Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism v8.0.2 (GraphPad Software, San Diego, CA, USA). For each strain, differences among prebiotic treatments were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test. A value of $p < 0.05$ was considered statistically significant.

2.7. Genome Assembly and Taxonomic Identification

Whole-genome sequence data of *B. animalis* BR2-5, *B. breve* BS2-PS1, *B. breve* BS2-PS2, and *B. breve* BS2-PB3, previously generated using Oxford Nanopore Technologies (Oxford, United Kingdom) by PT Genetika Science (Tangerang, Indonesia), were used for downstream bioinformatic analyses. De novo genome assembly was performed without the use of a reference genome. WGS data quality was assessed using Filtlong v0.2.1 (https://github.com/rrwick/Filtlong) and NanoPlot v1.40.2 (De Coster *et al.* 2018). Read correction and assembly were conducted using Canu v2.2 (Koren *et al.* 2017) and Flye v2.8.3-b1695 (Kolmogorov *et al.* 2019), respectively. The resulting assemblies were polished using Racon v1.5.0 (Vaser *et al.* 2017) for four rounds and Medaka v1.11.1 (Oxford Nanopore Technologies) for three rounds. Genome annotation and taxonomic identification were performed using the DDBJ Fast Annotation and Submission Tool (dFAST) (Tanizawa *et al.* 2018).

2.8. Carbohydrate-Active Enzymes

Carbohydrate-active enzyme analysis was performed using the Carbohydrate-Active Enzyme (CAZy) database through the dbCAN3 meta server (Zheng *et al.* 2023). The CAZy database catalogs glycoside hydrolases (GHs), carbohydrate esterases, polysaccharide lyases, glycosyltransferases, and other enzyme families involved in carbohydrate metabolism (Lombard *et al.* 2014). Particular attention was given to glycoside hydrolases (GHs), which are involved in the degradation of oligosaccharides and other carbohydrate substrates. Results were analyzed based on complete hits obtained from three prediction tools: HMMER, DIAMOND, and dbCAN_sub. Functional annotation of non-CAZyme genes was performed using the Rapid Annotations using Subsystems Technology (RAST) server (Brettin *et al.* 2015).

3. Results

3.1. Fermentation Activity and Growth Performance of *Bifidobacterium* spp.

Fermentation activity of *B. animalis* BR2-5 and the three *B. breve* strains (BS2-PS1, BS2-PS2, and BS2-PB3) was evaluated through pH reduction in TPY media supplemented with different prebiotics (Table 1). Across all strains, the no-sugar negative controls exhibited the highest pH values, ranging from 6.89 ± 0.09 in *B. animalis* BR2-5 to 6.95 ± 0.02 in *B. breve* BS2-PS1. All strains showed significant pH decreases in media containing FOS, GOS, inulin, and maltodextrin.

For *B. animalis* BR2-5, all tested prebiotics, including XOS (4.63 ± 0.08) and L-arabinose (4.78 ± 0.15), resulted in lower pH values than the negative control (6.89 ± 0.09). In contrast, the *B. breve* strains exhibited limited pH reductions in XOS- and L-arabinose-supplemented media, with values comparable to those of the negative controls. Among all tested substrates, GOS consistently produced the lowest pH values across the *B. breve* strains (4.19-4.26). FOS, inulin, and maltodextrin also supported marked acidification, whereas XOS and L-arabinose produced minimal changes in culture pH (Table 1).

After 72 h of incubation, colony-forming unit (CFU) counts revealed distinct growth responses among the tested

Table 1. pH values of TPY broth supplemented with 2% prebiotics after inoculation with *Bifidobacterium* strains following 72 h of anaerobic incubation at 37°C

	pH			
	B. breve BS2-PS1	*B. breve* BS2-PS2	*B. breve* BS2-PB3	*B. animalis* BR2-5
Negative control	6.95 ± 0.02^{A}	6.94 ± 0.03^{A}	6.94 ± 0.02^{A}	6.89 ± 0.09^{A}
Positive control (glucose)	4.58 ± 0.06^{E}	4.63 ± 0.05^{D}	4.31 ± 0.11^{C}	4.23 ± 0.01^{DE}
Malto-dextrin	5.34 ± 0.06^{C}	5.95 ± 0.19^{B}	4.89 ± 0.11^{B}	4.83 ± 0.03^{BC}
L-arabinose	6.36 ± 0.09^{B}	6.60 ± 0.19^{A}	6.66 ± 0.20^{A}	4.78 ± 0.15^{C}
XOS	6.84 ± 0.03^{A}	6.69 ± 0.03^{A}	6.77 ± 0.04^{A}	4.63 ± 0.08^{C}
Inulin	5.10 ± 0.01^{D}	5.48 ± 0.25^{C}	4.58 ± 0.10^{BC}	5.09 ± 0.16^{B}
FOS	4.22 ± 0.08^{F}	4.29 ± 0.18^{DE}	4.27 ± 0.06^{C}	4.54 ± 0.12^{CD}
GOS	4.18 ± 0.08^{F}	4.14 ± 0.06^{E}	4.16 ± 0.04^{C}	4.19 ± 0.02^{E}

