

# Genomic insights into the beneficial potential of *Bifidobacterium* and *Enterococcus* strains isolated from Cameroonian infants

Pierre Marie Kaktcham<sup>1,\*</sup>, Magdalena Kujawska<sup>2,3</sup>, Edith Marius Foko Kouam<sup>4</sup>, Laverdure Tchamani Piame<sup>1</sup>, Michele Letitia Tchabou Tientcheu<sup>1</sup>, Julia Mueller<sup>2</sup>, Angela Felsl<sup>2</sup>, Bastian-Alexander Truppel<sup>2</sup>, François Zambou Ngoufack<sup>1,4</sup> and Lindsay J. Hall<sup>2,3,5,6,\*</sup>

## Abstract

A healthy early-life gut microbiota plays an important role in maintaining immediate and long-term health. Perturbations, particularly in low- to middle-income communities, are associated with increased infection risk. Thus, a promising avenue for restoring a healthy infant microbiota is to select key beneficial bacterial candidates from underexplored microbiomes for developing new probiotic-based therapies. This study aimed to recover bifidobacteria and lactic acid bacteria from the faeces of healthy Cameroonian infants and unravel the genetic basis of their beneficial properties. Faecal samples were collected from 26 infants aged 0–5 months recruited in Dschang (Cameroon). Recovered bacterial isolates were subjected to whole-genome sequencing and *in silico* analysis to assess their potential for carbohydrate utilization, their antimicrobial capacities, host-adaptation capabilities and their safety. From the range of infant-associated *Bifidobacterium* and *Enterococcus* strains identified, *Bifidobacterium* species were found to harbour putative gene clusters implicated in human milk oligosaccharide metabolism. Genes linked to the production of antimicrobial peptides such as class IV lanthipeptides were found in *Bifidobacterium pseudocatenulatum*, while those implicated in biosynthesis of cytolysins, enterolysins, enterocins and propeptins, among others, were identified in enterococci. Bifidobacterial isolates did not contain genes associated with virulence; however, we detected the presence of putative tetracycline resistance genes in several strains belonging to *Bifidobacterium animalis* subsp. *lactis* and *Bifidobacterium longum* subsp. *longum*. Among the enterococci, *Enterococcus mundtii* PM10 did not carry any genes associated with antimicrobial resistance or virulence. The latter, together with all the *Bifidobacterium* strains, also encoded several putative adaptive and stress-response-related genes, suggesting robust gastrointestinal tract colonization potential. This work provides the first genomic characterization of *Bifidobacterium* and *Enterococcus* isolates from Cameroonian infants. Several strains showed the genomic potential to confer beneficial properties. Further phenotypic and clinical investigations are needed to confirm their suitability as customized probiotics.

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**Author affiliations:** <sup>1</sup>Research Unit of Biochemistry of Medicinal Plants, Food Science and Nutrition (URBPMAN) – Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon. P.O. Box 67, Dschang, Cameroon; <sup>2</sup>Intestinal Microbiome, ZIEL – Institute for Food & Health, Technical University of Munich, Freising, 85354, Germany; <sup>3</sup>Department of Microbes, Infection and Microbiomes, School of Infection, Inflammation and Immunology, College of Medicine and Health, University of Birmingham, Birmingham, B15 42TT, UK; <sup>4</sup>Department of Physiological Sciences and Biochemistry, Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Dschang, Cameroon; <sup>5</sup>Food, Microbiome & Health, Quadram Institute Bioscience, Norwich Research Park, Norwich, NR4 7UQ, UK; <sup>6</sup>Norwich Medical School, University of East Anglia, Norwich Research Park, NR4 7TJ, Norwich, UK.

**\*Correspondence:** Pierre Marie Kaktcham, pierre.kaktcham@univ-dschang.org; Lindsay J. Hall, l.hall.3@bham.ac.uk

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**Abbreviations:** AMR, antimicrobial resistance; ANI, average nucleotide identity; ARG, AMR genes; CARD, Comprehensive Antibiotic Resistance Database; GH, glycoside hydrolase; GIT, gastrointestinal tract; HMOs, human milk oligosaccharides; IS, insertion sequences; LAB, lactic acid bacteria; LMICs, low- and middle-income countries; LNnT, lacto-*N*-neotetraose; LNT, lacto-*N*-tetraose; MRS, de Man, Rogosa and Sharpe; NCBI, National Center for Biotechnology Information; RiPP, ribosomally synthesized and posttranslationally modified peptide; SCFAs, short-chain fatty acids; T3PKS, type III polyketide synthase.

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### Impact Statement

The underexplored ecological niches, e.g. the gut microbiota of infants from low- and middle-income countries (LMICs), could represent a reservoir of novel population-specific beneficial microbes. In this study, we recovered bifidobacteria and enterococci from the intestinal microbiota of Cameroonian infants. Their genomic characterization revealed that strains belonging to *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum* subsp. *longum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium animalis* subsp. *lactis* and *Enterococcus mundtii* have the potential to be considered for further studies aiming at the development of customized probiotics from Cameroon, in particular, and LMICs in general. Not only does our work constitute the first report of the isolation and genomic characterization of bifidobacteria and enterococci from Cameroonian infants, but it also contributes to the reduction of the scarcity of information available on the bacterial members of the infant intestinal microbiota in LMICs.

### DATA SUMMARY

The draft genomes of 36 infant-associated isolates sequenced here have been deposited to the National Center for Biotechnology Information (NCBI) database under the BioProject number PRJNA1091569. Individual accession numbers are available in Table 1. Here is the DOI number of the material stored in figshare: <https://doi.org/10.6084/m9.figshare.28278884> [1].

