Comparative genomics of *Bifidobacterium* species isolated from marmosets and humans

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**Abstract**
The genus *Bifidobacterium* is purported to have beneficial consequences for human health and is a major component of many gastrointestinal probiotics. Although species of *Bifidobacterium* are generally at low relative frequency in the adult human gastrointestinal tract, they can constitute high proportions of the gastrointestinal communities of adult marmosets. To identify genes that might be important for the maintenance of *Bifidobacterium* in adult marmosets, ten strains of *Bifidobacterium* were isolated from the feces of seven adult marmosets, and their genomes were sequenced. There were six *B. reuteri* strains, two *B. callitrichos* strains, one *B. myosotis* sp. nov. and one *B. tissieri* sp. nov. among our isolates. Phylogenetic analysis showed that three of the four species we isolated were most closely related to *B. bifidum, B. breve* and *B. longum*, which are species found in high abundance in human infants. There were 1357 genes that were shared by at least one strain of *B. reuteri, B. callitrichos, B. breve* and *B. longum*, and 987 genes that were found in all strains of the four species. There were 106 genes found in *B. reuteri* and *B. callitrichos* but not in human bifidobacteria, and several of these genes were involved in nutrient uptake. These pathways for nutrient uptake appeared to be specific to *Bifidobacterium* from New World monkeys. Additionally, the distribution of *Bifidobacterium* in fecal samples from captive adult marmosets constituted as much as 80% of the gut microbiome, although this was variable between individuals and colonies. We suggest that nutrient transporters may be important for the maintenance of *Bifidobacterium* during adulthood in marmosets.
1 | INTRODUCTION

Members of the genus *Bifidobacterium* are purported to have beneficial consequences for human health (Backhed, Ley, Sonnenburg, Peterson, & Gordon, 2005; Guarné & Malagelada, 2003; Nowak, Paliwoda, & Blasiak, 2018; Tojo et al., 2014). Currently, GenBank Taxonomy recognizes 71 species of *Bifidobacterium* that have been isolated from the gastrointestinal tracts of mammals, birds and social insects as well as human-impacted environments (Bottacini, Ventura, van Sinderen, & O’Connell Motherway, 2014; Killer et al., 2010; Michelini, Modesto et al., 2016; Milani et al., 2017). Bifidobacteria are noted for their ability to degrade ingested polysaccharides and proteins, and to synthesize vitamins and other nutrients that are beneficial to their hosts (Coakley et al., 2006; Kiyohara et al., 2012; Lugli et al., 2018; Milani et al., 2016; Milani, Lugli et al., 2015; O’Connell Motherway, Kinsella, Fitzgerald, & van Sinderen, 2013; Pompei et al., 2007). Species that are often found in the human gut microbiome have been intensively studied and are transmitted from mothers to infants where they occur at relatively high frequency until weaning. The bifidobacteria of the infant gut are proposed to play crucial roles in protecting infants against pathogenic bacteria, contributing to priming the mucosal immune system, and consequently protecting against susceptibility to diverse diseases later in life (Eckburg et al., 2005; Fukuda et al., 2011; Lee & O’Sullivan, 2010; Marco, Pavan, & Kleebezechem, 2006; O’Hara & Shanahan, 2007; Schell et al., 2002). After weaning, the frequencies of different species of bifidobacteria change over an individual’s life span (Bäckhed et al., 2015; Stewart et al., 2018; Turroni et al., 2012) and while their absolute abundance remains constant, their relative abundance decreases to around 4% due to colonization by other bacteria (Bäckhed et al., 2015; Milani, Mancabelli et al., 2015; Stewart et al., 2018; Tanaka & Nakayama, 2017; Turroni et al., 2012; Turroni et al., 2018).

Comparative genomics studies of members of the genus *Bifidobacterium* have provided valuable information regarding the characteristics that are unique to, shared between, or distinguish *Bifidobacterium* species from other gut microbiota. These characteristics likely evolved as a consequence of interspecies interactions among bacteria and adaptation to the host environment (Bottacini et al., 2018; Bottacini, O’Connell Motherway et al., 2014; Lee & O’Sullivan, 2010; Lugli et al., 2018; Schell et al., 2002; Sela et al., 2008). In view of what is known about the role of *Bifidobacterium* species in animal health, comparisons between species with different host specificity can provide information relevant to understanding adaptation to the host environment.

Species of *Bifidobacterium* have been isolated from the feces of adult and infant common marmosets (*Callithrix jacchus*; Endo, Futagawa-Endo, Schumann, Pukall, & Dicks, 2012; Lugli et al., 2017; Michelini et al., 2015; Michelini, Oki et al., 2016; Modesto et al., 2014; Toh et al., 2015). As found in humans (Stewart et al., 2018; Yatsunenko et al., 2012), the relative abundance of bifidobacteria is highly variable between individuals and populations. However, unlike humans the abundance of *Bifidobacterium* in marmosets can remain quite high well into adulthood (Ross et al., 2017). Genomic comparisons between *Bifidobacterium* isolated from marmosets and those isolated from humans may provide information that could be exploited in the development of probiotic strains of bifidobacteria that persist in high numbers in adult humans.

