The metabolic profile of *Bifidobacterium dentium* reflects its status as a human gut commensal

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**Abstract**

**Background:** Bifidobacteria are commensal microbes of the mammalian gastrointestinal tract. In this study, we aimed to identify the intestinal colonization mechanisms and key metabolic pathways implemented by *Bifidobacterium dentium*.

**Results:** *B. dentium* displayed acid resistance, with high viability over a pH range from 4 to 7; findings that correlated to the expression of Na+/H+ antiporters within the *B. dentium* genome. *B. dentium* was found to adhere to human MUC2+ mucus and harbor mucin-binding proteins. Using microbial phenotyping microarrays and fully-defined media, we demonstrated that in the absence of glucose, *B. dentium* could metabolize a variety of nutrient sources. Many of these nutrient sources were plant-based, suggesting that *B. dentium* can consume dietary substances. In contrast to other bifidobacteria, *B. dentium* was largely unable to grow on compounds found in human mucus; a finding that was supported by its glycosyl hydrolase (GH) profile. Of the proteins identified in *B. dentium* by proteomic analysis, a large cohort of proteins were associated with diverse metabolic pathways, indicating metabolic plasticity which supports colonization of the dynamic gastrointestinal environment.

**Conclusions:** Taken together, we conclude that *B. dentium* is well adapted for commensalism in the gastrointestinal tract.

**Keywords:** Bifidobacteria, Metabolism, Carbohydrates, Glycans, Acid stress, Intestine, Commensal

**Introduction**

Bifidobacteria are important members of the Actinobacteria phylum within the human intestinal microbiota [1–10]. The establishment of bifidobacteria in the intestine is connected with beneficial health effects, including immune development, neuromodulation, inhibition of pathogens, and modulation of the intestinal microbiota composition [11–23]. To produce these beneficial effects, bifidobacteria must be able to survive gastrointestinal (GI) transit and persist in the dynamic environment of the intestine. Thus, analysis of the mechanisms of intestinal survival and colonization are pivotal to understand the functional activities of bifidobacteria.

Nutrient availability and utilization shapes the composition and gene expression of the intestinal microbiota [11, 24–30]. Broad genomic approaches have predicted that bifidobacteria can use a wide variety of nutrient sources to colonize the human GI tract [5, 25, 31–35]. More direct studies that have examined growth parameters of bifidobacteria have largely focused on carbohydrate metabolism [36]. As a result, information about the physiology and metabolic profiles of any one *Bifidobacterium*...
species is fragmented. Identifying the strategies used by specific bifidobacteria to harvest dietary nutrients is important for defining the metabolic properties that underpin ecological fitness in and adaptation to the human intestinal environment. Moreover, this information could be employed to increase the presence of select bifidobacteria in the intestine and harness their associated health benefits.

The aim of this study was to identify key pathways in ecological niche development of Bifidobacterium dentium. B. dentium is a member of the oral and intestinal microbiome. It is frequently isolated from healthy infant stool [3, 6–8] and has an approximate relative abundance of 0.7% in healthy human adults according to the Human Microbiome Project consortium [37–41]. We have previously demonstrated that B. dentium colonizes gnotobiotic mice, promotes goblet cell maturation, secretion of the mucin protein MUC2, stimulates intestinal serotonin production, generates the neurotransmitter γ-aminobutyric acid (GABA), alleviates visceral hypersensitivity and regulates the gut-brain-axis [21–23, 38, 42]. The importance of these functions in GI health motivated us to characterize the metabolic profile of B. dentium to identify environmental queues that can influence intestinal colonization.

We sought to characterize the metabolic capacity of B. dentium using microbial phenotype microarray technology, genome analysis and proteomics. This work is among the first to delineate the metabolic profile of B. dentium ATCC 27678. Our data suggest that B. dentium adheres to the intestinal mucus layer, exhibits acid resistance, and utilizes a wide range of physiologically abundant dietary nutrient sources commonly found in the intestine. These data suggest that B. dentium is well-adapted for life in the gastrointestinal tract.

Methods
Bacterial culture conditions
Bifidobacterium dentium ATCC 27678 (ATCC, American Type Culture Collection) was grown in de Man, Rogosa and Sharpe (MRS) medium (Difco) in an anaerobic workstation (Anaerobe Systems AS-580) at 37 °C overnight in a mixture of 5% CO2, 5% H2, and 90% N2. Bacterial growth was measured by optical density (OD600nm) using a spectrophotometer. For intestinal adhesion assays, B. dentium was grown overnight in MRS anaerobically at 37 °C and bacterial cells were pelleted by centrifugation at 5000 x g for 5 min. Cell pellets were washed three times with sterile anaerobic PBS to remove residual MRS and the bacterial pellet was resuspended in anaerobic PBS containing 10 μM carboxyfluorescein diacetate succinimidyl ester (CFDA-SE; Thermo Fisher Scientific, Waltham, MA; #V12883) and incubated for 1 h anaerobically at 37 °C. Following incubation, bacterial cells were pelleted by centrifugation at 5000 x g for 5 min, and were washed 3-5x with sterile anaerobic PBS. B. dentium fluorescence was confirmed by microscopy and were used for adhesion with the HT29-PTX mammalian cell cultures.

For an acid stress test, B. dentium was grown in MRS anaerobically at 37 °C for 8 h to exponential phase and bacterial cells were pelleted by centrifugation at 5000 x g for 5 min. B. dentium was resuspended at an OD600nm = 2.0 in MRS at a pH of 7.6, 7.0, 6.0, 5.0, 4.0, and 3.0 to simulate the different regions of the GI tract. B. dentium was incubated anaerobically at 37 °C for 2 h in the various pH conditions. Following incubation, B. dentium cells were pelleted by centrifugation at 5000 x g for 5 min, washed 2x to remove residual MRS and then resuspended in anaerobic PBS. Cells were stained with the LIVE/DEAD BacLight Bacterial Viability Stains (Thermo Fisher Scientific cat# L7012) according to the manufacturer’s details. Briefly, B. dentium was mixed with a 2x LIVE/DEAD BacLight staining reagent mixture and incubated for 15 min in the dark at 37 °C anaerobically. Then a 100 μL volume of each of the B. dentium cell suspensions were added to a black-walled 96-well flat-bottom microplate. Fluorescence was recorded using the following excitation (ex) and emission (em) wavelengths: ex: 485 nm/em: 530 nm (green) and ex: 485 nm/em: 630 nm (red) on a Synergy H1 Microplate Reader (Bio-Tek Instruments, Inc.). Viabilities were calculated with the following equation: (ex:485/em: 530 values)/(ex:458/em: 630 values) × 100% (Ratio green/red × 100%).

Intracellular pH assay
B. dentium was grown in MRS for 24 h from a starter culture inoculated at OD600nm = 0.1. From this starter culture, a 100 μL volume of bacterial suspension was transferred to a conical bottomed 96-well plate and pelleted by centrifugation at 2000 x g for 5 min. Cell pellets were washed twice in live cell imaging solution (LCIS, Molecular Probes) and then resuspended in LCIS containing 1x pHrodo Red AM dye (provided as 1000x in dimethyl sulfoxide, DMSO) and 1x PowerLoad (provided as 100x) (Molecular Probes). B. dentium was incubated anaerobically at 37 °C for 30 min. Following incubation, bacterial cells were pelleted by centrifugation at 2000 x g for 5 min to remove excess staining solution and then were resuspended in a 100 μL volume of LCIS. Using a vacuum manifold with ~ 5 in Hg vacuum pressure, B. dentium cells were immobilized on a 0.22 μm-pore polycarbonate filter plate (Millipore Sigma, Burlington, MA). Filters were washed once by vacuum and wells were refilled with LCIS. The filter plate was then loaded into a Synergy HT plate reader with incubation at 37 °C. A citrate buffer series was used to examine intracellular pH due to the wide pH range and its successful application with other lactic acid bacteria [43].
Fluorescence (ex: 560 nm/em: 590 nm) was recorded every 5 min over a 50 min timeframe, first in a common buffer (pH 7.6, min 0–10), then in the test buffers at pH 3–8 (min 10–50). Higher relative fluorescence unit (RFU) values indicate more acidic conditions. Standard curves were generated from fluorescence readings taken over 10 min in potassium citrate buffers at pH 4.5, 5.5, 6.5, and 7.5 in the presence of 10 μM valinomycin and 10 μM nigericin to equilibrate intra- and extracellular pH. Intracellular pH was calculated at the final test buffer time point (t = 50 min) from linear regression lines.