### INTRODUCTION

The gut microbiota plays an essential role in human health, principally in host metabolism, physiology, nutrition and immune function [2, 3]. Especially during early life, the microbiota performs crucial roles in infant development that determine their health status in childhood and adulthood [4–6]. For example, members of the early-life microbiota can break down non-fermentable sugars such as human milk oligosaccharides (HMOs) and complex plant carbohydrates to produce short-chain fatty acids (SCFAs) [7, 8]. These compounds have been shown to contribute to the shaping of the immune system and the metabolic, endocrine and other host developmental pathways, as well as preventing colonization by pathogenic micro-organisms [9, 10].

The very dynamic infant-associated microbiota only begins to stabilize towards the third year of life, and its perturbation has been associated with conditions such as atopy, asthma, diabetes and gastrointestinal conditions such as diarrhoea, inflammatory bowel diseases and necrotizing enterocolitis [11, 12]. Within the African context, this imbalance may be caused by several factors including host genetics, maternal diet and maternal microbiota composition, antibiotic exposure and malnutrition and poor hygiene and sanitation practices [13, 14]. To mitigate this serious health problem in low- and middle-income countries (LMICs), probiotics, prebiotics and synbiotics are widely used as therapeutic options [15, 16].

Probiotics are ‘live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host’ [17]. The most widely studied and used probiotic strains belong to lactic acid bacteria (LAB) of genera *Lactobacillus*, *Lactocaseibacillus*, *Lactiplantibacillus*, *Levilactobacillus*, *Ligilactobacillus*, *Limosilactobacillus* and to the genus *Bifidobacterium*, which have been shown to dominate the early-life microbiota of humans and animals [18, 19] and to display health-promoting and disease-preventing traits. Another member of the LAB group and a common gut commensal is *Enterococcus*, which demonstrates both probiotic and potentially pathogenic characteristics [20]. Despite this duality, many *Enterococcus* strains have been shown to be effective and safe and have thus been used as probiotics in humans and animals. Mounting evidence has reported the success of particular strains of LAB and *Bifidobacterium* in modulating the gut microbiota and preventing, treating or reducing the risk of microbiota-associated perturbation-related disorders or diseases [21, 22]. Among the mechanisms by which they exert beneficial effects are their abilities to degrade particular dietary components, modulation of gut epithelial activity, increase in mucin production, microbial communication, production of antimicrobial substances (mainly SCFAs and bacteriocins) and immune system modulation [23–26].

Given the pivotal role of bifidobacteria and LAB in improving/maintaining health, coupled with the acute rise in antimicrobial resistance (AMR) among pathogenic bacteria, there is an increasing trend in the probiotic market for new target strains with more efficient probiotic potential, antimicrobial activity [but lack of AMR genes (ARG)] and immunomodulatory anti-infection capacities. In this respect, a promising avenue for novel strain development is an exploration of different geographical regions and ecological niches. One such niche is the early-life microbiota of non-Western communities that remain understudied, from which data and information on microbial composition are scarce. To the best of our knowledge, the microbiomes of Cameroonian infants living in rural areas have not yet been extensively profiled and may constitute a reservoir of key bifidobacteria and LAB candidates.

The search for novel probiotics currently involves the use of *in silico*-based approaches alongside traditional culture-dependent ones as they provide a deeper understanding and insight into the full breadth of strains’ biological capabilities in terms of their

**Table 1.** Putative gene clusters encoding for secondary metabolites in *Bifidobacterium* and *Enterococcus* genomes based on antiSMASH and BAGEL4 analyses

	Strain	Accession no.	Sample ID	Bacteriocin/RiPP identified (antiSMASH)	Bacteriocin/RiPP identified (BAGEL4)
1	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> LH-CAM-01	JBDXST000000000	V11	No	No
2	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> LH-CAM-04	JBDXSQ000000000	V22	No	No
3	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> LH-CAM-58	JBDXSD000000000	V21-2	No	No
4	<i>Bifidobacterium bifidum</i> LH-CAM-42	JBDXSJ000000000	V14-5	No	No
5	<i>Bifidobacterium breve</i> LH-CAM-38	JBDXSK000000000	V14-1	No	No
6	<i>Bifidobacterium breve</i> LH-CAM-52	JBDXSG000000000	V17-5	No	No
7	<i>Bifidobacterium breve</i> LH-CAM-63	JBDXSC000000000	V23-2	No	No
8	<i>Bifidobacterium longum</i> subsp. <i>longum</i> LH-CAM-03	JBDXSR000000000	V20	No	No
9	<i>Bifidobacterium longum</i> subsp. <i>longum</i> LH-CAM-33	JBDXSL000000000	V13-1	No	No
10	<i>Bifidobacterium pseudocatenulatum</i> LH-CAM-02	JBDXSS000000000	V15	Lanthipeptide class IV	No
11	<i>Enterococcus faecalis</i> LH-CAM-21	JBDXSP000000000	V8	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs)	Cytolysin ClyLI Enterolysin A Enterocin SE-K4
12	<i>Enterococcus faecalis</i> LH-CAM-23	JBDXSO000000000	V7-2	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs)	Cytolysin ClyLI Enterolysin A Enterocin SE-K4
13	<i>Enterococcus faecalis</i> LH-CAM-25	JBDXSN000000000	V22	Sactipeptides	No
14	<i>Enterococcus faecalis</i> LH-CAM-32	JBDXSM000000000	V12-2	Sactipeptides	Enterolysin A Enterocin SE-K4 Propeptin 2
15	<i>Enterococcus faecalis</i> LH-CAM-43	JBDXSI000000000	V16	No	No
16	<i>Enterococcus faecalis</i> LH-CAM-51	JBDXSH000000000	V17-4	No	Enterolysin A
17	<i>Enterococcus faecalis</i> LH-CAM-54	JBDXSF000000000	V18-2	No	Enterolysin A
18	<i>Enterococcus faecalis</i> LH-CAM-56	JBDXSE000000000	V19	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs)	Enterocin SE-K4 Enterocin 96 Cytolysin ClyLI Enterolysin A Propeptin 2
19	<i>Enterococcus faecalis</i> LH-CAM-67	JBDXSB000000000	V24-1	02 sactipeptides	Enterocin SE-K4 Enterocin P
20	<i>Enterococcus faecalis</i> LH-CAM-70	JBDXSA000000000	V25-2	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs) Sactipeptides	Enterocin SE-K4 Cytolysin ClyLI Enterolysin A Propeptin
21	<i>Enterococcus faecalis</i> PM1	JBDXRZ000000000	V1	No	Propeptin 2
22	<i>Enterococcus faecalis</i> PM11	JBDXRX000000000	V16	No	No
23	<i>Enterococcus faecalis</i> PM12	JBDXRW000000000	V17	No	No
24	<i>Enterococcus faecalis</i> PM14	JBDXRV000000000	V18	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs) Sactipeptides	Enterocin SE-K4 Enterocin 96 Cytolysin ClyLI Enterolysin A Propeptin 2
25	<i>Enterococcus faecalis</i> PM2	JBDXRU000000000	V1	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs)	Enterocin SE-K4 Cytolysin ClyLI Enterolysin A Propeptin 2
26	<i>Enterococcus faecalis</i> PM20	JBDXRT000000000	V21	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs) Sactipeptides	Enterocin SE-K4 Enterocin 96 Cytolysin ClyLI Enterolysin A