Data are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among prebiotic treatments for each strain (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$). The positive control consisted of TPY broth supplemented with 2% (w/v) glucose to support bacterial growth, whereas the negative control consisted of TPY broth without an added carbon source to assess basal growth in the absence of fermentable carbohydrates

prebiotics (Table 2). For *B. animalis* BR2-5, the positive control containing glucose yielded 11.36 ± 2.61 × 10^8 CFU/mL, whereas the negative control yielded 2.91 ± 0.35 × 10^8 CFU/mL. All prebiotic treatments supported increased growth compared with the negative control. The highest CFU value was observed in GOS-supplemented media (310.06 ± 0.49 × 10^8 CFU/mL), followed by FOS (221.23 ± 1.70 × 10^8 CFU/mL) and inulin (196.23 ± 2.11 × 10^8 CFU/mL).

Similarly, the *B. breve* strains exhibited significantly higher CFU counts in GOS-, FOS-, inulin-, and maltodextrin-supplemented media than in the negative controls. In contrast, XOS and L-arabinose resulted in little or no increase in growth among the *B. breve* isolates. Overall, the highest cell densities across all isolates were generally observed in GOS-containing media (Table 2).

3.2. Genome Assembly Quality Statistics

The assembly quality statistics demonstrated that all genomes were assembled with high contiguity (Table 3). The three *B. breve* strains (PS1, PS2, and PB3) were each assembled into a single sequence, with total genome sizes of approximately 2.27 Mb and N50 values equivalent to the total assembly length. In addition, no assembly gaps were detected, as indicated by a gap ratio of 0%. These results reflect highly contiguous genome assemblies with near-complete chromosomal reconstruction.

In contrast, *B. animalis* BR2-5 possessed a smaller genome size (1.96 Mb) and was assembled into two sequences, with an N50 value of 1.87 Mb. Despite the presence of two assembled sequences, the assembly remained highly contiguous and gap-free, supporting its suitability for downstream genomic analyses. The GC content of the three *B. breve* strains was identical (58.9%), whereas *B. animalis* BR2-5 exhibited a slightly higher GC content (60.5%), consistent with species-level genomic differences.

Genome annotation revealed comparable genomic features among the three *B. breve* strains, including similar numbers of predicted coding sequences (1,983-1,997 CDSs), coding ratios (86.5-86.8%), and RNA genes. In contrast, *B. animalis* BR2-5 contained fewer predicted coding sequences (1,705 CDSs) and a distinct rRNA profile. No CRISPR loci were identified in the *B. breve* strains, whereas one CRISPR locus was detected in *B. animalis* BR2-5. Furthermore, no plasmid sequences were identified in any of the assembled genomes.

Although raw sequencing quality metrics, such as total read numbers, sequencing depth, and Q20/Q30 scores, were not available in the final assembly report, the assembly-level quality indicators, including contig number, N50, gap ratio, genome size, and GC content, collectively demonstrate that the assembled genomes were of high quality and suitable for comparative genomic analyses.

3.3. Distribution of Glycoside Hydrolase (GH) Families Among *Bifidobacterium* Isolates

The distribution and abundance of glycoside hydrolase (GH) gene families are presented in Figure 1. The three *B. breve* strains exhibited highly similar GH-family profiles, with comparable gene counts across detected GH families. In contrast, *B. animalis* BR2-5 displayed a distinct GH distribution pattern. Several GH families identified in the *B. breve* genomes were absent or present at lower copy numbers in BR2-5. Figure 1 further shows that multiple GH families were detected across all isolates, although their abundance varied among species.