In this study, we tested the hypothesis that bifidobacteria from adult marmosets have genes that are not found in closely related bifidobacteria from humans. To do so, we isolated bifidobacteria from adult animals and determined their genome sequences. We proceeded to identify protein coding sequences from the genomes of these isolates that were not found in bifidobacteria that reside in humans, several of which were components of nutrient transport systems.

2 | METHODS

2.1 | Biological sources

The *Bifidobacterium* strains characterized in this study were originally isolated from fecal samples of adult common marmosets (*Callithrix jacchus*) raised in either barrier conditions at the University of Texas Health Science Center San Antonio (UTHSCSA) or a conventional colony at Southwest National Primate Research Center (SNPRC; Ross et al., 2017; Table 1). To determine whether the high abundance of *Bifidobacterium* in adult marmosets was a common phenomenon, additional fecal samples from marmosets over 1 year of age were provided by the SNPRC (*n* = 34), the Wisconsin National Primate Research Center (WNPRC; *n* = 45) and the New England Primate Research Center (NEPRC; *n* = 43). Animals were housed in family groups and underwent routine health and prevention procedures. They had free access to water. The UTHSCSA barrier colony of marmosets receives irradiated Teklad purified marmoset diet with Harlan irradiated primate enrichment mix and no other dietary enrichment items. SNPRC provides a mix of Teklad Purified and Mazuri Regular diets to their primate enrichment mix and no other dietary enrichment items. WNPRC provides a mix of Teklad Purified and Mazuri Regular diets to their conventional colony. NEPRC provides a mix of Teklad NWM and ZuPreem diets, and WNPRC provides the Mazuri High Fiber diet. Each of the conventional colonies provide different combinations of protein sources (eggs, peanuts, garbanzo beans, yogurt or cottage cheese), fruits, vegetables, and sweets ad libitum.

2.2 | Analysis of microbial communities by 16S rRNA gene sequencing

The first step toward obtaining *Bifidobacterium* isolates for genomic sequencing was to identify fecal samples that had high proportions of bifidobacteria. The composition of bacterial communities in all fecal samples were analyzed by sequencing the V1-V3 region of 16S rRNA genes that had been amplified from each sample as follows. Fecal samples were taken from marmosets by inserting a mini e-swab
Samples were mailed to the University of Idaho on dry ice and stored in Amies transport medium and immediately frozen at 80°C. Genomic DNA was extracted from fecal samples, and the V1-V3 region of the 16S rRNA gene was amplified and sequenced by 454 pyrosequencing using our standard protocols (Hickey et al., 2015), while the 16S rRNA genes from the WNPRC were sequenced using an Illumina MiSeq platform (Shen et al., 2016).

For the bacterial communities from which Bifidobacterium isolates were purified, raw sequence reads were demultiplexed and processed sequences according to our standard protocols (Hickey et al., 2015). The RDP Bayesian classifier was used to assign sequences to genus or higher (RDP 2.5). An alternative strategy was needed to identify Bifidobacterium sequences to species level, and the following protocol was used for sequences from the three colonies. Raw reads were demultiplexed with QIIME (Caporaso et al., 2010), adapter trimmed using Cutadapt (Martin, 2011), and quality filtered using DADA2 (truncQ = 2, maxN = 0, maxEE = 2, truncLen = 450; Callahan et al., 2016). Unique sequence variants were quantified using DADA2 and classified using DADA2 with the silva_species_assignment_v128 database or SPINGO (Allard, Ryan, Jeffery, & Claesson, 2015) with RDP 11.2 species database. Before taxonomic assignment, we manually added the 16S rRNA gene sequences of our four Bifidobacterium species (B. reuteri, B. myosotis, B. callitrichos, and B. tissieri). Classifications from DADA2 and SPINGO were merged in phyloseq (McMurdie & Holmes, 2013) so that the lowest classification was retained. In some instances the classification of sequences differed between DADA2 and SPINGO at the genus level. In these instances, we trimmed back the classification to the family level, where the methods never disagreed. Amplicon sequence variants (ASV) with 100% identity to the 16S rRNA gene sequences for our four bifidobacteria were combined for each species. All other ASVs that were designated as Bifidobacterium were combined into a single count for the Bifidobacterium genus.