Continued

Table 1. Continued

	Strain	Accession no.	Sample ID	Bacteriocin/RiPP identified (antiSMASH)	Bacteriocin/RiPP identified (BAGEL4)
27	<i>Enterococcus faecalis</i> PM27	JBDXRQ000000000	V23	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs) Sactipeptides	Enterocin SE-K4 Enterocin 96 Cytolysin ClyLI Enterolysin A
28	<i>Enterococcus faecalis</i> PM3	JBDXRP000000000	V16	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs)	Enterocin SE-K4 Cytolysin ClyLI Enterolysin A Propeptin 2
29	<i>Enterococcus faecalis</i> PM30	JBDXRO000000000	V25	Lanthipeptide class II (cytolysin ClyLI/cytolysin ClyLs) Sactipeptides	Enterocin SE-K4 Enterocin 96 Cytolysin ClyLI Enterolysin A
30	<i>Enterococcus faecalis</i> PM5	JBDXRL000000000	V6	No	No
31	<i>Enterococcus faecalis</i> PM6	JBDXRK000000000	V8	Sactipeptides	No
32	<i>Enterococcus faecium</i> PM21	JBDXRS000000000	V21	T3PKS	Bac 32 Sactipeptides Enterocin A Enterolysin A
33	<i>Enterococcus faecium</i> PM25	JBDXRR000000000	V22	T3PKS Enterocin A Sactipeptides	Enterocin A UViB Enterolysin A
34	<i>Enterococcus faecium</i> PM32	JBDXRN000000000	V26	T3PKS	Bac 32 Sactipeptides Enterocin A Enterolysin A
35	<i>Enterococcus faecium</i> PM34	JBDXRM000000000	V17	T3PKS	Bac 32 Sactipeptides Enterocin A Enterolysin A
36	<i>Enterococcus mundtii</i> PM10	JBDXRY000000000	V16	T3PKS Terpene	Sactipeptides UViB Enterolysin A

probiotic, functional and safety-associated capacities [27, 28]. Indeed, the analysis of genomic data helps to understand the potential mechanisms by which bacteria adapt to the specific environment of the gastrointestinal tract (GIT), while also revealing genetic functions that mediate specific microbe-microbe and microbe-host interactions – features claimed to be useful in the genome-inspired development of new probiotic formulations.

In this study, *Bifidobacterium* and *Enterococcus* isolates were recovered from the faeces of Cameroonian infants, and their whole-genome sequences were examined to unravel the genetic basis for their prospective beneficial abilities, antimicrobial activities and safety.

## METHODS

### Study participants, faecal sample collection and isolation of bacteria

Mothers (without gastrointestinal disorders, illness in the previous 7 days or antibiotic treatment in the previous 14 days) and infants aged 0–5 months were recruited in the health district of Dschang (Menoua Division, West Region, Cameroon) following parental written informed consent. The infant cohort consisted of vaginally delivered full-term healthy babies, breast-fed and/or mixed-fed (but not receiving probiotic formula) (Table S1, available in the online version of this article).

Fresh infant faecal samples (from diapers immediately after defecation) were aseptically collected in labelled faeces collectors and kept in iceboxes. They were subsequently transported to the laboratory within 2 h and frozen at  $-80^{\circ}\text{C}$  in de Man, Rogosa and Sharpe (MRS, Lab M, UK) broth supplemented with glycerol (30% v/v) until shipping to the Technical University of Munich for processing (Intestinal Microbiome, ZIEL-Institute for Food and Health, TUM, Germany).

For isolation, 26 faecal glycerol stocks were tenfold serially diluted (1 g, up to  $10^{-5}$ ), and the dilutions were plated (100  $\mu\text{l}$ ) onto MRS agar (BD Difco™, Becton, Dickinson and Company, USA) without any additives or supplemented with L-cysteine hydrochloride (0.05%, Sigma-Aldrich, UK) and mupirocin (0.005%, Sigma-Aldrich, UK). The plates were then incubated at  $37^{\circ}\text{C}$  for 48 h, aerobically or in an anaerobic chamber (Whitley A95 Anaerobic Workstation, Don Whitley Scientific Limited, UK). For each sample, three colonies from each dilution were randomly selected, visually examined for colony and cell morphology

characteristics and streaked to purity on the isolation medium. Pure cultures were stored at  $-80^{\circ}\text{C}$  in cryogenic tubes containing MRS with 30% glycerol.

### Genomic DNA extraction, whole-genome sequencing and data processing

Total DNA was extracted from bacterial cells (pellet of 48-h-old culture) using the FastDNA™ SPIN kit (MP Biomedicals, USA) after lysis in the FastPrep-24 instrument (MP Biomedicals, USA) at a speed of  $6.0\text{ m s}^{-1}$  for 40 s. Partial 16S rRNA gene sequencing was performed using primers 5'-AGA GTT TGA TCC TGG CTC AG-3', 5'-AGA GTT TGA TCA TGG CTC AG-3' and 5'-ACG GTT ACC TTG TTA CGA CTT-3' at Eurofins Genomics, Ebersberg, Germany. Preliminary identification of the isolates was performed with *sina* aligner (v.1.2.12) [29].