Biolog phenotypic microarray
For Biolog assays, B. dentium was grown overnight (~16 h) in MRS as described above. Cells were then diluted 1:20 in a fully-defined medium, termed LDM4 (Lactic Acid Bacteria Defined Media 4) [44], lacking glucose. Each well of Biolog NPGM2 and PM1 microarrays (Biolog, Inc., Haywood, CA, USA) was seeded with a 100 μL volume of cell suspension. Growth was monitored by Optical density (OD_{600nm}) readings at 10 min intervals for 16 h. Growth was assessed compared to a negative control well lacking any carbon substrate and a value of OD_{600nm} ≥ 0.2 was considered positive (n = 2 independent biological replicates per plate).

Bacterial genome analysis
The genome of B. dentium ATCC 27678 (GCF_00017213 5.1) was downloaded from NCBI and functionally assessed using the web-based tools NCBI Conserved Domain Database, Carbohydrate Active Enzymes (CAZy; www.cazy.org), and KEGG [45–48].

Mammalian culture conditions
HT29-MTX cells were obtained from Millipore-Sigma (#12040401). Cells were maintained in Gibco Dulbecco’s Modified Eagle Medium (Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS) in a humidified atmosphere at 37 °C, 5% CO₂. Cells were tested for Mycoplasma using the Mycoplasma Detection Kit (Lonza, cat # LT07–518). For adhesion assays, HT29-MTX cells were seeded at 2 × 10⁵ cells on poly-L-lysine coated round coverslips and incubated for 3–5 days until confluent. When monolayers were confluent, HT29-MTX cells were incubated with Hoechst 33342 staining dye solution (Invitrogen) in PBS for 10 min at 37 °C, washed, and treated with 1 × 10⁷ cells of CFDA-tagged B. dentium for 1 h aerobically at 37 °C. After the incubation, non-adhered cells were removed with 3x washes of PBS and cells were fixed with Clarke’s Fixative to maintain the mucus architecture. A subset of cells were used for Scanning Electron Microscopy (SEM) imaging using a FEI XL-30FEG microscope. Cells that were reserved for immunostaining were permeabilized with 0.1% Triton-X for 30 min at room temperature, blocked with PBS containing 10% donkey serum, and incubated with an anti-human MUC2 antibody (Santa Cruz, cat # sc-515,032; 1: 200 dilution) overnight at 4 °C. Following PBS washes, cells were incubated with donkey-anti-mouse AlexaFluor 555 (Life Technologies, cat # A11004; 1:1000 dilution) for 1 h at room temperature. Coverslips were mounted to slides using FluoroMount (Thermo Fisher Scientific) and slides were imaged on the Nikon Eclipse TiE inverted microscope.

Scanning electron microscopy (SEM)
Following imaging, the wells of the slides were washed gently with PBS containing Mg²⁺ and Ca²⁺ (2x) and fixed in 2.5% glutaraldehyde in PBS for 1 h at room temperature as previously described [42]. The black compartment of the CELLview slide was detached, the slide was dehydrated with ethanol, and coated in 20 nm of gold using a desktop sputtering system (Denton Desk II). All slides were viewed in a FEI XL-30FEG SEM microscope operated with an electron beam acceleration voltage of 12 kV [42].

Proteomic analysis
Chemical and reagents
Optima LC/MS-grade acetonitrile (ACN), formic acid (FA), and water, and Promega™ porcine trypsin protease were all purchased from Thermo Fisher Scientific. Ammonium bicarbonate (BioUltra-grade) was purchased from Millipore-Sigma.

Proteomics sample preparation
Bacterial sample pellets were suspended in a 200-μL volume of water and samples were sonicated in an ultrasonic bath for 30 min. Afterwards, the samples were centrifuged for 5 min at 10,000 rpm. The resulting sample supernatants containing bacterial protein were removed from the pellet of cellular debris, and the samples were dried in a SpeedVac overnight to yield pelleted protein in the sample tubes. A 100-μL volume of a 10 μg/mL solution of porcine trypsin prepared in a 25 mM ammonium bicarbonate solution was added to the pelleted protein contained in each sample tube, and the samples were vortex-mixed for 1 min and incubated at 37 °C for 8 h.

Chromatography
Tryptic digest samples were chromatographically separated on a Dionex Ultimate 3000 RSLC nano-system (Thermo Scientific) using an Acclaim PepmapTM C-18 capillary column (75 μm (ID) × 150 mm (L), Thermo Scientific) outfitted with an Acclaim PepmapTM C18 trap column (100 μm (ID) × 20 mm (L), Thermo Scientific). Chromatography was performed as previously described [22]. Elution gradients were prepared from an aqueous...
mobile phase (A) of H2O:ACN:FA (94.9:5:0.1 v/v/v) and an organic mobile phase (B) of ACN:FA (99.9:0.1 v/v). Sample elution onto the trap column was carried out using a trap column buffer of H2O:ACN:FA (94.9:5:0.1 v/v/v). Samples (5 μL) were injected onto the trap column with a flow rate of 5 μL/min. After 5 min, the loading valve was switched to allow the sample to elute off the trap column at a flow rate of 300 nL/min and onto the capillary column for separation. The elution gradient used was specified as follows: Started at 1% B, ramped up linearly to 45% B over 37 min; ramped up linearly to 80% B over 1 min; held at 80% B for 1 min; ramped back to 1% B over 1 min and held for 16 min to re-equilibrate.

Mass spectrometric conditions
Samples were analyzed using an Orbitrap Fusion mass spectrometer (Thermo Scientific) using a nanoionization source operated in positive ion mode with the following source conditions: ionspray voltage, static at 1.6 kV; ion transfer tube temperature, 275 °C. Global MS acquisition parameters were specified as follows: precursor ion scan range, mass-to-charge (m/z) 200 - m/z 1000; S-lens RF level, 60%; data type, profile; MIPS, true; charge states, 2–4; data dependent mode, top speed; precursor priority, most intense; exclude after n times, 1: exclusion duration, 200 μs; CID activation, true; CID collision energy, 35%; detector type, Orbitrap; scan range mode, auto; orbitrap resolution, 120,000; automatic gain control (AGC) target, 5.0e4; maximum injection time, 60 ms; microscans, 1; and, tandem MS data format, profile. Data were acquired with the Thermo Scientific Xcalibur software package (v4.1.50).

Mass spectrometric data analysis
Data were analyzed using Proteome Discoverer (Thermo Scientific). Data were searched against the Uniprot Bifidobacterium database (8 Aug 2020) which also included a common contaminant database. The following parameters were used for protein identification: minimum precursor mass, 350 Dalton (Da); maximum precursor mass, 5000 Da; minimum peak count, 1; minimum peptide length, 6; precursor mass tolerance, 10 ppm; fragment mass tolerance, 0.02 Da; dynamic modifications included oxidation for methionine and acetylation for protein N-terminus; target and decoy database, concatenated; validation based on q-Value; and, FDR targets were 0.01 for strict and 0.05 for relaxed.

Statistics and graphs
Graphs and heat maps were created using GraphPad Prism software (version 8) (GraphPad Inc.). Comparisons were made with either One-way ANOVA or Repeated Measures ANOVA with the Holm-Sidak post-hoc test. The data are presented as mean ± standard deviation, with P < 0.05 (*) considered statistically significant. See Tables 3 and 4 and Supplemental Table 1 and Supplemental Table 2 for statistical analysis.