### 2.3 | Isolation of Bifidobacterium strains and growth conditions

The second step in identifying Bifidobacterium isolates for genomic sequencing was to isolate putative Bifidobacterium from the fecal samples for which Bifidobacterium phylotypes represented more than 50% of the sequence reads. This was done by streaking fecal material on modified DB agar plates (g/L; Columbia agar base 42.5, glucose 2.5, lactulose 2.5, L-cysteine 0.5, riboflavin 0.01, propionic acid 5.0 ml, pH 5.5+/−0.2). The plates were incubated anaerobically at 37°C for 72 hr (Anaerobe Systems Model: AS-580, (www.aanerobesystems.com) supplied with 90% N2, ~5% CO2, and ~5% H2. Colonies with typical bifid, gram-positive rods were restreaked onto modified DB agar plates and well-isolated colonies were used to inoculate de Man-Rogosa-Sharpe broth supplemented with 500 mg/L L-cysteine (MRS medium) then incubated at 37°C for 24 hr under anaerobic condition. Aliquots of these cultures were stored at 80°C.

### 2.4 | Genomic DNA extraction and amplification of 16S rRNA genes for Bifidobacterium isolates

The third step in identifying isolates for genomic sequencing was to extract genomic DNA from each isolate and identify different strains of Bifidobacterium by sequencing their 16S rRNA genes. To do this, colonies grown on modified DB agar plates were used to inoculate MRS medium and the cultures were incubated at 37°C for 24 hr under anaerobic conditions. Bacterial genomic DNA was isolated from each culture using the QIAamp DNA mini kit (Qiagen, Valencia, CA) following the manufacturer’s protocol. Briefly, 500 µl of each bacterial cell suspension was incubated with 100 µl of lytic enzymes (50 µl lysozyme (10 mg/ml; Sigma-Aldrich, St Louis, MO), 6 µl mutanolysin (25,000 U/ml; Sigma-Aldrich), 3 µl lysostaphin (4,000 U/ml in sodium acetate; Sigma-Aldrich) and 41 µl TE buffer (10 mmol/l Tris-HCl; 50 mmol/l EDTA; pH 8.0) at 37°C for 1 hr. Bacterial cells were then mechanically disrupted by adding
The genome sequences of 10 strains were determined using the Illumina MiSeq platform in the IBEST Genome Resources Core at the University of Idaho. Reference-guided genome assembly was performed using our in-house program, ARC (https://ibest.github.io/ARC/). Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The genome sequence data for these genomes listed in Table S1. Additionally, the protein sequences listed in Table S2 were downloaded from GenBank (https://www.ncbi.nlm.nih.gov). These protein sequences were used to infer the phylogenetic relationships among 76 bifidobacteria. The sequences were aligned using MUSCLE (Edgar, 2004), and the phylogeny was inferred using the maximum likelihood algorithm and the WAG scoring matrix as implemented in RAxML (Stamatakis, 2014).

2.7 | Ethics statement

This study was carried out in adherence with the guidelines put forth by the American Society of Primatologists’ Principles for the Ethical Treatment of Primates. All procedures were reviewed and approved by the respective Institutional Animal Care and Use Committees at UTHSCSA, SNPRC, NEPRC, and WNPRC.

3 | RESULTS

3.1 | Isolation and genome sequences of Bifidobacterium strains from marmoset fecal samples

The purpose of our study was to determine whether Bifidobacterium from marmosets carry genes that are not found in Bifidobacterium from humans. The first step in answering this question was to isolate and sequence the genomes of Bifidobacterium strains from marmoset fecal samples so that gene content could be compared to human-derived bifidobacteria. Eleven adult marmosets were identified as having greater than 50% bifidobacteria in their bacterial communities. Multiple isolates were purified from the fecal samples of these individuals. Isolates were identified as Bifidobacterium based on their 16S rRNA gene sequences, and ten isolates from seven individuals were chosen for genome sequencing based upon having greater than 50% bifidobacteria. Isolates were identified as Bifidobacterium based on their 16S rRNA gene sequences, and ten isolates from seven individuals were chosen for genome sequencing based on having greater than 50% bifidobacteria.

All ten genomes were greater than 2.7 Mbp and the genome content varied from 1984 to 2191 inferred coding sequences (Table 1). All but one of the genomes had three or more copies of ribosomal RNA genes, and all genomes had >50 tRNA genes. Although the sequence coverage was >100× for nine of the ten isolates, it was not possible to assemble scaffolds into closed genome sequences.

Analysis of 16S rRNA gene sequences showed that we had isolated six B. reuteri strains, two B. callitrichos strains, one B. myosotis sp. nov. and one B. tissier sp. nov. (Endo et al., 2012; Michelin et al., 2015; Michelin, Oki et al., 2016; Table 1). Two of the six B. reuteri
isolates came from the same animal and were very similar in gene content and nucleotide sequence (UTCIF-1, UTCIF-2). Two other marmoset fecal samples provided two isolates each, and these were different species.

There was little variation in the gene content of strains belonging to the same species. The genomes of B. reuteri and B. callitrichos type strains had been previously sequenced (Table S1). The gene content of our isolates of these species were similar to those of their type strains, but differences were also evident. In general, the type strains had larger genomes and more coding sequences. Five of our B. reuteri strains had only one to seven unique coding sequences, although samples came from separately housed colonies, the barrier colony was started with animals from the conventional colony 2 to 5 years before sampling for this study (Ross et al., 2017). The genome size and gene content of B. reuteri strain UTCIF-68 was greater than that of other isolates. Of the 118 unique genes, some were putative prophage sequences. Our two B. callitrichos isolates were also quite similar, sharing more than 98% of their genes.