Based on the results of the preliminary identification, we selected *Bifidobacterium* and *Enterococcus* isolates and subjected them to whole-genome sequencing on either Illumina MiSeq (samples labelled 'PM') or Illumina NextSeq2000 platform (samples labelled 'LH-CAM') (read length  $2\times 150\text{ bp}$ , average sequencing coverage of  $216\times$ ) at the Quadram Institute Bioscience (Norwich, UK). Sequencing reads were pre-processed with *fastp* v0.20 (samples 'PM') and *fastp* v0.23 (samples 'LH-CAM') [30]. Draft genome assemblies were generated using SPAdes v.3.14.1 with '--careful' option ('PM' samples) [31] and Unicycler v.0.4.9 with the '--mode conservative' option ('LH-CAM' samples) [32]. Contigs below 1000 bp were filtered out of all assemblies. We estimated genome completeness and contamination using CheckM v.1.2.0 [33] and retained sequences with completeness  $>98\%$  and contamination  $<1\%$ . GTDB-Tk v.2.3.2 [34] was used to classify all genomic sequences to the strain level, and python3 module pyANI v.0.2.10 with default settings was used to calculate the average nucleotide identity (ANI) values [35]. Species delineation cut-off was set at 95% identity [36]. Sequences showing identity values above 99.9% from the same sample were considered identical and were removed from further analysis [37]. After such processing, the final dataset comprised 36 unduplicated *Bifidobacterium* and *Enterococcus* genomes (Table S2). All assemblies were annotated using Prokka v.1.14.6 [38]. In addition, previously assembled publicly available sequences of selected type strains *Bifidobacterium* and *Enterococcus* were retrieved from the NCBI Genome database.

### Computational analysis

The multiple sequence alignment generated with GTDB-Tk was used to assess the phylogenetic relationship of strains included in the analysis. Model testing and subsequent maximum likelihood estimation (best-fit model LG+F+R3) were performed in IQ-TREE v.2.0.5 [39]. For selected *Bifidobacterium* and *Enterococcus* isolates from different infants showing ANI values above 99.9%, *snippy* v.4.6.0 [40] was used to map the raw sequencing reads against reference assemblies, namely *Bifidobacterium animalis* subsp. *lactis* ATCC 22563<sup>T</sup> and *Enterococcus faecalis* PM12. Carbohydrate metabolism properties of strains included in the dataset were profiled using a standalone version of dbCAN3 [41] with '--hmm\_cov 0.50' option. Prediction of the presence of HMO clusters in *Bifidobacterium* was performed by comparing genomes included in the final dataset to known bifidobacterial protein sequences using local BLASTP (e-value  $<1e^{-50}$ ). HMO clusters were annotated 'present' if all cluster components were identified at the above homology level. Incomplete clusters (more than three locally clustered genes) were annotated as 'partially present'.

The presence of putative bacteriocin and secondary metabolite biosynthesis gene clusters in the assemblies was predicted using antiSMASH v.7.0 [42] and BAGEL4 [43]. MobileElementFinder v.1.0.3 [44] and VirulenceFinder 2.0 database tool [45] (with a threshold of at least 99% similarity) were used to assess the presence of plasmids, insertion sequences (IS) and virulence factors in our dataset.

The Comprehensive Antibiotic Resistance Database (CARD, v.3.2.9) (perfect or strict hits with at least 95% amino acid homology considered significant) [46, 47] and the ResFinder v.4.4.2 tool incorporated in the MobileElementFinder package [48] (at least 99% identity threshold) were used for the *in silico* identification of antibiotic resistance genes in the analysed genomes. The standalone version of PHAge Search Tool with Enhanced Sequence Translation [49] implemented in Docker Compose v.2.30.3 [50] was employed as a predictive tool for the identification of prophages within bacterial genomes. The completeness level of all predicted prophage regions was evaluated using CheckV v.1.0.3 [51] with an 'end-to-end' parameter and the database v.1.5. To identify potential ARG and virulence factors in prophage sequences, we compared them to the CARD and VirulenceFinder databases, as above.

The annotated files produced by Prokka were used to manually search for the presence of genes and gene clusters involved in tolerance to acid and bile salts, general stress resistance, adhesion and host GIT adaptations.

R v.4.2.3 [52] with packages *factoextra* v.1.0.7 [53] and *ComplexHeatmap* [54], as well as iTOL v.6.8.1 [55], were used for data visualization.