Results
B. dentium is acid resistant and can adhere to intestinal mucus suggesting its efficacy to persist in the gastrointestinal tract
To colonize the gastrointestinal tract microbes must overcome the acidic pH found in the stomach and upper GI to gain access to the lower parts of the intestine. In general, bifidobacteria are considered to have a weak acid tolerance with the exception of B. animalis [49] and B. longum [50–52]. Using the NCBI Conserved Domain Database to assess the functional annotation of the B. dentium ATCC 27678 (GCF_000172135.1) proteins, we noted the presence of three Na+/H+ antiporter proteins that may contribute to acid tolerance in B. dentium [45–48] (Table 1). To address the ability of B. dentium to survive transit through the low pH environment of the stomach and small intestine experimentally, we incubated overnight cultures of B. dentium in MRS with pH of 3, 4, 5, 6, and 7 for 2 h. After incubation, cell viability was obtained by live/dead cell staining using a BACLight kit as examined by microscopy (Fig. 1a) and fluorescence plate reader quantification (Fig. 1b). B. dentium exhibited high viability over a pH range from 4 to 7, as denoted by green staining, and > 90% viability levels. Even in highly acidic conditions (pH 3), B. dentium still maintained 41.8% ± 2.4 viability, indicating acid tolerance. Intracellular pH analysis by pHrodo Red AM dye demonstrated that surviving B. dentium were able to regulate their intracellular pH over time (Fig. 1c, d). These data suggest that B. dentium is acid-tolerant, similar to findings with gastrointestinal colonizers B. animalis and B. longum [53], and thus likely able to survive the transit through the upper GI system.

The ability to adhere to the intestinal mucus layer is an important aspect of bifidobacterial colonization [54]. Mucus adhesion is proposed to enhance epithelial integrity and pathogen exclusion [55], as well as provide closer access for metabolite delivery and immune

Table 1 Notable ion antiporters identified from the genome of Bifidobacterium dentium ATCC 27678

| Accession No. | Description                  | Proposed Function |
|---------------|------------------------------|-------------------|
| WP_003840740.1 | Na+/H+ antiporter            | Acid tolerance    |
| WP_003837813.1 | cation:proton antiporter     | Acid tolerance    |
| WP_003838459.1 | Na+/H+ antiporter            | Acid tolerance    |
stimulation [56, 57]. Investigation of the functional annotation of B. dentium ATCC 27678 indicated the presence of glycosyltransferase enzymes that promote bacterial capsular formation along with pilin and fimbrial proteins (Table 2). These proteins have been previously associated with mucus adherence and GI colonization and may also facilitate mucus adhesion for B. dentium. To assess adhesion of B. dentium to intestinal mucus, we added fluorescently-tagged B. dentium to human mucin-producing HT29-MTX monolayers for 1 h and examined adhesion by immunostaining (Fig. 2). Similar to other well characterized Bifidobacterium strains, we observed robust adhesion of B. dentium to MUC2 mucin by immunostaining (Fig. 2a) and SEM imaging (Fig. 2b). The ability of B. dentium to withstand acidic conditions and adhere to intestinal mucus highlights its potential to inhabit the intestine.

**B. dentium** metabolism of dietary sugars and select host derived carbon sources promote growth

Previous work examining microbe metabolism have relied on adding nutritional components to rich media, such as MRS. However, the complexity of this medium, poses challenges to identification of the dietary requirements of these microbes. To circumvent this challenge we used LDM4 media, a fully-defined medium which can be prepared to exact nutrient composition [44]. Using LDM4 prepared without glucose, we examined the ability of B. dentium to grow on a number of nutrients as individual primary carbon sources by Biolog phenotype analysis (Fig. 3, Tables 3 and 4). Growth of B. dentium was examined in the presence of 50 different sugars, including hexoses (Fig. 3a), pentoses (b), ketoses (c), disaccharides (d), trisaccharides (e), sugar alcohols (f), deoxy sugars (g) and amino sugars (h). As shown in

![Image](image-url)
the graphs and heat map (Fig. 3i), *B. dentium* exhibits robust growth with a variety of carbohydrates, with substantial growth found on galactose, mannose, maltose, xylose, sucrose, truanose, D-raffinose, maltotriose, stachyose, D-melibiose, gentiobiose, sedoheptulosan and D-mannitol. These findings are consistent with the *B. dentium* Bd1 genome analysis [33], which indicated that *B. dentium* encoded a wide variety of enzymes for the fermentation of pentose sugars. The utilization of sucrose by *B. dentium* ATCC 27678 was reflected by our proteomic analysis, in which we identified 24 proteins involved in sucrose metabolism (Table 5). We also identified proteins involved in maltose-binding (MalE), maltose transport systems (MalG), xylose isomerases, xylose ABC transporters, raffinose-binding, mannose metabolism, and ABC sugar transports (Table 5); findings which reflect our growth profiles. No appreciable growth was observed on many sugars, including D- or L-arabitol, lactitol, maltitol, D-lactose, D-cellboiose, D-trehalose, lactulose, fucose, among others. The inability of *B. dentium* ATCC 27678 to use fucose is consistent a previous study that demonstrate that *B. dentium* DSM 20436 and VBif10D2 are unable to use fucose in mYCF medium [58]. In this capacity, *B. dentium* resembles most *Bifidobacterium* species which are largely unable to use fucose [58].

The *B. dentium* ATCC 27678 genome contains 88 glycosyl hydrolase (GH) genes from 25 different GH families (Fig. 4). The majority of the *B. dentium* ATCC 27678 GH genes are found in families GH3 (14%), GH13 (14%), and GH43 (16%). The GH13 family

Table 2 Notable glycosyltransferases and proteins involved in adhesion identified from the genome of *Bifidobacterium dentium* ATCC 27678

| Accession No. | Description | Proposed Function |
|---------------|-------------|------------------|
| WP_003837192.1 | Glycosyltransferase family 2 | Bacterial capsule biosynthesis |
| WP_003837196.1 | Glycosyltransferase family 2 | Bacterial capsule biosynthesis |
| WP_003836797.1 | Glycosyltransferase family 2 | Bacterial capsule biosynthesis |
| WP_003836799.1 | Glycosyltransferase family 1 | Exopolysaccharide biosynthesis |
| WP_003837542.1 | Glycosyltransferase family A | Mannose-1-phosphate guanylyltransferase |
| WP_003837819.1 | Glycosyltransferase family 2 | Bacterial capsule biosynthesis |
| WP_003838069.1 | Glycosyltransferase family B | GT transferase |
| WP_034257238.1 | Glycosyltransferase family 4 protein | Cell wall biosynthesis |
| WP_034489000.1 | Glycosyltransferase | Anthranilate phosphoribosyltransferase |
| WP_034257219.1 | Nucleotidytransferases | 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase |
| WP_003837207.1 | Isopeptide-forming fimbrial protein | Pilus formation |

![Fig. 2](image_url) *B. dentium* adheres to mucus-producing human intestinal epithelial cells. a Representative immunofluorescence images of *B. dentium* ATCC 27678 (yellow) co-localization with MUC2 (blue) in mucin-producing human HT29-MTX colonic cells after 1 h incubation (scale bar = 50 μm). b Scanning electron micrograph of *B. dentium* and HT29-MX cells after 1 h incubation (scale bar = 5 μm).
encodes enzymes which degrade α-glucoside linkages, such as α-amylases [59, 60], while the GH43 family contains xylanase (which break down plant-derived hemicellulose into xylose and arabinose) as well as arabinases (which degrade complex polysaccharides or arabino-oligosaccharides and liberate L-arabinose). The GH3 family notably contains β-glucosidases, β-xylosidases, N-acetylhexosaminidase, and other enzymes. The presence of these GHs suggests a high propensity to degrade dietary plant polysaccharides.

Interestingly, we observed few GHs associated with human milk oligosaccharide (HMOs) or mucin degradation. *B. dentium* did possess genes in GH2 (6.8%; galactosidase); GH 29 (1.1%; fucosidase), and GH125 (1.1%; mannosidase). Surprisingly, *B. dentium* lacked GH33, GH101, GH129, GH84, GH85, GH89, GH95, GH20, and GH38; which are involved in HMO and mucin degradation and common in some *Bifidobacterium* species [42]. Consistent with these findings, the experimental carbohydrate utilization profile (Fig. 3) indicates poor or absent growth.