The gene content of all four species was fairly conserved (Figure 1). For the criteria used here (50% identity, 50% coverage), the four species shared 1216 genes, and another 313 genes were shared among three of the four species. Between 128 and 258 genes were unique to each species. Finally, between 62 and 376 genes were found in only one marmoset Bifidobacterium species, but these genes were shared with other Bifidobacterium species of human origin (see below).

3.2 | Phylogenetic relationships of Bifidobacterium from marmosets and humans

Meaningful comparisons between bacterial genomes require a clear understanding of the evolutionary relationships among the bacteria. To place the bifidobacteria from marmosets within an evolutionary context of bifidobacteria from humans and other primates, their Type I polyketide synthase amino acid sequences were used to infer the phylogenetic relationships of these species (Figure 2). Our phylogenetic analysis shows that B. myosotis shared a common ancestor with B. reuteri, B. breve, and B. longum, whereas the most recent common ancestor of B. tissieri shares a more distant common ancestor with these four, B. bifidum and B. callitrichos (Figure 2). Note that Bifidobacterium species isolated from marmosets and tamarins, which are New World monkeys with overlapping species ranges, occurred intermingled within clades (Figure 2; green and blue labels). Also, the Bifidobacterium species from Eulemur macaco and Lemur catta were outliers to the bifidobacteria from humans and New World monkeys, reflecting the phylogeny of the primate host.

3.3 | Comparison of Bifidobacterium genomes from marmosets and humans

The genomes of bifidobacteria isolated from marmosets were compared to the genomes of 47 Bifidobacterium strains from the species B. adolescentis (n = 3), B. animalis (n = 16), B. bifidum (n = 3), B. breve (n = 8), B. callitrichos (n = 1), B. dentium (n = 1), B. longum (n = 13), B. reuteri (n = 1), and B. thermophilum (n = 1) (Table S1; www.ncbi.nlm.nih.gov). The genomes of the human-derived Bifidobacterium species were generally smaller than those from marmosets and accordingly, had fewer genes (Table S1). There were 937 genes found in at least one strain of each species. Of these, 712 were found in every strain of every species. Genetic distance plays a large role in detecting evolutionary relationships; the faster a protein evolves, the less likely homologs will be found among distantly related organisms. Thus, the core genome of the Bifidobacterium genus has only 413 genes when all species and strains were considered (Milani et al., 2016).

We sought to determine if there were features of Bifidobacterium species in marmosets that allow them to persist in the adult gastrointestinal tract. To explore this we compared the gene content of strains of bifidobacteria isolated from humans, which do not persist at high relative frequency in adults, to those from marmosets that can be found at high relative frequency in adults. We minimized the probability of declaring a difference that is only due to genetic distance, we chose to compare the genomes of two marmoset-derived species with multiple strains, B. reuteri and B. callitrichos, to the genomes of the two most closely related human-derived species, B. longum and B. breve (Figure 3). Using our criteria, there were 1357 genes that were found in at least one strain from each of the four species, and 987 genes that were found in all strains of the four species. More important, there were 106 genes in B. reuteri and B. callitrichos that were not found in B. breve and B. longum. Forty five of the 106 genes encoded hypothetical proteins, the rest had sufficient similarity to known proteins that they were functionally annotated.

We investigated the 61 annotated genes in greater depth using BLAST to find homologous sequences in other bacteria. Twenty of
The 61 genes were found in human-derived bifidobacteria other than \textit{B. breve} and \textit{B. longum}, and we excluded them from further consideration. The remaining 41 genes encoded proteins with various functions, including transcriptional regulation, membrane proteins and a range of enzymes (Table S3). Seven proteins were listed as components of ABC transporters, suggesting that differences in nutrient uptake might distinguish marmoset bifidobacteria from human bifidobacteria.

**FIGURE 2** Maximum likelihood phylogeny of \textit{Bifidobacterium} species based upon the type 1 polyketide synthase protein. \textit{Alloscardovia macaca} was used to root the phylogeny; \textit{Gardnerella vaginalis} has recently been recognized as a member of the genus \textit{Bifidobacterium}. Species used in the comparative genome analysis are underlined. Names of \textit{Bifidobacterium} isolated from nonhuman primates are colored: blue=marmosets, green=tamarins, orange=lemurs. Branches with more than two strains for a single species are collapsed. The error bar indicates the mean number of nucleotide substitutions per site. The table shows the distribution of genes associated with three ABC transport systems: A) 2-aminooethylphosphonate ABC transporter (0. ATP binding protein, 1. periplasmic 2-aminooethylphosphonate-binding and permease protein, 2. 2-aminooethyl-phosphonate:pyruvate aminotransferase, 3. phosphono-acetaldehyde hydrolase); B) peptide ABC transport (4. peptide-binding protein, 5. two permease proteins, 6. two ATP binding proteins); C) aliphatic sulfonate ABC transporter (7. alphatic sulfonate binding protein, 8. permease protein, 9. ATP binding protein). Complete type 1 polyketide synthase sequences were not available for \textit{B. catulorum} and \textit{B. stellensboschense}, but their ABC transporter contents are shown at the bottom of the phylogeny.