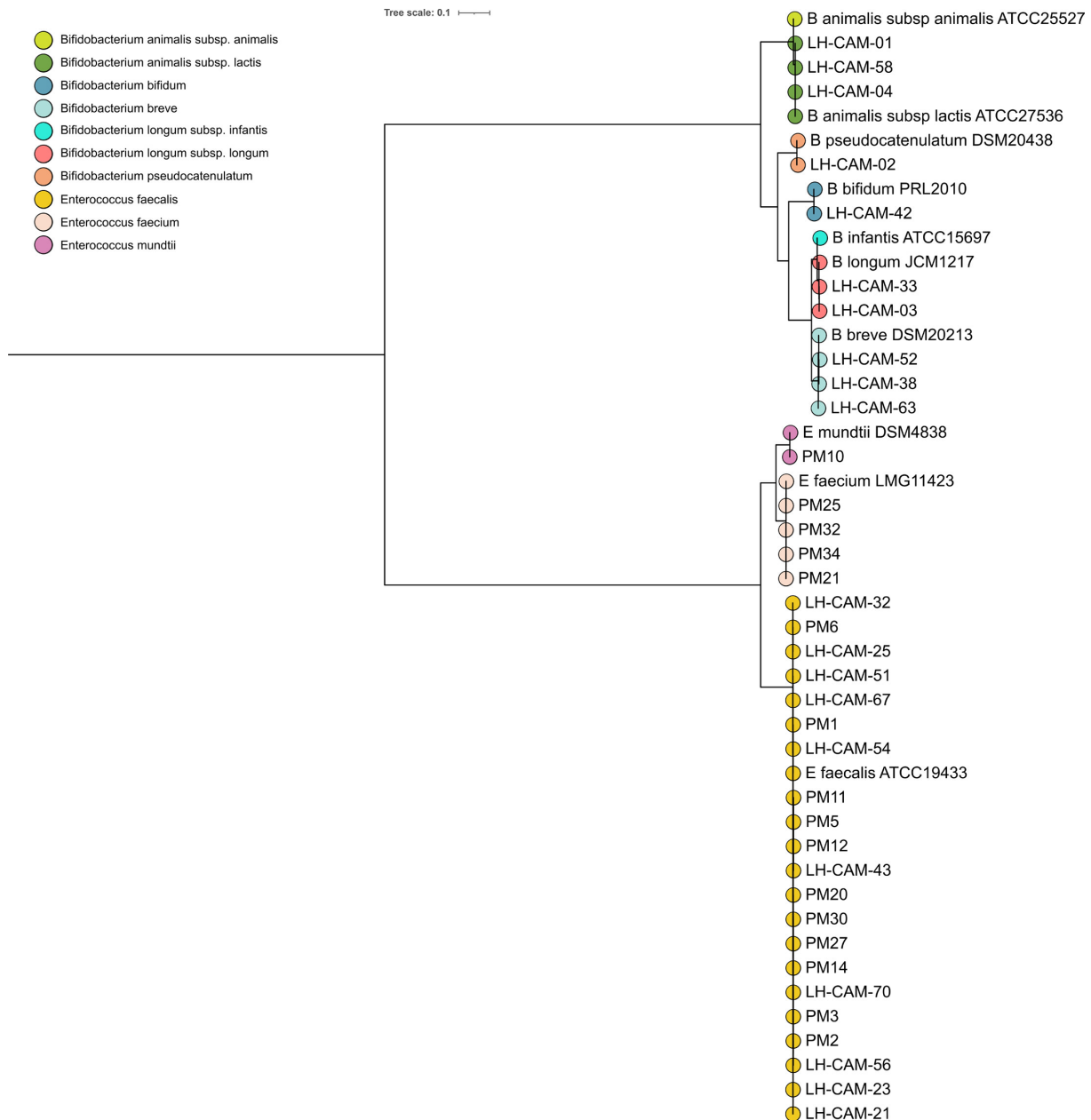
## RESULTS

### General genomic features and relatedness of isolates recovered from Cameroonian infants

The purpose of this study was to define the beneficial potential of bifidobacteria and enterococci isolated from Cameroonian infants using an *in silico* approach. A total of 150 isolates were obtained from the faecal samples. After preliminary identification

based on partial 16S rRNA gene sequencing, we performed whole-genome sequencing on *Enterococcus* and *Bifidobacterium* isolates, which constituted the majority of the recovered isolate pool (55 and 30%, respectively). Following processing and dereplication, we included 36 genomic sequences in the final dataset. Genome assembly produced sets of contigs ranging from 11 to 138 per strain (Table S2). The G+C content ranged from 37.0 to 38.2% for *Enterococcus* strains and 56.2 to 62.3% for *Bifidobacterium* strains. Consistent with previous reports [56], genome sizes ranged from 1.89 to 2.46 Mb for bifidobacteria (average of 2.22 Mb) and 2.68 to 3.21 Mb for enterococci (average of 2.91 Mb). Differences in genome sizes were reflected in the number of predicted CDS, which was lower in bifidobacteria compared to enterococci, with an average of 1858 and 2751, respectively.

Together with ten genomic sequences representative of type strains *Bifidobacterium* and *Enterococcus* species, genomes constituting our final dataset were subjected to relatedness and gene content assessment to gain insights into their global genomic traits. The phylogeny (Fig. 1) and the ANI analysis (Table S3) indicated clustering into eight main groups: *B. animalis*, with



**Fig. 1.** Relatedness of isolates recovered in this study to bacterial type strains representative of infant-associated species of *Bifidobacterium* and *Enterococcus*. The phylogeny was constructed from the GTDB-tk marker gene alignment using the maximum likelihood estimation based on the best-fit model LG+F+R3. The tree was rooted mid-point. The node colours denote cluster membership of analysed isolates.