Table 2. Growth of *Bifidobacterium* strains in TPY broth supplemented with 2% prebiotics, expressed as viable cell counts (CFU/mL) after 72 h of anaerobic incubation at 37°C

	Cell number (10^8 CFU/mL)			
	B. breve BS2-PS1	*B. breve* BS2-PS2	*B. breve* BS2-PB3	*B. animalis* BR2-5
Negative control	3.17 ± 0.57^{E}	3.39 ± 0.82^{E}	2.95 ± 0.50^{EF}	2.91 ± 0.35^{F}
Positive control (glucose)	11.69 ± 2.68^{D}	11.83 ± 2.35^{D}	9.28 ± 2.71^{DE}	11.36 ± 2.61^{E}
Malto-dextrin	8.94 ± 3.20^{D}	10.86 ± 4.91^{D}	10.86 ± 4.91^{D}	14.54 ± 1.46^{DE}
L-arabinose	3.31 ± 0.95^{E}	4.93 ± 2.17^{DE}	2.31 ± 0.14^{F}	15.84 ± 2.39^{DE}
XOS	3.54 ± 0.91^{E}	6.07 ± 2.56^{DE}	2.43 ± 0.43^{F}	17.73 ± 2.62^{D}
Inulin	155.24 ± 1.16^{C}	44.91 ± 0.66^{C}	87.63 ± 2.40^{C}	196.23 ± 2.11^{C}
FOS	177.37 ± 1.15^{B}	134.99 ± 2.07^{B}	138.73 ± 2.06^{B}	221.23 ± 1.70^{B}
GOS	279.23 ± 1.11^{A}	268.79 ± 2.02^{A}	241.80 ± 1.14^{A}	310.06 ± 0.49^{A}

Data are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among prebiotic treatments for each strain (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$). The positive control consisted of TPY broth supplemented with 2% (w/v) glucose to support bacterial growth, whereas the negative control consisted of TPY broth without an added carbon source to assess growth in the absence of carbohydrate supplementation

Table 3. Genome assembly quality statistics and genomic features of *B. breve* PS1, PS2, PB3, and *B. animalis* BR2-5

	B. breve PS2	*B. breve* PS1	*B. breve* PB3	*B. animalis* BR2-5
Total sequence length (bp):	2269407	2269504	2269404	1964884
Number of sequences:	1	1	1	2
Longest sequences (bp):	2269407	2269504	2269404	1866183
N50 (bp):	2269407	2269504	2269404	1866183
Gap ratio (%):	0	0	0	0
GC content (%):	58.9	58.9	58.9	60.5
Number of CDSs:	1997	1986	1983	1705
Average protein length:	327.6	329.9	331.1	331.2
Coding ratio (%):	86.5	86.6	86.8	86.2
Number of rRNAs:	4	4	4	9
Number of tRNAs:	54	54	54	54
Number of CRISPRs:	0	0	0	1

N50 represents the contig length at which 50% of the total assembled genome is contained in contigs of equal or greater length