![Image](envevik-et-al-bmc-microbiology-2021-21-154-page-7-of-26.png)

**Fig. 3** *B. dentium* grows on select sugars in the absence of glucose. *B. dentium* ATCC 27678 was grown anaerobically at 37 °C in Biolog plates with a fully-defined media (LDM4) preparation that lacked glucose. Growth was monitored over 16 h by plate reader in plate containing a hexoses, b pentoses, c ketoses, d disaccharides, e trisaccharides, f sugar alcohols, g deoxy sugars and h amino sugars. i For visualization, heat maps were generated for all sugars at time 0, 8.3 and 16.0 h. All data are presented as mean ± stdev.
### Table 3

Statistics from growth curves at time point, 8.3 h. Significant $p$ values are denoted as follows: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

| Comparison                  | 95.00% CI of diff.             | Significant? | $P$ Value |
|-----------------------------|--------------------------------|--------------|-----------|
| Negative vs. D-Galactose    | -0.7584 to -0.09959            | **           | 0.0022    |
| Negative vs. D-Mannose      | -0.8634 to -0.2046             | ****         | <0.0001   |
| Negative vs. alpha-D-Glucose| -0.8554 to -0.1966             | ****         | <0.0001   |
| Negative vs. Sedoheptulosen | -0.7294 to -0.07059            | **           | 0.0061    |
| Negative vs. D-Xylose       | -0.6594 to -0.0005865          | *            | 0.0492    |
| Negative vs. D-Mannitol     | -0.7794 to -0.1206             | **           | 0.0011    |
| Negative vs. D-Mannose      | -0.7194 to -0.06059            | **           | 0.0084    |
| Negative vs. D-Mannitol     | -0.7994 to -0.1406             | ***          | 0.0005    |
| Negative vs. Maltoolose     | -0.9494 to -0.2906             | ****         | <0.0001   |
| Negative vs. Sucrose        | -1.049 to -0.3906              | ****         | <0.0001   |
| Negative vs. Turanose       | -0.8294 to -0.1706             | ***          | 0.0002    |
| Negative vs. D-Raffinose    | -0.8694 to -0.2106             | ****         | <0.0001   |
| Negative vs. Maltotriose    | -0.9094 to -0.2506             | ****         | <0.0001   |
| Negative vs. Stachyose      | -0.8794 to -0.2206             | ****         | <0.0001   |
| Negative vs. D-Gluconic Acid| -0.5014 to -0.03863            | **           | 0.0097    |
| Negative vs. L-Proline      | -0.7237 to -0.03635            | *            | 0.0206    |
| Negative vs. Sec-Butylamine | 0.004907 to 0.03509            | *            | 0.013     |
| Negative vs. Amygdalin      | -0.4010 to -0.09401            | ****         | <0.0001   |
| Negative vs. Arbutin        | -0.3325 to -0.02551            | *            | 0.0107    |
| Negative vs. Salicin        | -0.3335 to -0.02651            | *            | 0.01      |

| Comparison                  | 95.00% CI of diff.             | Significant? | $P$ Value |
|-----------------------------|--------------------------------|--------------|-----------|
| Negative vs. D-Mannose      | -0.8481 to -0.2299             | ****         | <0.0001   |
| Negative vs. alpha-D-Glucose| -0.9221 to -0.3039             | ****         | <0.0001   |
| Negative vs. Sedoheptulosen | -0.6891 to -0.07092            | **           | 0.0052    |
| Negative vs. D-Mannitol     | -0.6491 to -0.03092            | *            | 0.0198    |
| Negative vs. Gentiose       | -0.8191 to -0.2009             | ****         | <0.0001   |
| Negative vs. Maltoolose     | -0.7591 to -0.1409             | ***          | 0.0004    |
| Negative vs. Sucrose        | -0.8091 to -0.1909             | ****         | <0.0001   |
| Negative vs. Turanose       | -0.8791 to -0.2609             | ****         | <0.0001   |
| Negative vs. D-Raffinose    | -0.7291 to -0.1109             | **           | 0.0012    |
| Negative vs. Maltotriose    | -0.8391 to -0.2209             | ****         | <0.0001   |
| Negative vs. Stachyose      | -0.7891 to -0.1709             | ***          | 0.0001    |
| Negative vs. D-Gluconic Acid| -0.3574 to -0.08257            | ****         | <0.0001   |
| Negative vs. Amygdalin      | -0.6605 to -0.4385             | ****         | <0.0001   |
| Negative vs. Inosine        | -0.2255 to -0.003522           | *            | 0.038     |
| Negative vs. Arbutin        | -0.4595 to -0.2375             | ****         | <0.0001   |
| Negative vs. Salicin        | -0.4375 to -0.2155             | ****         | <0.0001   |
on components of host-derived glycans as a sole carbon source, including lactose, N-acetylgalactosamine, N-acetylglucosamimic acid or N-acetylneuraminic acid that would most likely require GH genes from families GH33, GH95, and GH101 (Fig. 3a, d, g, h and i). Consistent with the presence of GH43 and GH125, host-associated galactose and mannose supported growth of *B. dentium* (Fig. 3a), indicating select host factors influence *B. dentium* colonization and growth. As expected, *B. dentium* growth (i.e., a final OD 600nm of > 0.2) was also observed on certain plant-derived carbohydrates such as maltose, melibiose, sucrose, ribose, fructose, and turanose (Fig. 3i). These data suggest that in the absence of glucose, *B. dentium* is able to support its growth via 14 different sugars, most of which are plant-derived and may have variable availability depending on the host diet.

*B. dentium* has limited ability to use amino acids, nucleosides and polymers as a sole carbon source

The ability to metabolize peptides and amino acids is a common feature among gut microbiota [61]. However, amino acids and nucleotides are often studied as nitrogen sources rather than a primary carbon source. Currently, little information is available on the ability of bifidobacteria to use these substrates as both primary carbon and nitrogen sources in the absence of additional carbohydrates. We examined the growth of *B. dentium* during a time course on a panel of 32 amino acids and amino acid derivatives in LDM4 lacking glucose (Fig. 5, Tables 3 and 5). Surprisingly, *B. dentium* could use 14 amino acids as sole carbon sources to support limited growth over short time periods ≤8.3 h (OD 600nm > 0.2, representing growth) (Fig. 5a, b). These amino acids included D-aspartic acid, D-serine, D-threonine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-glutamine, L-serine, L-threonine, tyramine, Glycyl-L-aspartic acid, Glycyl-L-glutamic acid, and Glycyl-L-Proline. Next, we examined *B. dentium* on glycosides and specifically nucleosides (Fig. 6a-d). *B. dentium* had significant growth using amygdalin, arbutin and salicin (Fig. 6a, c), consistent with findings in pigs that these glycosides promote the growth of certain *Bifidobacterium* strains [62]. In contrast, no growth was observed with nucleosides (Fig. 6b, d), cyclodextrin polymers (Fig. 6e, h), or polysorbates (Fig. 6g, h). Interestingly, we observed no significant growth with several polysaccharides (Fig. 6f, h), including inulin, which has been shown in mouse studies and human clinical trials to lead to an increase in bifidobacteria [13, 63–68]. To simulate the diverse number of carbon sources in the GI tract, we supplemented inulin containing LDM4 with glucose and we observed that the combination of carbon sources supported more growth than glucose alone (at 2.5 h: LDM4 glucose control OD 600nm = 0.39 ± 0.11, Inulin = 0.54 ± 0.12; mean ± stdev). Finally, we analyzed the ability of *B. dentium* to grow with 59 different organic acid sources. Of the organic acids