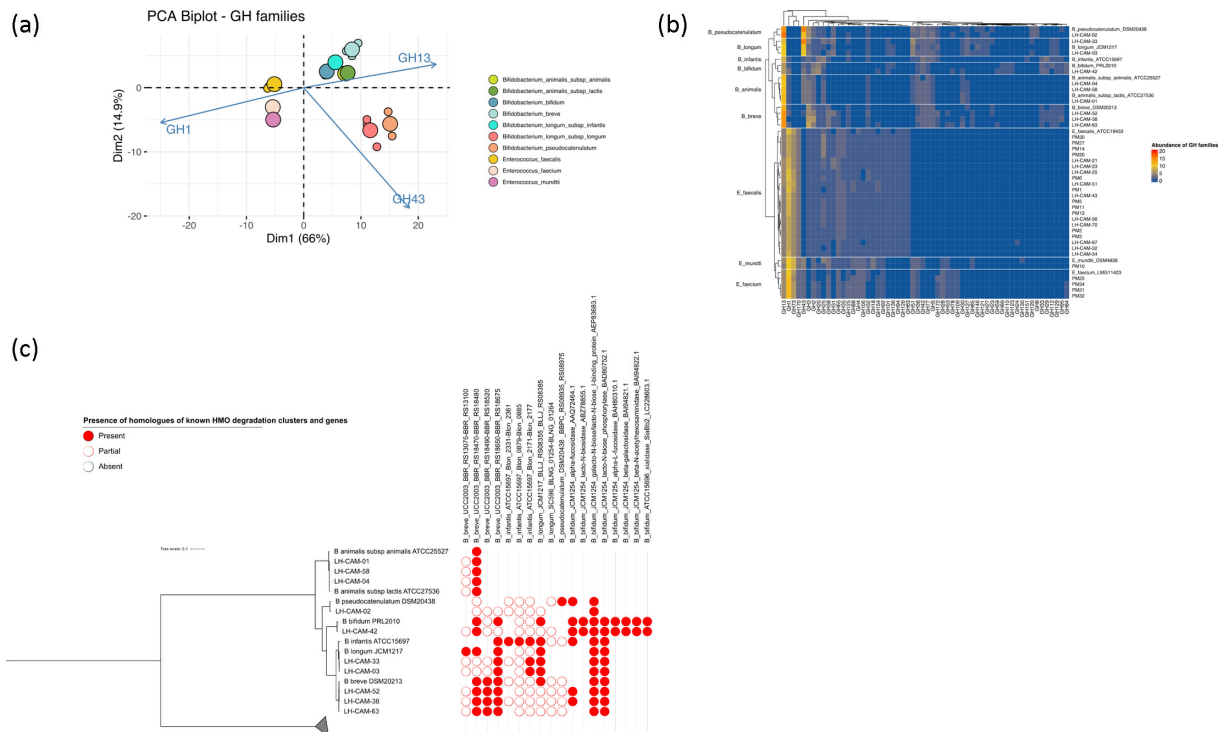
strains belonging to subsp. *lactis* identified in our dataset, *Bifidobacterium longum* subsp. *longum*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium pseudocatenulatum*, *E. faecalis*, *Enterococcus faecium* and *Enterococcus mundtii*. Based on the ANI values (ANI >99.9%) and snippy analysis of selected genomes, we identified what we considered to be clonal strains belonging to *B. animalis* subsp. *lactis* (<26 SNPs) and *E. faecalis* (1 SNP) taxa in samples from different infants (Table S4).

### Functional annotation of bifidobacterial and enterococcal genomes – potential for carbohydrate utilization

Different infant-associated bacterial taxa have previously been shown to possess particular carbohydrate metabolism capabilities, which are important for dietary substrate degradation and the development of cooperative microbial communities [57–60]. We therefore investigated carbohydrate-active enzyme family repertoires, and specifically the presence of glycoside hydrolases (GH), in *Bifidobacterium* and *Enterococcus* genomes, as well as the genomic potential of bifidobacteria to metabolize HMOs (Fig. 2, Tables S5 and S6).

On average, we identified 18 different GH families in *Bifidobacterium* sequences, with an average of 46 GH genes (2.1% of CDS) per genome, consistent with previous reports [26, 61]. The predominant GH family identified in *Bifidobacterium* was GH13, comprising enzymes capable of hydrolysing  $\alpha$ -1,4-glycosidic linkages in starches and similar substrates [62], while the second most abundant GH family detected in the genomes of *B. longum* and *B. pseudocatenulatum*, but not in other bifidobacterial species, was GH43 containing enzymes involved in the degradation of aryl glycosides [63]. Other notable GH families included GH3, GH2 and GH42, which encompass enzymes capable of hydrolysing a wide variety of plant cell wall glycans, with members of GH2 and GH42 families also implicated in the metabolism of lactose, a disaccharide abundant in human milk. We also identified putative GH33 exo-sialidases in the genomes of *B. bifidum* and *B. breve*, which indicates that these strains may be able to directly utilize host glycans and metabolize free sialic acid (Fig. 2a, b, Table S5) [64].

In enterococci, an average of 36 different GH families were identified, with an average of 47 genes per genome (1.3% of CDS). Genes identified as members of the GH1 family, which contains enzymes with  $\beta$ -glucosidase and  $\beta$ -galactosidase activity, were the most abundant. Consistent with previous findings, other highly represented GH families included GH73, which represents enzymes with suggested peptidoglycan hydrolase activity [65, 66] and GH13 (Fig. 2a, b, Table S5).



**Fig. 2.** Genomic prediction of carbohydrate metabolism properties of strains analysed in this study. (a) Principal component analysis based on the predicted GH family abundance data grouped by bacterial species. The top three contributing variables are marked on the biplot. (b) Heatmap showing the abundance of predicted GH families in the strains included in the dataset. (c) Diagram depicting the presence and absence of homologues of known HMO degradation clusters and genes in *Bifidobacterium* isolates used in this study (BLASTP, e-value <1e<sup>-50</sup>). HMO clusters were annotated 'present' if all cluster components were identified at the above homology level. Incomplete clusters (more than three locally clustered genes) were annotated as 'partially present'.

Since the ability to metabolize HMOs is an important functional property of several early life-associated *Bifidobacterium* species, namely *B. breve*, *B. infantis*, *B. longum*, *B. pseudocatenulatum* and *B. bifidum* [67–71], we investigated the presence of known bifidobacterial HMO metabolism genes and gene clusters in our genomic dataset. Consistent with previous reports, the results of this analysis revealed strain- and species-specific differences in the distribution of putative homologues of clusters and genes implicated in HMO metabolism in our *Bifidobacterium* strains [26, 72].

The metabolism of lacto-*N*-tetraose (LNT) and lacto-*N*-neotetraose (LNnT) has been linked to specific metabolic pathways in different bifidobacterial species and strains. The ability of type strain *B. infantis* ATCC 15697<sup>T</sup> to degrade these HMOs has been shown to be regulated by a global transcription factor NagR acting as a regulator for multiple gene clusters, including *nag* (Blon\_0879–0885), *lnp* (Blon\_2717–2177) and H1 (Blon\_2331–2361) [73]. We identified homologues to the *lnp* cluster in the two isolated Cameroonian *B. longum* strains (LH-CAM-03 and LH-CAM-33), with partial homology to the H1 cluster recorded only in *B. longum* LH-CAM-33. Additionally, *B. pseudocatenulatum* LH-CAM-02 and *B. breve* LH-CAM-38 showed partial homology to all three clusters (Fig. 2c, Table S6).