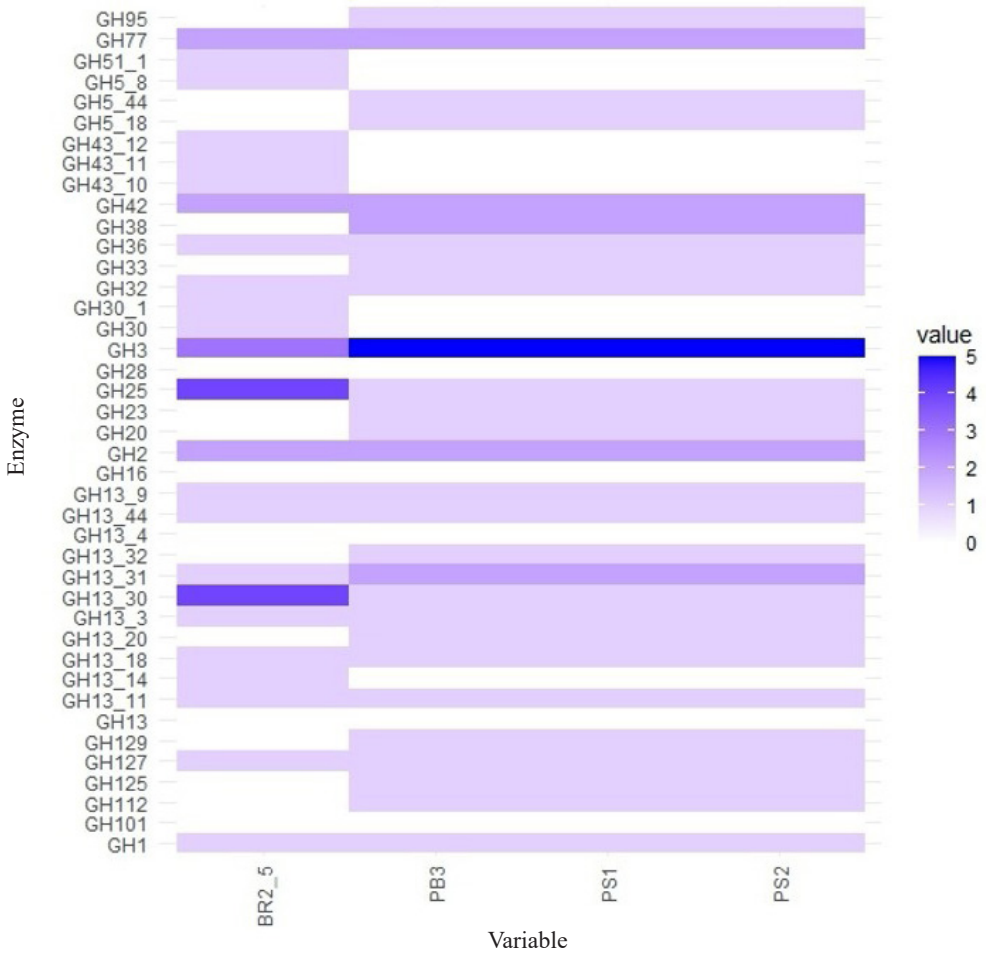


Figure 1. Heatmap showing the distribution and abundance of glycoside hydrolase (GH) families among *B. animalis* BR2-5, *B. breve* BS2-PB3, *B. breve* BS2-PS1, and *B. breve* BS2-PS2

3.4. Genome Analysis of Prebiotic Utilization Pathways

Genome annotation identified genes associated with the utilization of the prebiotics tested in this study (Table 4). For XOS utilization, *B. animalis* BR2-5 possessed genes encoding β-D-xylosidase, xylulose kinase, and xylose isomerase, whereas the three *B. breve* strains encoded only xylulose kinase. For maltodextrin metabolism, all isolates contained genes encoding 4-α-glucanotransferase, α-amylase, α-glucosidase, and pullulanase.

Genes associated with L-arabinose metabolism showed species-specific distributions. *B. animalis* BR2-5 possessed genes encoding L-arabinose isomerase, L-ribulokinase, and L-ribulose-phosphate epimerase, whereas the *B. breve* strains contained only the gene encoding L-ribulose-phosphate epimerase. In addition, all isolates encoded β-galactosidase and β-fructofuranosidase, enzymes associated with GOS, FOS, and inulin utilization.

Overall, all strains possessed genes corresponding to complete pathways for GOS, FOS, inulin, and maltodextrin metabolism, whereas complete pathways for XOS and L-arabinose utilization were found only in *B. animalis* BR2-5.

3.5. Proposed Carbohydrate Utilization Pathways in *Bifidobacterium* spp.

Figure 2 illustrates the proposed metabolic pathways for the utilization of GOS, FOS/inulin, XOS, maltodextrin, and L-arabinose in *Bifidobacterium* spp. GOS is hydrolyzed by β-D-galactosidase into galactose, whereas FOS and inulin are degraded into fructose. XOS is converted into D-xylose and subsequently metabolized through D-xylulose and D-xylulose-5-phosphate. Maltodextrin is hydrolyzed into smaller oligosaccharides and glucose, while L-arabinose is metabolized through L-ribulose and L-ribulose-5-phosphate prior to entering central carbohydrate metabolism.

4. Discussion

The present study demonstrated clear strain-specific differences in prebiotic utilization among human-derived *Bifidobacterium* isolates. Fermentation and growth assays consistently showed that GOS, FOS, and inulin supported the highest growth of all isolates, whereas XOS and L-arabinose supported growth primarily in *B. animalis* BR2-5. Because the tested prebiotics were supplied as the sole carbohydrate source, the observed increases in viable cell counts and medium acidification indicate that these substrates could be metabolized by the respective strains under the experimental conditions. The close relationship between pH reduction and CFU increase observed in this study is consistent with previous reports indicating that acidification assays are reliable indicators of carbohydrate metabolism and growth in bifidobacteria (Dong *et al.* 2024). However,

Table 4. Distribution of genes associated with prebiotic utilization pathways in *Bifidobacterium* isolates