| Comparison                  | 95.00% CI of diff.     | Significant? | P Value     |
|-----------------------------|------------------------|--------------|-------------|
| Negative vs. D-Mannose      | –0.8481 to –0.2299     | ****         | < 0.0001    |
| Negative vs. alpha-D-Glucose| –0.9221 to –0.3039     | ****         | < 0.0001    |
| Negative vs. Sedoheptulosan | –0.6891 to –0.07092    | **           | 0.0052      |
| Negative vs. D-Mannitol     | –0.6491 to –0.30902    | *            | 0.0198      |
| Negative vs. Gentiose       | –0.8191 to –0.2009     | ****         | < 0.0001    |
| Negative vs. Maltose        | –0.7591 to –0.1409     | ***          | 0.0004      |
| Negative vs. Sucrose        | –0.8091 to –0.1909     | ****         | < 0.0001    |
| Negative vs. Turanose       | –0.8791 to –0.2609     | ****         | < 0.0001    |
| Negative vs. D-Raffinose    | –0.7291 to –0.1109     | **           | 0.0012      |
| Negative vs. Maltotriose    | –0.8391 to –0.2209     | ****         | < 0.0001    |
| Negative vs. Stachyose      | –0.7891 to –0.1709     | ***          | 0.0001      |
| Negative vs. D-Gluconic Acid| –0.3574 to –0.08257    | ****         | < 0.0001    |
| Negative vs. Amygdalin      | –0.6605 to –0.4385     | ****         | < 0.0001    |
| Negative vs. Inosine        | –0.2255 to –0.003522   | *            | 0.038       |
| Negative vs. beta-Methyl-DXyloside| –0.06148 to 0.1605 | ns          | 0.9514      |
| Negative vs. Arbutin        | –0.4595 to –0.2375     | ****         | < 0.0001    |
| Negative vs. Salicin        | –0.4375 to –0.2155     | ****         | < 0.0001    |
Table 5 Proteins identified in *B. dentium* ATCC 27678 by proteomic analysis

| Pathway Description                      | Pathway Accession | # Proteins |
|------------------------------------------|-------------------|------------|
| 2-Oxocarboxylic acid metabolism          | bde01210          | 12         |
| 2-Oxocarboxylic acid metabolism          | bks01210          | 9          |
| 2-Oxocarboxylic acid metabolism          | blf01210          | 5          |
| 2-Oxocarboxylic acid metabolism          | blx01210          | 1          |
| 2-Oxocarboxylic acid metabolism          | bln01210          | 1          |
| ABC transporters                         | bde02010          | 26         |
| ABC transporters                         | bks02010          | 6          |
| ABC transporters                         | blf02010          | 4          |
| Acarbose and validamycin biosynthesis    | bde00525          | 1          |
| Acarbose and validamycin biosynthesis    | bks00525          | 1          |
| Acarbose and validamycin biosynthesis    | boa00525          | 1          |
| Alanine, aspartate and glutamate metabolism | bde00250   | 9          |
| Alanine, aspartate and glutamate metabolism | bks00250   | 4          |
| Alanine, aspartate and glutamate metabolism | blf00250   | 4          |
| Alanine, aspartate and glutamate metabolism | bln00250   | 3          |
| Amino sugar and nucleotide sugar metabolism | bde00520   | 13         |
| Amino sugar and nucleotide sugar metabolism | bks00520   | 9          |
| Amino sugar and nucleotide sugar metabolism | blf00520   | 7          |
| Amino sugar and nucleotide sugar metabolism | bln00520   | 2          |
| Amino sugar and nucleotide sugar metabolism | boa00520   | 2          |
| Aminoacyl-tRNA biosynthesis              | bde00970          | 19         |
| Aminoacyl-tRNA biosynthesis              | bks00970          | 11         |
| Aminoacyl-tRNA biosynthesis              | blf00970          | 5          |
| Aminoacyl-tRNA biosynthesis              | bln00970          | 4          |
| Aminoacyl-tRNA biosynthesis              | boa00970          | 2          |
| Arginine biosynthesis                    | bde00220          | 8          |
| Arginine biosynthesis                    | bks00220          | 7          |
| Arginine biosynthesis                    | blf00220          | 6          |
| Arginine biosynthesis                    | bln00220          | 3          |
| Bacterial secretion system               | bde03070          | 6          |
| Bacterial secretion system               | bks03070          | 3          |
| Bacterial secretion system               | blf03070          | 2          |
| Bacterial secretion system               | bln03070          | 1          |
| beta-Alanine metabolism                  | bde00410          | 1          |
| beta-Lactam resistance                   | bde01501          | 3          |
| beta-Lactam resistance                   | blf01501          | 1          |
| Biosynthesis of amino acids              | bde01230          | 38         |
| Biosynthesis of amino acids              | bks01230          | 23         |
| Biosynthesis of amino acids              | blf01230          | 18         |
| Biosynthesis of amino acids              | bln01230          | 7          |
| Biosynthesis of amino acids              | boa01230          | 4          |
| Biosynthesis of amino acids              | blx01230          | 1          |
| Biosynthesis of antibiotics              | bde01130          | 51         |
| Biosynthesis of antibiotics              | bks01130          | 31         |
Table 5 Proteins identified in *B. dentium* ATCC 27678 by proteomic analysis (Continued)

| Pathway Description                             | Pathway Accession | # Proteins |
|-------------------------------------------------|-------------------|------------|
| Biosynthesis of antibiotics                     | blf01130          | 19         |
| Biosynthesis of antibiotics                     | bln01130          | 9          |
| Biosynthesis of antibiotics                     | boa01130          | 7          |
| Biosynthesis of antibiotics                     | bx01130           | 2          |
| Biosynthesis of antibiotics                     | blm01130          | 1          |
| Biosynthesis of secondary metabolites           | bde01110          | 62         |
| Biosynthesis of secondary metabolites           | bks01110          | 37         |
| Biosynthesis of secondary metabolites           | blf01110          | 24         |
| Biosynthesis of secondary metabolites           | bln01110          | 10         |
| Biosynthesis of secondary metabolites           | boa01110          | 8          |
| Biosynthesis of secondary metabolites           | bx01110           | 2          |
| Biosynthesis of secondary metabolites           | blm01110          | 1          |
| Butanoate metabolism                            | bde00650          | 4          |
| Butanoate metabolism                            | bks00650          | 2          |
| Butanoate metabolism                            | blf00650          | 1          |
| Butanoate metabolism                            | boa00650          | 1          |
| CS-Branced dibasic acid metabolism              | bde00660          | 4          |
| CS-Branced dibasic acid metabolism              | bks00660          | 3          |
| Carbon metabolism                               | bde01200          | 25         |
| Carbon metabolism                               | bks01200          | 15         |
| Carbon metabolism                               | blf01200          | 10         |
| Carbon metabolism                               | boa01200          | 5          |
| Carbon metabolism                               | bln01200          | 3          |
| Chloroalkane and chloroalkene degradation       | bks00625          | 1          |
| Chloroalkane and chloroalkene degradation       | bde00625          | 1          |
| Citrate cycle (TCA cycle)                       | bde00020          | 5          |
| Citrate cycle (TCA cycle)                       | bks00020          | 2          |
| Citrate cycle (TCA cycle)                       | boa00020          | 2          |
| Citrate cycle (TCA cycle)                       | blf00020          | 1          |
| Cyanamoano acid metabolism                      | bde00460          | 1          |
| Cyanamoano acid metabolism                      | boa00460          | 1          |
| Cysteine and methionine metabolism              | bde00270          | 8          |
| Cysteine and methionine metabolism              | bks00270          | 6          |
| Cysteine and methionine metabolism              | blf00270          | 3          |
| Cysteine and methionine metabolism              | boa00270          | 2          |
| Cysteine and methionine metabolism              | blm00270          | 1          |
| Degradation of aromatic compounds               | bks01220          | 1          |
| Degradation of aromatic compounds               | bde01220          | 1          |
| DNA replication                                 | bde03030          | 2          |
| DNA replication                                 | bks03030          | 1          |
| Fatty acid biosynthesis                         | boa00061          | 2          |
| Fatty acid biosynthesis                         | bks00061          | 1          |
| Fatty acid biosynthesis                         | blf00061          | 1          |
| Fatty acid biosynthesis                         | bde00061          | 1          |
Table 5 Proteins identified in *B. dentium* ATCC 27678 by proteomic analysis (Continued)