**FIGURE 2** Maximum likelihood phylogeny of \textit{Bifidobacterium} species based upon the type 1 polyketide synthase protein. Of the 61 genes found in human-derived bifidobacteria other than \textit{B. breve} and \textit{B. longum}, and we excluded them from further consideration. The remaining 41 genes encoded proteins with various functions, including transcriptional regulation, membrane proteins and a range of enzymes (Table S3). Seven proteins were listed as components of ABC transporters, suggesting that differences in nutrient uptake might distinguish marmoset bifidobacteria from human bifidobacteria.

### 3.4 ATP-binding cassette transport systems

We looked more closely at the genes encoding ATP-binding cassette (ABC) proteins that appeared to be specific for \textit{Bifidobacterium} species isolated from marmosets. ABC importers are complexes of two or three proteins, with the following functions: 1) an ATPase that provides energy for transport across the cell membrane, 2) a permease that mediates passage of the nutrient into the cell and 3) an extracellular substrate binding protein that recognizes the
and its presence in diverse genomes suggests that lateral gene distribution of this metabolic pathway has been noted previously, source (Kamat & Raushel, 2013). The wide bonds in organophosphate compounds, releasing the P as a nutrient β including Firmicutes, bifidobacteria isolated from marmosets and tamarins. On the other bifidobacteria even though by our phylogenetic analysis, these proteins were not found in any of the human fecal isolates of rabbit feces and two from chicken feces. Genes for these four Clostridium scardovii feces (Figure 2; green labels), one species from human blood (B. tissieri, B. callitrichos, B. catulorum, and the tamarin-derived B. primatum. Proteins from clostridia had the closest sequence similarity to these transport proteins. The sporadic distribution of these two transport systems suggests that lateral gene transfer has played an important role in their distribution. The other three ABC transport components that were not found in human-derived bifidobacteria were ABC exporters that are putatively involved in multidrug export.

**3.5 | Phosphotransferase systems**

Phosphotransferase systems (PTS) are used by bacteria to actively import carbohydrates at the expense of ATP and concomitantly catalyze their phosphorylation (Kotrba, Inui, & Yukawa, 2001). PTS are found in many bacterial species, however, there are also many species that lack PTS homologs. Differences in their distributions sometimes exist even among strains of the same species (Barabote & Saier, 2005). Because this system seems to evolve rapidly, possibly in response to nutrient availability, we categorized the types of PTS that were found in all of the genomes that we studied (Tables 1 and S1).

The strains of B. animalis that we studied did not have any PTS genes, whereas strains of the other species had all three PTS genes necessary for both β-glucoside and glucose transport. B. bifidum and B. callitrichos had PTS genes for sugar and cellobiose-specific transfer. In contrast, B. bifidum, B. breve, and B. callitrichos had PTS genes for N-acetylgalactosamine and ascorbate, whereas B. breve also had PTS genes for fructose. The distribution of the PTS genes in both marmoset-and human-derived bifidobacteria suggests that this is not a system associated with the persistence of bifidobacteria into adulthood.

**3.6 | Distribution of Bifidobacterium species in adult marmoset fecal samples**

Our goal was to test the hypothesis that bifidobacteria from adult marmosets carry genes that allow them to persist at high levels, as
FIGURE 4  Relative abundance of *Bifidobacterium* species in marmoset fecal samples from three primate research centers. Each fecal sample was from a different individual, who was over one year old.
compared to bifidobacteria in humans that have low abundances in adults. This hypothesis was based upon our previous results showing that the gastrointestinal tracts of adult marmosets often have high proportions of bifidobacteria (Ross et al., 2017). To confirm the high proportion of bifidobacteria in a larger sample, we determined the relative abundance of *Bifidobacterium* species in bacterial communities of 122 adult marmosets. This was done by sequencing and classifying amplicons of 16S rRNA genes in fecal samples. As seen previously, there was wide variation in the relative abundance of *Bifidobacterium* species ranging from zero to more than 80% (Figure 4). About 72% of our adult marmosets had a relative abundances of *Bifidobacterium* greater than 10%, which is more than three times what is commonly seen in adult humans (Yassour et al., 2016), and over half had abundances greater than 25%.