Substrate	Enzyme name	Corresponding CAZymes	Presence of putative gene(s) (RAST and dbCAN3)	
			B. animalis BR2-5	*B. breve* (BS2-PS1, BS2-PS2, and BS2-PB3)
XOS	β- d-xylosidases (EC 3.2.1.37)	GH3/51	Yes	No
	Xylulose kinase (EC 2.7.1.17)	N/A	Yes	Yes
	Xylose isomerase (EC 5.3.1.5)	N/A	Yes	No
Malto-dextrin	4-α-glucanotransferase (amylomaltase) (EC 2.4.1.25)	GH77	Yes	Yes
	α-amylase (EC 3.2.1.1)	GH13	Yes	Yes
	α-glucosidase (EC 3.2.1.20)	GH13	Yes	Yes
	Pullulanase (EC 3.2.1.41)	GH13	Yes	Yes
L-arabinose	L-arabinose isomerase (EC 5.3.1.4)	N/A	Yes	No
	L-ribulokinase (EC 2.7.1.16)	N/A	Yes	No
	L-ribulose-phosphate epimerase (EC 5.1.3.4)	N/A	Yes	Yes
GOS	β-galactosidase	GH42	Yes	Yes
FOS, Inulin	β-fructofuranosidase	GH32	Yes	Yes

N/A means the corresponding enzyme is not considered as glycosyl hydrolase (GH)

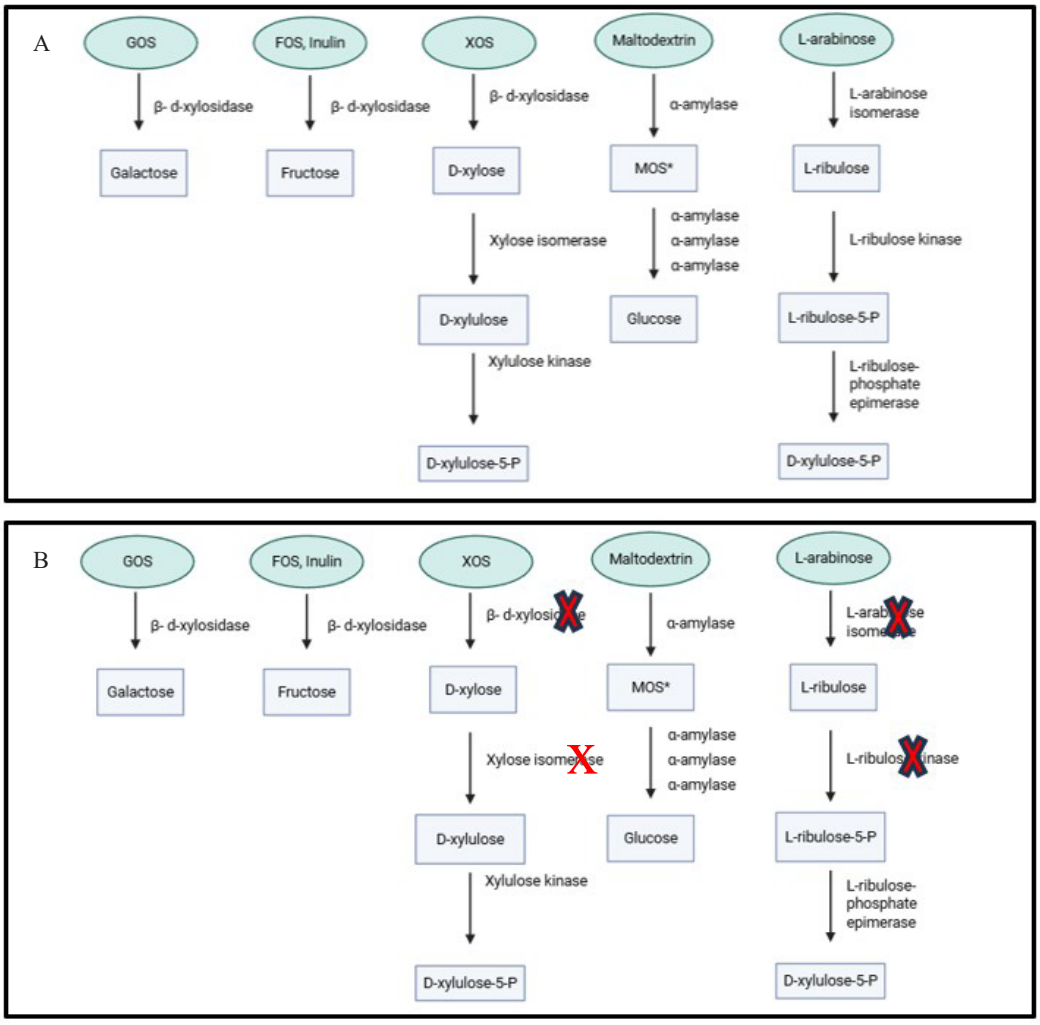


Figure 2. Proposed metabolic pathways for the utilization of prebiotic carbohydrates by *Bifidobacterium* spp. based on genome annotation results. (A) Proposed prebiotic utilization pathway for *B. animalis*, (B) Proposed prebiotic utiliz

direct quantification of substrate consumption was not performed, and therefore the extent of carbohydrate utilization could not be determined.