| Pathway Description | Pathway Accession | # Proteins |
|---------------------|-------------------|------------|
| Fatty acid degradation | bks00071 | 1 |
| Fatty acid degradation | bde00071 | 1 |
| Fatty acid metabolism | boa01212 | 2 |
| Fatty acid metabolism | bks01212 | 1 |
| Fatty acid metabolism | bfo1212 | 1 |
| Fatty acid metabolism | bde01212 | 1 |
| Fructose and mannose metabolism | bde00051 | 2 |
| Fructose and mannose metabolism | bks00051 | 1 |
| Fructose and mannose metabolism | boa00051 | 1 |
| Galactose metabolism | bde00052 | 6 |
| Galactose metabolism | bks00052 | 4 |
| Galactose metabolism | bfo00052 | 3 |
| Galactose metabolism | boa00052 | 1 |
| Glutathione metabolism | bde00480 | 3 |
| Glutathione metabolism | bks00480 | 2 |
| Glutathione metabolism | bfo00480 | 1 |
| Glycerolipid metabolism | bks00561 | 1 |
| Glycerolipid metabolism | bfo00561 | 1 |
| Glycerolipid metabolism | bde00561 | 1 |
| Glycerolipid metabolism | bks00564 | 3 |
| Glycerolipid metabolism | bfo00564 | 2 |
| Glycerolipid metabolism | bln00564 | 1 |
| Glycine, serine and threonine metabolism | bde00260 | 9 |
| Glycine, serine and threonine metabolism | bks00260 | 3 |
| Glycine, serine and threonine metabolism | bln00260 | 1 |
| Glycine, serine and threonine metabolism | bfo00260 | 1 |
| Glycine, serine and threonine metabolism | boa00260 | 1 |
| Glycolysis / Gluconeogenesis | bde00010 | 11 |
| Glycolysis / Gluconeogenesis | bks00010 | 10 |
| Glycolysis / Gluconeogenesis | bfo00010 | 5 |
| Glycolysis / Gluconeogenesis | bln00010 | 3 |
| Glycolysis / Gluconeogenesis | boa00010 | 3 |
| Glycolysis / Gluconeogenesis | bln00010 | 1 |
| Glycolysis / Gluconeogenesis | bfo00010 | 1 |
| Glycolysis / Gluconeogenesis | bde00630 | 6 |
| Glycolysis / Gluconeogenesis | bks00630 | 2 |
| Glycolysis / Gluconeogenesis | bfo00630 | 2 |
| Histidine metabolism | bfo00340 | 1 |
| Histidine metabolism | bde00340 | 1 |
| Homologous recombination | bde03440 | 2 |
| Homologous recombination | bks03440 | 1 |
| Inositol phosphate metabolism | boa00562 | 2 |
| Inositol phosphate metabolism | bks00562 | 1 |
| Inositol phosphate metabolism | bde00562 | 1 |
Table 5 Proteins identified in *B. dentium* ATCC 27678 by proteomic analysis (Continued)

| Pathway Description                              | Pathway Accession | # Proteins |
|--------------------------------------------------|-------------------|------------|
| Lipopolysaccharide biosynthesis                  | boa00540          | 1          |
| Lysine biosynthesis                              | bde00300          | 4          |
| Lysine biosynthesis                              | bks00300          | 3          |
| Metabolic pathways                               | bde01100          | 118        |
| Metabolic pathways                               | bks01100          | 71         |
| Metabolic pathways                               | blf01100          | 48         |
| Metabolic pathways                               | boa01100          | 19         |
| Metabolic pathways                               | bln01100          | 17         |
| Metabolic pathways                               | bks01100          | 7          |
| Metabolic pathways                               | bks00680          | 4          |
| Methane metabolism                               | bde00680          | 7          |
| Methane metabolism                               | bks00680          | 2          |
| Methane metabolism                               | bln00680          | 2          |
| Methane metabolism                               | blf00680          | 2          |
| Methane metabolism                               | boa00680          | 1          |
| Microbial metabolism in diverse environments     | bde01120          | 36         |
| Microbial metabolism in diverse environments     | bks01120          | 24         |
| Microbial metabolism in diverse environments     | blf01120          | 13         |
| Microbial metabolism in diverse environments     | boa01120          | 6          |
| Microbial metabolism in diverse environments     | bln01120          | 3          |
| Microbial metabolism in diverse environments     | blm01120          | 1          |
| Mismatch repair                                  | bde03430          | 3          |
| Mismatch repair                                  | bks03430          | 2          |
| Monobactam biosynthesis                          | bks00261          | 2          |
| Monobactam biosynthesis                          | bde00261          | 2          |
| Naphthalene degradation                          | bks00626          | 1          |
| Naphthalene degradation                          | bde00626          | 1          |
| Nicotinate and nicotinamide metabolism           | blf00760          | 2          |
| Nicotinate and nicotinamide metabolism           | bks00760          | 2          |
| Nicotinate and nicotinamide metabolism           | bde00760          | 2          |
| Nitrogen metabolism                              | bde00910          | 3          |
| Nitrogen metabolism                              | bks00910          | 2          |
| Nitrogen metabolism                              | blf00910          | 2          |
| One carbon pool by folate                        | bde00670          | 2          |
| One carbon pool by folate                        | blf00670          | 2          |
| One carbon pool by folate                        | bks00670          | 1          |
| Other glycan degradation                         | bde00511          | 1          |
| Oxidative phosphorylation                        | bde00190          | 9          |
| Oxidative phosphorylation                        | bks00190          | 6          |
| Oxidative phosphorylation                        | blf00190          | 4          |
| Oxidative phosphorylation                        | bln00190          | 3          |
| Oxidative phosphorylation                        | boa00190          | 2          |
| Pantothenate and CoA biosynthesis                | bde00770          | 6          |
Table 5 Proteins identified in *B. dentium* ATCC 27678 by proteomic analysis (Continued)

| Pathway Description                                   | Pathway Accession | # Proteins |
|-------------------------------------------------------|-------------------|------------|
| Pantothenate and CoA biosynthesis                     | bks00770          | 4          |
| Pantothenate and CoA biosynthesis                     | blf00770          | 3          |
| Pantothenate and CoA biosynthesis                     | bks00770          | 1          |
| Pantothenate and CoA biosynthesis                     | bln00770          | 1          |
| Pantothenate and CoA biosynthesis                     | boa00770          | 1          |
| Pentose and glucuronate interconversions              | bde00040          | 4          |
| Pentose and glucuronate interconversions              | blf00040          | 1          |
| Pentose and glucuronate interconversions              | bks00040          | 1          |
| Pentose phosphate pathway                             | bks00030          | 10         |
| Pentose phosphate pathway                             | blf00030          | 5          |
| Peptidoglycan biosynthesis                            | bde00550          | 4          |
| Peptidoglycan biosynthesis                            | bks00550          | 1          |
| Peptidoglycan biosynthesis                            | blf00550          | 1          |
| Phenylalanine, tyrosine and tryptophan biosynthesis    | bde00400          | 2          |
| Phenylalanine, tyrosine and tryptophan biosynthesis    | blf00400          | 1          |
| Phenylalanine, tyrosine and tryptophan biosynthesis    | bks00400          | 1          |
| Phosphotransferase system (PTS)                       | bks02060          | 1          |
| Phosphotransferase system (PTS)                       | bde02060          | 1          |
| Polyketide sugar unit biosynthesis                    | bks00523          | 1          |
| Polyketide sugar unit biosynthesis                    | bde00523          | 1          |
| Polyketide sugar unit biosynthesis                    | boa00523          | 1          |
| Purine metabolism                                     | bde00230          | 19         |
| Purine metabolism                                     | bks00230          | 10         |
| Purine metabolism                                     | blf00230          | 6          |
| Purine metabolism                                     | bln00230          | 4          |
| Purine metabolism                                     | bks00230          | 2          |
| Pyrimidine metabolism                                 | bde00240          | 11         |
| Pyrimidine metabolism                                 | bks00240          | 7          |
| Pyrimidine metabolism                                 | blf00240          | 5          |
| Pyrimidine metabolism                                 | bln00240          | 3          |
| Pyrimidine metabolism                                 | boa00240          | 2          |
| Pyrimidine metabolism                                 | bks00240          | 1          |
| Pyruvate metabolism                                   | bde00620          | 10         |
### Table 5 Proteins identified in *B. dentium* ATCC 27678 by proteomic analysis (Continued)