Additionally, we determined the relative abundance of different species of bifidobacteria. *B. reuteri* and *B. myositis* were found in fairly high proportions in the SNPRC colonies, which is the center from which the isolates originated. *B. tissieri* was found at less than 10% relative frequency in samples from NEPRC and WNPRC. Oddly, it was not found at the SNPRC from which it was originally isolated. *B. callitrichos* predominates in samples from the WNPRC colony (Figure 4). Sequences from species other than our four species of *Bifidobacterium* predominated in samples from the NEPRC colony. The sequence with the highest frequency at NEPRC was 100% identical to GenBank accession EU459101, which was originally isolated from Geoffrey's marmoset (*C. geoffroyi*) feces (Ley et al., 2008).

4 | DISCUSSION

4.1 | Genome comparisons between *Bifidobacterium* species from marmosets and from humans

Our goal in this study was to compare *Bifidobacterium* from the common marmoset with those from humans with the intent of identifying mechanisms that allow bifidobacteria to persist at high levels in adult marmosets. There may be multiple reasons for the differential effect of age on the frequency of human-derived and marmoset-derived bifidobacteria. The reasons for differences are not known, but diet, host physiology, exposure to different microbes and genetic differences between marmosets and humans seem like reasonable possibilities. The results of our genomic comparison suggested that diet might be a major influence. There were only 106 proteins found in *B. reuteri* and *B. callitrichos* genomes that were not found in genomes of *B. breve* and *B. longum*. Forty one of these proteins had been assigned putative functions and were not found in any other human-derived bifidobacteria strain (Table S3). Because diet influences the composition of the gut microbiome and adult diets are different from infant diets, we focused on nutritional transporters in the marmoset-derived bifidobacteria that might explain the persistence of bifidobacteria in the gut microbiomes of adult marmosets. We found examples of ABC transporters, but not PTS transporters, that distinguished bifidobacteria from marmosets and humans.

An important component of nutritional transport systems are the substrate binding proteins that are secreted into the environment to capture nutrients and facilitate nutrient uptake. In a study that examined the secretomes of *Bifidobacterium*, *B. biavatii* and *B. aesculapii* isolated from New World monkeys were predicted to encode the largest arsenal of secreted proteins (Lu et al., 2018). Indeed, with few exceptions, New World *Bifidobacterium* have the highest number of predicted extracellular proteins. These results suggest that there are multiple genomic differences between New World primate bifidobacteria and human bifidobacteria. An evolutionary comparison of *Bifidobacterium* from different primates may provide insights to the evolution of this interesting genus.

We found several examples of ABC transporters that were found in certain species of *Bifidobacterium* and not others. In our analysis, the bacteria outside of *Bifidobacterium* with protein sequences most similar to these transporters were *Firmicutes*, which are often found

| TABLE 2 Differences among colonies in content of prepared diets |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                               | NEPRC            | SNPRC            | WNPRC           |
|                               | Teklad NWM       | ZuPreem          | Teklad purified | Mazuri regular  |
| Top five ingredients in order by weight |                  |                  |                 |                 |
| Ground corn                   | Cracked wheat    | Dextrin          | Glucose         | Glucose         |
| Ground wheat                  | Soybean meal     | Sucrose          | Soybean meal    | Ground corn     |
| Wheat middlings               | Sugar            | Lactalbumin or   | Ground corn     | Soybean meal    |
| Wheat germ                    | Dried egg product| Whey protein     | Casein          | Casein          |
| Soybean meal                  | Vegetable oil    | Soybean oil      | Wheat middlings| Corn gluten meal|
| Gelling agent                 | None             | Agar or gelatin  | Gelatin         | Gelatin         |
|                               | None             |                  | Xanthum gum     | Xanthum gum     |
| Crude DM protein %            | 20               | 14              | 20              |                 |
| Crude DM fat %                | 7.5              | 10              | 5.6             | 7.0             |
| Kcal/kg DW                    | 3,200            | 3,600           | 3,600           | 3,410           |

Abbreviations: NEPRC: New England Primate Research Center; SNPRC: Southwest National Primate Research Center; WNPRC: Wisconsin National Primate Research Center.
in the gut microbiomes of adult humans. We propose that these transporters allow the marmoset-derived *Bifidobacterium* to use resources in the marmoset gut that are also used by *Firmicutes* in the human gut, and this may permit the persistence of bifidobacteria at high relative abundance in the adult marmoset.

### 4.2 Phylogenetic relationships of *Bifidobacterium* from marmosets and humans

Our analysis of the taxonomic distribution of ABC transporters led us to include species of *Bifidobacterium* obtained from other primates in our phylogenetic analysis. An interesting pattern was seen in the phylogeny inferred using the type 1 polyketide synthase protein. The clade that includes *B. bifidum*, *B. breve*, and *B. longum*, the first colonizers of the human infant gut, contains many species that are found in New World monkeys. Thus, it appears that New World monkeys share bifidobacteria with a common ancestor of the human bifidobacteria, but the genus has undergone evolutionary radiation and multiple species have emerged in the gut microbiomes of marmosets and tamarins. Although these species were isolated from specific primates, it would be interesting to determine whether the New World monkeys share these species or if they are specific to the host from which they were derived.