The superior performance of GOS is consistent with the established bifidogenic properties of this prebiotic. Since growth and acid production occurred only when prebiotics were supplied as the carbohydrate source, while the negative control showed minimal growth and negligible acidification, the results indicate that the tested strains were able to metabolize these substrates under the experimental conditions. Genome analyses showed that all isolates possessed the β-galactosidase gene within the lacS operon, enabling efficient hydrolysis and utilization of galactooligosaccharides (Akiyama *et al.* 2015). Similarly, the presence of β-fructofuranosidase in all strains explains the strong growth responses observed in FOS- and inulin-supplemented media. These findings agree with previous studies reporting that GOS and FOS are among the most effective prebiotics for stimulating bifidobacterial growth and short-chain fatty acid production (Kaewarsar *et al.* 2023). In particular, Marbun *et al.* (2026) demonstrated that *Bifidobacterium breve* BS2-PS1 efficiently utilized both GOS and FOS and possessed the corresponding genes involved in oligosaccharide metabolism. The present study extends those observations by demonstrating that three human milk-derived *B. breve* isolates (BS2-PS1, BS2-PS2, and BS2-PB3) exhibited similar substrate preferences and genomic capacities for GOS, FOS, and inulin utilization.

Maltodextrin also supported growth in all isolates, although to a lesser extent than GOS and FOS. The presence of genes encoding 4-α-glucanotransferase, α-amylase, α-glucosidase, and pullulanase suggests that all strains possess the enzymatic machinery necessary

for maltodextrin degradation (Khoroshkin *et al.* 2016). Similar observations were reported by McLaughlin *et al.* (2015), who found that *B. breve* UCC2003 was capable of utilizing maltodextrin, although the efficiency varied between strains. This characteristic may be advantageous for functional food applications because maltodextrin has also been reported to improve probiotic stability during microencapsulation (Corpas-Iguarán *et al.* 2023).

A notable finding of this study was the broader substrate utilization profile of *B. animalis* BR2-5. Unlike the *B. breve* strains, BR2-5 efficiently utilized both XOS and L-arabinose. This finding is supported by genome annotation, which revealed the presence of complete pathways for both substrates. For XOS metabolism, BR2-5 encoded β-D-xylosidase, xylose isomerase, and xylulose kinase, whereas the *B. breve* strains lacked key enzymes required for complete conversion of D-xylose into D-xylulose-5-phosphate. This observation agrees with previous reports describing variability in XOS utilization among bifidobacterial species and strains (Gupta *et al.* 2022; Dong *et al.* 2024).

Similarly, only BR2-5 possessed the complete set of genes required for L-arabinose metabolism, namely L-arabinose isomerase, L-ribulokinase, and L-ribulose-phosphate epimerase. The absence of L-arabinose isomerase and L-ribulokinase in the *B. breve* strains likely explains their limited growth in L-arabinose-supplemented media. These findings are consistent with previous genomic analyses showing that *B. animalis* generally possesses a more complete L-arabinose utilization pathway than *B. breve* (Milani *et al.* 2015).

Comparison of phenotypic and genomic data further demonstrated the value of integrating growth assays with genome-based predictions. Although several glycoside hydrolase families were identified in all isolates, not all predicted GHs corresponded to measurable utilization of the tested substrates. This indicates that the presence of GH genes alone may not fully predict metabolic activity under specific culture conditions. Instead, phenotypic assays remain essential for validating genomic predictions of prebiotic utilization.

The highly similar GH profiles observed among the three *B. breve* strains may explain their comparable growth responses across the tested prebiotics. In contrast, the distinct GH repertoire of *B. animalis* BR2-5 is consistent with its broader carbohydrate utilization capacity. Together, these findings demonstrate that genomic variation directly contributes to species-specific and strain-specific differences in substrate preference.

Overall, this study highlights the ecological and functional diversity of human-derived *Bifidobacterium* isolates from Indonesia. The broad metabolic versatility of *B. animalis* BR2-5 and the strong preference of *B. breve* strains for GOS, FOS, and inulin provide valuable information for the development of targeted synbiotic formulations. Future studies should evaluate short-chain fatty acid production, investigate synergistic combinations of prebiotics, and assess the performance of these isolates in food matrices and *in vivo* systems to further support their application as probiotic candidates.