Similarly, the metabolism of LNT and LNnT in *B. breve* UCC2003 has been shown to involve several clusters, namely *lnt* (BBR\_RS13075–BBR\_RS13100), *lac* (BBR\_RS18470–BBR\_RS18480), *nah* (BBR\_RS18490–BBR\_RS18520), and *lnp/lgt* (BBR\_RS18650–BBR\_RS18675) [69]. All Cameroonian *B. breve* genomes contained homologues to *lac*, *nah* and *lnp/lgt* clusters; however, only partial homology to the *lnt* cluster was detected in our *B. breve* strains (Fig. 2c, Table S6).

Degradation of fucosylated HMOs by bifidobacteria has been linked to the presence of particular enzymatic machinery containing an  $\alpha$ 1-3/4-fucosidase (GH29) and/or  $\alpha$ 1-2-fucosidase (GH95). A specific gene cluster for 2'-fucosyllactose metabolism has been described in *B. longum* SC569 (BLNG\_01254–BLNG\_01264), with homologous genes identified in *B. infantis* ATCC 15697<sup>T</sup> and *B. pseudocatenulatum* DSM 20438<sup>T</sup> [70]. In our dataset, we identified putative GH29 and GH95 fucosidases in *B. breve* LH-CAM-38, *B. breve* LH-CAM-52 and *B. bifidum* LH-CAM-42, with all three strains showing partial homology to the *B. longum* SC569 cluster (Fig. 2b, c, Tables S5 and S6).

Previous studies in *B. bifidum* have shown that six extracellular enzymes, namely  $\alpha$ 1,2-fucosidase,  $\alpha$ 1-3/4-fucosidase, lacto-*N*-biosidase,  $\beta$ 1,4-galactosidase,  $\beta$ 1,3-*N*-acetylglucosaminidase and sialidase, as well as one intracellular phosphorylase and one transporter, play crucial roles in the metabolism of neutral HMOs in this species [74]. We detected the presence of homologues of all these genes in *B. bifidum* LH-CAM-42, with homology to the intracellular phosphorylase and the transporter alone identified in strains of other early-life-associated bifidobacteria (Fig. 2c, Table S6).

### Genomic antimicrobial, adhesion, stress-resistance and safety features of bifidobacteria and enterococci isolated from Cameroonian infants

A significant aspect of the initial probiotic strain selection is the assessment of antibiotic resistance patterns. In addition, a candidate strain must be able to tolerate acidic and bile-rich conditions of the GIT [75] and be able to adhere to the epithelium to successfully colonize the gut, thereby facilitating displacement of potentially pathogenic micro-organisms from host cells [76, 77]. The production of antimicrobial compounds (e.g. bacteriocins) may enhance a strain's ability to compete against other bacteria and potentially inhibit pathogenic microbes [78]. Therefore, we investigated the genomic potential of our bifidobacterial and enterococcal isolates to survive the passage through the GIT and their safety features.

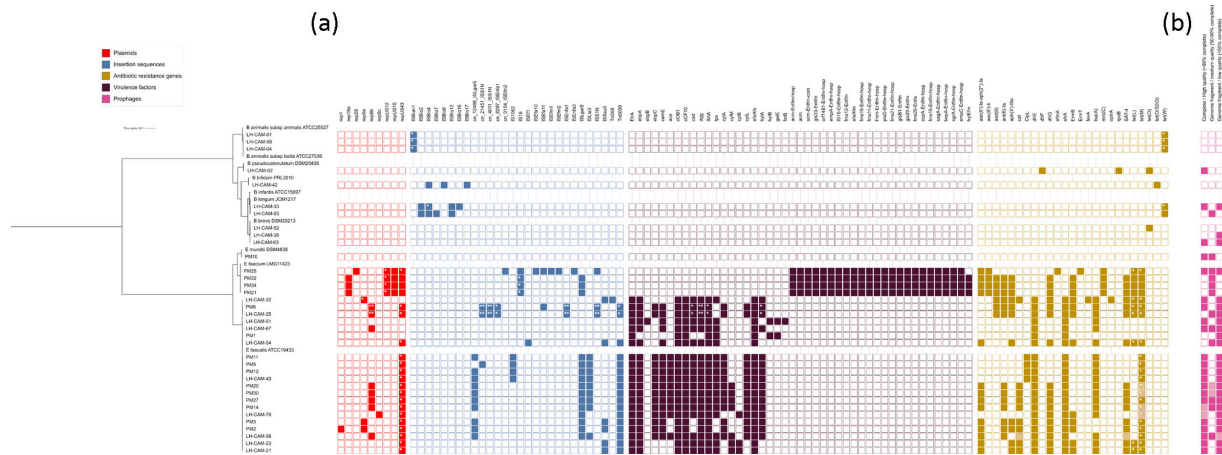
#### Putative secondary metabolite production capabilities

We used antiSMASH and BAGEL4 to predict the presence of putative secondary metabolite biosynthesis gene clusters in our dataset (Table 1). In bifidobacteria, only one hit indicating putative production of class IV lanthipeptides was recorded in the genome of *B. pseudocatenulatum* LH-CAM-02 after antiSMASH analysis. Class IV lanthipeptides are young members of ribosomally synthesized and posttranslationally modified peptide (RiPP) subfamilies, described more recently and having no or only weak antibacterial activity [79]. In contrast, the presence of several different secondary metabolite biosynthesis gene clusters was predicted in enterococci. Overall, gene clusters for cytolysin ClyLI and CLyLs, enterolysin A, enterocin SE-K4, enterocin P, enterocin 96, propeptin, propeptin 2 and sactipeptides were found in *E. faecalis* genomes; enterocin A, enterolysin A, Bac 32, UViB, type III polyketide synthase (T3PKS) and sactipeptides in *E. faecium* genomes; and T3PKS, UViB, terpene, enterolysin A and sactipeptides in *E. mundtii* PM10 genome (Table 1).