### 4.3 Distribution of *Bifidobacterium* species in adult marmosets

Within each marmoset colony, the relative abundances of *Bifidobacterium* species vary widely from 0 to 80% (Figure 4). However, closer examination shows that the predominant species are different among the three marmoset colonies considered in this study (Figure 4). Studies that view microbiomes with similar distributions of phyla or enterotypes (Figure 3) may tend to overlook important variation within members of the enterotype (Arumugam et al., 2011; Koren et al., 2013; Liang et al., 2017; Wu et al., 2011). For instance, putative aliphatic sulfonate ABC transporter genes were found in *B. calitrichos* and not in *B. reuteri* (Figure 3), and there are >500 other proteins that are not shared between strains of these two species (Figure 2). Thus, simply identifying the frequency of *Bifidobacterium* in these colonies would not convey the underlying differences among them that could be important in defining their ecological roles.

Diet plays an important role in determining the species composition of the gut microbiome (David et al., 2014; Muegge et al., 2011; Wu et al., 2011). Our three marmoset colonies were fed different formulations of prepared diets that provided similar caloric and protein content, but from different sources (Table 2). All colonies were also provided supplemental foods, such as fruits, vegetables, and yogurt ad libitum, and these supplements also differed among colonies. These differences in diet may account for the distribution of the different *Bifidobacterium* species among the colonies. For instance, the NEPRC marmosets received far less simple carbohydrate by weight than the other two colonies (Table 2). On the other hand, the species composition of a particular colony may be maintained by the close proximity of marmosets in adjacent cages or by contact with their caregivers. Evaluating the effect of dietary differences on species composition in the gut is an important next step and the goal of our current research.

Although *B. myosotis* and *B. tissieri* were previously isolated from infant marmoset feces (Michelini et al., 2015; Michelini, Oki et al., 2016), our isolates of these two species came from fecal samples of adult animals. In addition, both species were found in multiple adults from different colonies (Figure 4). It is possible that—as with human infants (Stewart et al., 2018)—the frequency of *Bifidobacterium* species changes over time, and these two species may have greater prevalence in infant marmosets. Common marmosets are housed as family groups with multiple litters, thus adults are constantly exposed to the gut microbiomes of their offspring. Further work is needed to understand the development of the marmoset microbiome from infancy onwards.

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### CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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### REFERENCES

Allard, G., Ryan, F. J., Jeffery, I. B., & Claesson, M. J. (2015). SPINGO: A rapid species-classifier for microbial amplicon sequences. *BMC Bioinformatics*, 16, 324. https://doi.org/10.1186/s12859-015-0747-1

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., ... Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature*, 473(7346), 174–180. https://doi.org/10.1038/nature09944

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science*, 307(5717), 1915–1920. https://doi.org/10.1126/science.1104816
terium avesanii sp. nov. and Bifidobacterium ramosum sp. nov.: Three
species isolated from faeces of baby common marmosets (Callithrix jacchus). International Journal of Systematic and Evolutionary Microbiology, 66(1), 255–265. https://doi.org/10.1099/ijs.0.000708

Milani, C., Mancabelli, L., Lugli, G. A., Duranti, S., Turrioni, F., Ferrario, C., & Ventura, M. (2015). Exploring vertical transmission of Bifidobacteria from mother to child. Applied and Environmental Microbiology, 81(20), 7078–7087. https://doi.org/10.1128/AEM.02037-15

Milani, C., Lugli, G. A., Duranti, S., Turrioni, F., Mancabelli, L., Ferrario, C., & Ventura, M. (2015). Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut. Scientific Reports, 5, 15782. https://doi.org/10.1038/srep15782

Milani, C., Turrioni, F., Duranti, S., Lugli, G. A., Mancabelli, L., Ferrario, C., & Ventura, M. (2016). Genomics of the genus Bifidobacterium reveals species-specific adaptation to the glycan-rich gut environment. Applied and Environmental Microbiology, 82(4), 980–991. https://doi.org/10.1128/AEM.03500-15

Milani, C., Mangifesta, M., Mancabelli, L., Lugli, G. A., James, K., Duranti, S., & Ventura, M. (2017). Unveiling bifidobacterial biogeography across the mammalian branch of the tree of life. ISME Journal, 11, 2834–2847. https://doi.org/10.1038/isjme.2017.138

Modesto, M., Michelinis, S., Stafanin, I., Ferrara, A., Tagconi, S., Biavati, B., & Mattarelli, P. (2014). Bifidobacterium aesculapii sp. nov., from the faeces of the baby common marmoset (Callithrix jacchus). International Journal of Systematic and Evolutionary Microbiology, 64(PT 8), 2819–2827. https://doi.org/10.1099/ijs.0.056937-0

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., Gonzalez, A., Fontana, L., & Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science, 332(6032), 970–974. https://doi.org/10.1126/science.1198719