In conclusion, Prebiotic utilization among Indonesian *Bifidobacterium* isolates was strain-dependent and closely associated with genomic potential. *Bifidobacterium animalis* BR2-5 exhibited the broadest substrate utilization profile, including XOS and L-arabinose, whereas *B. breve* BS2-PS1, BS2-PS2, and BS2-PB3 preferentially utilized GOS, FOS, inulin, and maltodextrin. Genomic analyses corroborated the phenotypic observations by revealing complete metabolic pathways corresponding to the substrates utilized by each strain. These findings provide a foundation for the development of targeted synbiotic formulations using locally derived probiotic candidates.

Acknowledgements

This work was supported by the Institute of Research and Community Service of Universitas Pelita Harapan (P-16-FaST/VII/2023 and P-005-S-FaST/I/2024).

References

Abdelhamid, A.G., El-Masry, S.S., El-Dougdoug, N.K., 2019. Probiotic *Lactobacillus* and *Bifidobacterium* strains possess safety characteristics, antiviral activities and host adherence factors revealed by genome mining. *EPMA J.* 10, 337-350. https://doi.org/10.1007/s13167-019-00184-z

Akiyama, T., Kimura, K., Hatano, H., 2015. Diverse galactooligosaccharides consumption by bifidobacteria: Implications of β-galactosidase–LacS operon. *Biosci. Biotechnol. Biochem*. 79, 664-672. https://doi.org/10.1080/09168451.2014.987204

Arzamasov, A.A., Rodionov, D.A., Hibberd, M.C., Gugure, J.L., Kent, J.E., Kazanov, M.D., Lyen, S.A., Elane, M.L., Sejane, K., Furst, A., Bode, L., Barratt, M.J., Gordon, J.I., Osterman, A.L., 2025. Integrative genomic reconstruction reveals heterogeneity in carbohydrate utilization across human gut bifidobacteria. *Nat. Microbiol.* 10, 2031-2047. https://doi.org/10.1038/s41564-025-02056-x

Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J., Olson, R., Overbeek, R., Parrello, B., Pusch, G.D., Shukla, M., Thomason, J.A., Stevens, R., Vonstein, V., Wattam, A.R., Xia, F., 2015. RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci. Rep*. 5, 8365. https://doi.org/10.1038/srep08365

Corpas-Iguarán, E.J., Triviño-Valencia, J., Tapasco-Alzate, O., Garcés-Gómez, Y.A., 2023. Evaluation of viability and survival of free and maltodextrin microencapsulated *Bifidobacterium animalis* subsp. animalis through spray-drying process. *Commun. Sci. Technol*. 8, 190-197. https://doi.org/10.21924/CST.8.2.2023.1239

De Coster, W., D'Hert, S., Schultz, D.T., Cruts, M., Van Broeckhoven, C., 2018. NanoPack: Visualizing and processing long-read sequencing data. *Bioinformatics.* 34, 2666-2669. https://doi.org/10.1093/bioinformatics/bty149

Dong, Y., Han, M., Fei, T., Liu, H., Gai, Z., 2024. Utilization of diverse oligosaccharides for growth by *Bifidobacterium* and *Lactobacillus* species and their *in vitro* co-cultivation characteristics. *Int. Microbiol*. 27, 941-952. https://doi.org/10.1007/s10123-023-00446-x

Dosan, R., Mudana, S.O., Julyanto, C.M.P., Purnama, E.T., Sugata, M., Jo, J., Tan, T.J., 2024. Isolation and identification of *Bifidobacterium* species from human breast milk and infant feces in Indonesia. *Biodiversitas.* 25, 337-343. https://doi.org/10.13057/biodiv/d250139

Faghfoori, Z., Faghfoori, M.H., Saber, A., Izadi, A., Khosroushahi, A.Y., 2021. Anticancer effects of bifidobacteria on colon cancer cell lines. *Cancer Cell Int*. 21, 258. https://doi.org/10.1186/s12935-021-01971-3

Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K., Reid, G., 2017. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol*. 14, 491-502. https://doi.org/10.1038/nrgastro.2017.75

González-Herrera, S.M., Bermúdez-Quiñones, G., Ochoa-Martínez, L.A., Rutiaga-Quiñones, O.M., Gallegos-Infante, J.A., 2020. Synbiotics: A technological approach in food applications. *J. Food Sci. Technol*. 58, 811-818. https://doi.org/10.1007/s13197-020-04532-0

Gupta, M., Bangotra, R., Sharma, S., Vaid, S., Kapoor, N., Dutt, H.C., Bajaj, B.K., 2022. Bioprocess development for production of xylooligosaccharides prebiotics from sugarcane bagasse with high bioactivity potential. *Ind. Crops Prod*. 178, 114591. https://doi.org/10.1016/j.indcrop.2022.114591