| Pathway Description                  | Pathway Accession | # Proteins |
|--------------------------------------|-------------------|------------|
| Pyruvate metabolism                 | bks00620          | 5          |
| Pyruvate metabolism                 | boa00620          | 3          |
| Pyruvate metabolism                 | blf00620          | 2          |
| Pyruvate metabolism                 | blm00620          | 1          |
| Quorum sensing                      | bde02024          | 17         |
| Quorum sensing                      | bks02024          | 5          |
| Quorum sensing                      | blf02024          | 4          |
| Quorum sensing                      | bln02024          | 1          |
| Riboflavin metabolism               | boa00740          | 1          |
| Ribosome                             | bde03010          | 47         |
| Ribosome                             | bks03010          | 17         |
| Ribosome                             | bln03010          | 15         |
| Ribosome                             | blf03010          | 14         |
| Ribosome                             | boa03010          | 10         |
| Ribosome                             | blx03010          | 5          |
| RNA degradation                      | bde03018          | 6          |
| RNA degradation                      | bln03018          | 4          |
| RNA degradation                      | bks03018          | 4          |
| RNA degradation                      | blf03018          | 3          |
| RNA degradation                      | boa03018          | 2          |
| RNA degradation                      | bad03018          | 1          |
| RNA polymerase                       | bde03020          | 4          |
| RNA polymerase                       | bks03020          | 3          |
| RNA polymerase                       | blf03020          | 2          |
| RNA polymerase                       | blx03020          | 1          |
| RNA polymerase                       | bln03020          | 1          |
| Secondary bile acid biosynthesis    | bks00121          | 1          |
| Secondary bile acid biosynthesis    | bde00121          | 1          |
| Selenocompound metabolism           | bde00450          | 2          |
| Selenocompound metabolism           | bks00450          | 1          |
| Selenocompound metabolism           | blf00450          | 1          |
| Sphingolipid metabolism             | bde00600          | 1          |
| Starch and sucrose metabolism       | bde00500          | 11         |
| Starch and sucrose metabolism       | bks00500          | 6          |
| Starch and sucrose metabolism       | blf00500          | 4          |
| Starch and sucrose metabolism       | boa00500          | 2          |
| Starch and sucrose metabolism       | bln00500          | 1          |
| Streptomycin biosynthesis            | bks00521          | 3          |
| Streptomycin biosynthesis            | bde00521          | 3          |
| Streptomycin biosynthesis            | boa00521          | 2          |
| Streptomycin biosynthesis            | blf00521          | 1          |
| Taurine and hypotaurine metabolism  | bde00430          | 3          |
| Taurine and hypotaurine metabolism  | bks00430          | 2          |
| Taurine and hypotaurine metabolism  | blf00430          | 1          |
examined, *B. dentium* growth was only stimulated to statistical significance by D-glucuronic acid (Fig. 7a-f). These data point to the metabolic flexibility of *B. dentium* to use select amino acids, glycosidases and organic acids to support microbial growth in the absence of a carbohydrate source.

These findings of metabolic functionality are supported by our proteomic analysis of *B. dentium* ATCC 27678 from LDM4 cultures using glucose as a primary carbon source (Fig. 8, Table 5). Of the 319 proteins we identified, 52 (16.3%) were involved in metabolic pathways and 15 (4.7%) were involved in metabolism in diverse environments. We observed several proteins involved in carbon metabolism (3.4%), purine metabolism (2.5%), amino sugar and nucleotide sugar metabolism (1.6%), glycine/serine/threonine metabolism (1.6%), cysteine/methionine metabolism (1.6%), pyruvate metabolism (1.3%), starch and sucrose metabolism (1.3%) and

| Pathway Description                              | Pathway Accession | # Proteins |
|--------------------------------------------------|-------------------|------------|
| Thiamine metabolism                              | bde00730          | 1          |
| Two-component system                             | bde02020          | 4          |
| Two-component system                             | bfl02020          | 2          |
| Two-component system                             | bks02020          | 1          |
| Tyrosine metabolism                              | bks00350          | 1          |
| Tyrosine metabolism                              | bde00350          | 1          |
| Valine, leucine and isoleucine biosynthesis      | bde02900          | 7          |
| Valine, leucine and isoleucine biosynthesis      | bks00290          | 4          |
| Valine, leucine and isoleucine biosynthesis      | bfl00290          | 3          |
| Valine, leucine and isoleucine biosynthesis      | bks00290          | 1          |
| Valine, leucine and isoleucine biosynthesis      | bde00280          | 2          |
| Valine, leucine and isoleucine degradation       | bks00280          | 1          |
| Valine, leucine and isoleucine degradation       | bfl00280          | 1          |
| Vancorycin resistance                            | bde01502          | 1          |
| Thiamine metabolism                              | bde00750          | 1          |

Table 5 Proteins identified in *B. dentium* ATCC 27678 by proteomic analysis (Continued)

![Fig. 4](image-url) The *B. dentium* ATCC 27678 genome contains multiple glycosyl hydrolase (GH) genes. The *B. dentium* ATCC 27678 genome was found to harbor 88 GH-related genes, encoding for 25 different GH families.
alanine/aspartate/glutamate metabolism (0.9%). Consistent with our genome analysis, we observed proteins involved in the pentose phosphate pathway (1.3%) and large number of ABC transporters (4.3%). Together these findings indicate that \textit{B. dentium} can metabolize a wide range of growth substrates, including nutrient sources that are commonly found in the human diet and in the gut lumen.

**Discussion**

The human GI tract is a highly competitive environment characterized by fluctuations in nutrient source availability. As a result, metabolic versatility, which allows microbes to use multiple carbon, nitrogen, and other sources, is characteristic of successful commensal microbes. In this study, we provide an in-depth analysis of \textit{B. dentium} growth in a myriad of conditions, including varying acid conditions and nutrient sources (Fig. 9). We demonstrate that \textit{B. dentium} can survive conditions which mirror the transit through the GI tract and adheres to intestinal mucus, indicating adaptation as a commensal member of the GI tract. The data gathered in this study also provide a substantial amount of information on the growth-promoting properties of \textit{B. dentium}. We demonstrate that in the absence of glucose, \textit{B. dentium} can still use 14 sugars, 4 amino acids/amino acid derivatives/amines, 3 glycosides, and 1 organic acid to support its growth. These data reveal metabolic flexibility in nutrient utilization in \textit{B. dentium}, which likely is key to successful competition in the dynamic intestinal milieu. Despite some carbon sources supporting only modest/or short-term growth, according to Rolf Freter’s nutrient niche hypothesis, we interpret this finding as being both a necessary and a sufficient component of \textit{B. dentium} ecological fitness in the GI tract. It is highly unlikely that long term carbon utilization will depend on any single source in vivo, but short-term utilization of variable and transient nutrients is critical to successful colonization [69–71]. The data presented demonstrate \textit{B. dentium}'s ability to grow and thrive under varying conditions found in the gastrointestinal tract. These findings enlighten our understanding of the diverse sources that regulate \textit{B. dentium}'s ability to colonize the human intestine.

*Fig. 5* \textit{B. dentium} yields minimal growth on amino acids and amino acid derivatives in LDM4 preparations prepared without glucose. \textit{B. dentium} ATCC 27678 was grown anaerobically at 37 °C in Biolog plates with a fully-defined media (LDM4) preparation that lacked glucose. Growth was monitored over 16 h by plate reader in plate containing (a) 33 different amino acids. b For visualization, heat maps were generated for all amino acids at time 0, 8.3 and 16.0 h. All data are presented as mean ± stdev.
Fig. 6 (See legend on next page.)
Like other bifidobacteria, *B. dentium* is a recognized member of the infant and adult intestinal microbiome [3, 6–8, 37]. However, *B. dentium* species are also members of the oral microbiome and have been identified in dental caries [33, 50, 72–81]. In addition to *B. dentium*, *B. breve*, *B. adolescentis*, and *B. longum* have also been isolated from dental caries [33, 50, 72–81]. Although the precise role bifidobacteria plays in dental caries is unknown, *Bifidobacterium* species may be bystanders due to their adhesive properties and their resistance to acidity [50, 82–84]. In gnotobiotic animals, *B. dentium* was found to have beneficial effects on the host, with no adverse effects noted [21–23, 42]; suggesting that *B. dentium* also participates as a commensal intestinal microbe.