Nowak, A., Paliwoda, A., & Blasiak, J. (2018). Anti-proliferative, pro-apoptotic and anti-oxidative activity of Lactobacillus and Bifidobacterium strains: A review of mechanisms and therapeutic perspectives. Critical Reviews in Food Science and Nutrition, https://doi.org/10.1080/10408398.2018.1494539

O’Connell Motherway, M., Kinsella, M., Fitzgerald, G. F., & van Sinderen, D. (2013). Transcriptional and functional characterization of genetic elements involved in galacto-oligosaccharide utilization by Bifidobacterium breve UCC2003. Microbial Biotechnology, 6(1), 67–79. https://doi.org/10.1111/1751-7915.12011

O’Hara, A. M., & Shanahan, F. (2007). Mechanisms of action of probiotics in intestinal diseases. TheScientificWorldJournal, 7, 31–46. https://doi.org/10.1101/tsw.2007.26

Pompei, A., Cordisco, L., Amaretti, A., Zanon, S., Matteucci, D., & Rossi, M. (2007). Folate production by bifidobacteria as a potential probiotic property. Applied and Environmental Microbiology, 73(1), 179–185. https://doi.org/10.1128/AEM.01763-06

Ross, C. N., Austad, S., Brasky, K., Brown, C. J., Forney, L. J., Gelfond, J. A., & Tardif, S. D. (2017). The development of a specific pathogen free (SPF) barrier colony of marmosets (Callithrix jacchus) for aging research. Aging (Albany NY), 9(12), 2544–2558. https://doi.org/10.18632/aging.103140

Schelli, M. A., Karmirantzou, M., Snel, B., Vilanova, D., Berger, B., Pessi, G., & Arigoni, F. (2002). The genome sequence of Bifidobacterium longum reflects its adaptation to the human gastrointestinal tract. Proceedings of the National Academy of Sciences of the United States of America, 99(22), 14422–14427. https://doi.org/10.1073/pnas.212527599

Sela, D. A., Chapman, J., Adeyua, A., Kim, J. H., Chen, F., Whitehead, T. R., & Mills, D. A. (2008). The genome sequence of Bifidobacterium longum subsp. infantis reveals adaptations for milk utilization within the infant microbiome. Proceedings of the National Academy of Sciences of the United States of America, 105(48), 18964–18969. https://doi.org/10.1073/pnas.0809584105

Shen, J., Song, N., Williams, C. J., Brown, C. J., Yan, Z., Xu, C., & Forney, L. J. (2016). Effects of low dose estrogen therapy on the vaginal microbiomes of women with atrophic vaginitis. Scientific Reports, 6, 24380. https://doi.org/10.1038/srep24380

Stamatakis, A. (2014). RaXML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stewart, C. J., Aijami, N. J., O’Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., & Petrosino, J. F. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature, 562(7728), 583–588. https://doi.org/10.1038/s41586-018-0617-x

Tanaka, M., & Nakayama, J. (2017). Development of the gut microbiota in infancy and its impact on health in later life. Allergology International, 66(4), 515–522. https://doi.org/10.1016/j.alit.2017.07.010

Toh, H., Yamazaki, Y., Tashiro, K., Kawarai, S., Oshima, K., Nakano, A., & Morita, H. (2015). Draft genome sequence of Bifidobacterium aesculapii DSM 26737T, isolated from feces of baby common marmoset. Genome Announcements, 3(6), e01463–01415. https://doi.org/10.1128/genomeA01463-15

Tojo, R., Suarez, A., Clemente, M. G., de los Reyes-Gavilan, C. G., Margolles, A., Guimonde, M., & Ruas-Madiedo, P. (2014). Intestinal microbiota in health and disease: Role of bifidobacteria in gut homeostasis. World Journal of Gastroenterology, 20(41), 15163–15176. https://doi.org/10.3748/wjg.v20.i41.15163

Turrioni, F., Peano, C., Pass, D. A., Foroni, E., Severgnini, M., Claesson, M. J., & Ventura, M. (2012). Diversity of bifidobacteria within the infant gut microbiota. PLoS One, 7(5), e36957. https://doi.org/10.1371/journal.pone.0036957

Turrioni, F., Milani, C., Duranti, S., Ferrario, C., Lugli, G. A., Mancabelli, L., & Ventura, M. (2018). Bifidobacteria and the infant gut: An example of co-evolution and natural selection. Cellular and Molecular Life Science, 75(1), 103–118. https://doi.org/10.1007/s00018-017-2672-0

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. Science, 334(6052), 105–108. https://doi.org/10.1126/science.1208344

Yassour, M., Vatanen, T., Siljander, H., Hamalainen, A. M., Harkonen, T., Ryhanen, S. J., & Xavier, R. J. (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Science Translational Medicine, 8(343), 343ra381–343ra81ra381. https://doi.org/10.1126/scitranslmed.aad0917

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., & Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. Nature, 486(7402), 222–227. https://doi.org/10.1038/nature11053

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