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., Sanders, M.E., 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. *Hepatol*. 11, 506-514. https://doi.org/10.1038/nrgastro.2014.66

Kaewarsar, E., Chaiyasut, C., Lailerd, N., Makhamrueang, N., Peerajan, S., Sirilun, S., 2023. Effects of synbiotic *Lacticaseibacillus paracasei*, *Bifidobacterium breve*, and prebiotics on growth stimulation of beneficial gut microbiota. *Foods.* 12, 3847. https://doi.org/10.3390/foods12203847

Khoroshkin, M.S., Leyn, S.A., van Sinderen, D., Rodionov, D.A., 2016. Transcriptional regulation of carbohydrate utilization pathways in the *Bifidobacterium* genus. *Front. Microbiol*. 7, 120. https://doi.org/10.3389/fmicb.2016.00120

Kolmogorov, M., Yuan, J., Lin, Y., Pevzner, P.A., 2019. Assembly of long, error-prone reads using repeat graphs. *Nat. Biotechnol*. 37, 540-546. https://doi.org/10.1038/s41587-019-0072-8

Koren, S., Walenz, B.P., Berlin, K., Miller, J.R., Bergman, N.H., Phillippy, A.M., 2017. Canu: Scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res*. 27, 722-736. https://doi.org/10.1101/gr.215087.116

Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B., 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res*. 42, D490-D495. https://doi.org/10.1093/nar/gkt1178

Marbun, K.T., Figo, Purnomo, J.S., Dikson, Jo, J., 2026. *In vitro* and *in silico* evaluation of fructo-oligosaccharide and galacto-oligosaccharide utilisation by *Bifidobacterium breve* strain BS2-PS1. *J. Trop. Biodivers. Biotechnol*. 11, jtbb24842. https://doi.org/10.22146/jtbb.24842

McLaughlin, H.P., Motherway, M.O.C., Lakshminarayanan, B., Stanton, C., Ross, R.P., Brulc, J., Menon, R., O'Toole, P.W., van Sinderen, D., 2015. Carbohydrate catabolic diversity of bifidobacteria and lactobacilli of human origin. *Int. J. Food Microbiol*. 203, 109-121. https://doi.org/10.1016/j.ijfoodmicro.2015.03.008

Milani, C., Lugli, G.A., Duranti, S., Turroni, F., Mancabelli, L., Ferrario, C., Mangifesta, M., Hevia, A., Viappiani, A., Scholz, M., Arioli, S., Sanchez, B., Lane, J., Ward, D.V., Hickey, R., Mora, D., Segata, N., Margolles, A., van Sinderen, D., Ventura, M., 2015. Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut. *Sci. Rep*. 5, 15782. https://doi.org/10.1038/srep15782

O'Callaghan, A., van Sinderen, D., 2016. Bifidobacteria and their role as members of the human gut microbiota. *Front. Microbiol*. 7, 925. https://doi.org/10.3389/fmicb.2016.00925

Sugata, M., Kim, Y., Rachmah, A.F., Oei, J., Purnama, E.T., Nitbani, A.C.J., Victor, H., Jo, J., Tan, T.J., 2024. Assessment of probiotic characteristics of *Lactiplantibacillus plantarum* SU-KC1a isolated from human breast milk in Indonesia. *Appl. Food Biotechnol*. 11, e31. https://doi.org/10.22037/afb.v11i1.45707

Tanizawa, Y., Fujisawa, T., Nakamura, Y. 2018. DFAST: A flexible prokaryotic genome annotation pipeline for faster genome publication. *Bioinformatics.* 34, 1037-1039. https://doi.org/10.1093/bioinformatics/btx713

Vaser, R., Sović, I., Nagarajan, N., Šikić, M., 2017. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Res*. 27, 737-746. https://doi.org/10.1101/gr.214270.116

Wang, S., Xiao, Y., Tian, F., Zhao, J., Zhang, H., Zhai, Q., Chen, W., 2020. Rational use of prebiotics for gut microbiota alterations: Specific bacterial phylotypes and related mechanisms. *J. Funct. Foods.* 66, 103838. https://doi.org/10.1016/j.jff.2020.103838

Zheng, J., Ge, Q., Yan, Y., Zhang, X., Huang, L., Yin, Y., 2023. dbCAN3: Automated carbohydrate-active enzyme and substrate annotation. *Nucleic Acids Res*. 51, W115-W121. https://doi.org/10.1093/nar/gkad328