Dealing with acid stress is an important factor for colonizing gut microbes. Acid tolerance in bifidobacteria has been linked to the activity of the membrane H+-F1F0-ATPase enzyme responsible for maintaining pH homeostasis in most anaerobic microbes. Acid-resistant *Bifidobacterium* species like *B. animalis* activate the F1F0-ATPase complex upon acid exposure [86, 87]. *B. dentium* encodes the genes for the H+-F1F0-ATPase (KEGG) and based on the relative resistance of *B. dentium* to low pH, we speculate that the H+-F1F0-ATPase is likely activated. In *B. longum*, low extracellular pH is reflected by a low intracellular pH [85]. Similar to the literature, our data indicate that *B. dentium*’s intracellular pH can reach a low level without a significant loss in viability. Although these experiments were performed in rich bacterial media, we speculate that *B. dentium* would survive the transit of the gastrointestinal tract. Together, these findings suggest that *B. dentium* harbors compensatory mechanisms to withstand the various pH's of the gastrointestinal tract.

Nutrient availability may be limited in the intestinal lumen due to a variety of factors including competition by other microbes, absorption by the host, or transit through the GI system. Therefore, metabolic plasticity is key to successful microbial colonization. Recent analysis of multiple oral and intestinal derived *B. dentium* genomes identified 140 conserved genes among *B. dentium* strains, indicating a high degree of phylogenetic relatedness [88]. All *B. dentium* genomes shared 19 glycosyl hydrolases families, with the highest abundance observed in GH13. This is consistent with our *B. dentium* ATCC 27678 analysis, which revealed the highest expression of GH13. The glycobiome of *B. dentium* strains also indicated a degradation of a wide range of carbohydrates and plant-derived polysaccharides [88]. Using Biolog phenotyping arrays, we identified that in the absence of glucose, *B. dentium* ATCC 27678 readily uses mannose, xylose, mannotol, maltose, sucrose, melibiose, gentiobiose, trunose, raffinose, maltotriose, and stachyose, Sedoheptulosan. We also observed growth with galactose, which supports previous work indicating that galacto-oligosaccharides (GOS) supplementation bolsters the abundance of bifidobacteria [1, 2, 89]. This is also consistent with the *B. dentium* ATCC 27678 genome, which contains the GH enzyme for β-galactosidases (GH2 and GH42 families), likely allowing *B. dentium* to grow on galacto-oligosaccharides.

Interestingly, we found that in the absence of glucose, *B. dentium* was unable to use several polysaccharides which normally promote bifidobacterial growth. These included well characterized inulin, lactulose, and pectin. Prebiotic substrates, in particular inulin and lactulose, have been used in human trials where they have been observed to increase *Bifidobacterium* spp. and provide beneficial effects to the host [13, 36, 90–93]. The addition of glucose back into the LDM4 preparation in our studies showed that *B. dentium* ATCC 27678 growth was enhanced with inulin, confirming the dependence on glucose for inulin metabolism [63].

We also observed that in the absence of glucose, *B. dentium* was able to use amino acids to support baseline growth. Limited data are available on nitrogen assimilation in the gut lumen, particularly by *Bifidobacterium* species [94]. Herein, we provide evidence for metabolism of select amino acids in lieu of a carbohydrate-based carbon source which can be used as carbon or nitrogen substrates as needed. We also found that *B. dentium* was largely unable to use host glycan sugars. This finding is consistent with the GH profile of *B. dentium* and our previous work which found that *B. dentium* could not degrade intact MUC2 mucus [42]. Other bifidobacteria, such as *B. bifidum* (PRL2010, D119 and L22), *B. breve* NCIMB8807, and *B. longum* NCIMB8809, harbor a much larger repertoire of mucin-degrading glycosyl hydrolases [95–99]. These mucin- and HMO-degrading
Fig. 7 (See legend on next page.)
GHs likely provide these *Bifidobacterium* strains with a competitive edge, allowing these microbes to be found at greater abundance than *B. dentium* in vivo.

Consistent with GHs profile, we found that *B. dentium* exhibited substantial growth on β-glucans. *B. dentium* harbors GH 1, 3 and 30 which harbor β-glucosidases which can degrade plant based β-glucans and natural phenols, such as salicin, arbutin and amygdalin. Additionally, *B. dentium* had robust growth on these β-glucans, indicating that *B. dentium* may target plant-based nutrients. Our in vitro findings indicate that *B. dentium* supports it growth with several plant-derived compounds which closely mirrors dietary studies in humans. For example, consumption of pea and whey protein extract increases bifidobacteria levels in healthy subjects [100–102]. Consumption of date fruits, containing high levels of glucose, fructose, and sucrose, has also been reported to increase the relative abundance of bifidobacteria [103–105]. Diets rich in non-digestible carbohydrates, such as whole grain and wheat bran, are also linked to increases in bifidobacteria [106, 107]. In contrast, a Western diet (high in animal protein and fat, low in fiber) has been associated with decreased bifidobacteria [108–110]. These human studies support the important role of dietary compounds in modulating the microbial community and influencing the levels of bifidobacteria. Bifidobacteria have been associated with numerous health benefits, including immune-modulation, gut-brain-axis cross-talk, increasing intestinal mucus, enhancing epithelial integrity, pathogen exclusion, cancer prevention, and management of inflammatory bowel disease [16, 21, 23, 24].

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**Fig. 7** *B. dentium* has minimal growth on organic acids without glucose. *B. dentium* ATCC 27678 was grown anaerobically at 37 °C in Biolog plates with a fully-defined media (LDM4) preparation that lacked glucose. Growth was monitored over 16 h by plate reader in plate containing 59 different organic acids. Acids were separated into groups: a 12 acids, b 9, c 9, d 8 and e 21 acids. f Heat maps were generated for organic acids at time 0, 8.3 and 16.0 h. All data are presented as mean ± stdev

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**Fig. 8** Pathway analysis *B. dentium* by proteomic analysis. *B. dentium* ATCC 27678 were examined using high-resolution liquid chromatography-tandem mass spectrometry based proteomics and 319 proteins were identified from *B. dentium*. The functional classifications of these proteins are illustrated in the pie chart above.
Thus, maintenance of bifidobacteria is likely important for maintaining intestinal homeostasis. Based on our newly identified nutrient sources for *B. dentium*, we propose that these compounds could be implemented in the future to promote *B. dentium* abundance in the human gastrointestinal tract. Collectively this work provides novel insights into the proteome and metabolic profile of *B. dentium* and our findings point to *B. dentium* as a well-adapted member of the gastrointestinal tract.

**Supplementary Information**

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**Additional file 1.**

**Authors’ contributions**

Concept and design (MAE, JV); intellectual contribution (MAE, HAD, AH, KAE, TDH, SJH, KMH, BTE, MB, KWG, RAB, JKS, AMH, JV); data acquisition (MAE, HAD, AH, KAE, TDH, SJH, KMH, BTE); data analysis, statistics, and interpretation (MAE, HAD, AH, KAE, TDH, SJH, KMH, BTE, MB, KWG, JKS, AMH); drafting manuscript (MAE); editing manuscript (MAE, HAD, AH, KAE, TDH, SJH, KMH, BTE, MB, KWG, RAB, JKS, AMH); funding (MAE, RAB, JKS, JV). The author(s) read and approved the final manuscript.

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**Availability of data and materials**

The datasets generated in the current study are deposited in the NIH-funded Center for Computational Mass Spectrometry MassIVE database, Dataset Number MSV000086294. Link: massive.ucsd.edu/ProteoSAFe/private-dataset.jsp?task=9e9343b12d3540e7bc8c4702fde719d3.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

J. Versalovic serves on the scientific advisory boards of Biomica, Plexus Worldwide and Seed Health. RA. Britton serves on the scientific advisory board of Tenza, is a co-founder of Mikrovia, and consults for Takeda and Probiotech. J. Versalovic, J.K. Spinler, and RA. Britton have received unrestricted research support from BioGaia, AB. The remaining authors have no commercial or financial relationships that could be construed as a potential conflict of interest.
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