#### Prediction of plasmids, mobile genetic elements, antibiotic resistance genes, virulence factors and prophage sequences

Assessment of genomic safety features is a crucial step in the characterization of potentially beneficial bacterial strains. Our results from such analysis indicate that bifidobacteria isolated from Cameroonian infants do not possess plasmids nor virulence factors but harbour putative tetracycline resistance genes [*tet(W)* and *tet(O)*] in their genomes, except for two *B. breve* strains, LH-CAM-38 and LH-CAM-68 (Fig. 3, Table S7). In addition, *B. lactis*, *B. longum* and *B. bifidum* strains were found to harbour IS homologous to previously reported various strains of *B. animalis* subsp. *animalis* and *lactis* [80] and *B. longum*, in particular *B. longum* NCC2704 [81].





**Fig. 3.** Genomic prediction of features potentially associated with virulence and antimicrobial resistance in the strains recovered in this study. (a) The coloured squares denote the presence of DNA regions encoding putative plasmids (red; MobileElementFinder, 99% identity), insertion sequences (blue; MobileElementFinder, 99% identity), virulence factors (maroon; VirulenceFinder, 99% identity) and antibiotic resistance genes (mustard; combined results from CARD and ResFinder databases, 95 and 99% amino acid identity, respectively). Asterisks denote features located on the same contig. (b) The coloured squares mark the presence of identified prophage sequences at various genomic integrity levels (pink; CheckV quality score as follows: high quality, >90% complete; medium quality, 50–90% complete; low quality, <50% complete). Striped squares in both the antibiotic resistance panel and the prophage panel denote corresponding putative prophage-mediated antibiotic resistance genes.

Regarding enterococci, *E. mundtii* PM10 was interestingly found to be free of plasmids, IS, antibiotic resistance genes and virulence factors (Fig. 3, Table S7). Two *E. faecalis* strains (LH-CAM-51 and PM1) were found not to possess plasmids nor IS, but they harboured various ARG and virulence factors. The majority of *E. faecalis* genomes were found to contain homologues of genes encoding resistance to tetracyclines [*tet(M)*, *tet(L)*, *tet(S)*], lincosamides (*lsaA*), macrolides (*ermB*, *ermT*, *msrC*), aminoglycosides [*aac(6′)-Ii*, *aph(3′)-III*, *ant(6)-Ia*, *aac(6′)-aph(2′′)*], streptogramins (*lsaA*, *msrC*), trimethoprim (*dfgG*) and chloramphenicol (*cat*, *optA*). In addition, a plethora of virulence genes were detected, including biofilm formation-associated pili genes (*ebpA*, *ebpB*, *ebpC*, *SrtA*), adhesins (*ace*, *efaAfs*, *agg*, *acm*, *efaAfm*), sex pheromones (*cad*, *camE*, *cCF10*, *cOB1*), cytolysins (*cylB*, *cylL*, *cylM*), hyaluronidase (*hyla*, *hylB*), gelatinase (*gelE*), oxidative stress resistance (*tpx*), anti-phagocytic activity (*ElrA*) and quorum-sensing (*fsrB*). An even broader spectrum of virulence genes was detected in *E. faecium* genomes (Fig. 3, Table S7). All *Enterococcus* genomes were found to contain prophage sequences, with *E. faecium* strains harbouring 1.74 and *E. faecalis* strains harbouring 3.38 prophage fragments, on average (Fig. 3, Tables S9 and S10). We did not identify any virulence factors associated with prophage sequences, and it is worth noting, however, that we detected prophage-mediated ARGs in five out of 21 *E. faecalis* strains (23.8%), with tetracycline resistance gene *tet(M)* being the most commonly identified (Fig. 3, Table S11). Moreover, in the remaining *E. faecalis* and *E. faecium* strains, *tet(M)* and *tet(L)* genes were often located on plasmids.

### Prediction of probiotic and stress-related genes in strains selected as safe

Based on the results of our *in silico* functional and safety screens, we selected 11 Cameroonian strains of interest, namely all *Bifidobacterium* strains and the *E. mundtii* PM10, and assessed their genomic potential for gut survival and stress response. This analysis revealed the putative presence of a vast array of genes important for cell survival and intestinal adhesion in all analysed genomes (Table S8). Among the acid tolerance genes identified, ATP-dependent protease proteolytic subunits (ATPase subunits a, b, c, alpha, beta, gamma and delta), Na<sup>+</sup>/H<sup>+</sup> antiporter, L-lactate dehydrogenase, aspartate-semialdehyde dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate isomerase, pyruvate kinase and protein RecA were present in both *Bifidobacterium* and *Enterococcus*, with species-specific differences recorded for the components of phosphotransferase systems, i.e. cellobiose-specific enzymes, and ATP-dependent Clp protease ATP-binding subunits, among others. Not all bifidobacterial strains harboured the full spectrum of acid tolerance genes.

In terms of putative tolerance to bile salts, bile salt hydrolase, phosphoglycerate kinase, cytidine triphosphate synthase, chaperone protein DnaK and DnaJ, ornithine carbamoyltransferase and arginine repressor ArgR genes were found in all *Bifidobacterium* and the *Enterococcus*, while the arginine/ornithine antiporter ArcD was found exclusively in the *E. mundtii* PM10 genome. Among the genes responsible for adhesion, manganese ABC transporter substrate-binding lipoprotein, sortase D and tyrosine-protein kinases YveL and YwqD were only identified in *E. mundtii* PM10. Moreover, among the 12 adaptive stress response genes screened for universal stress protein, heat shock protein and cold-shock protein-encoding genes, eight were present in *E. mundtii* PM10, while only six of them were distributed among the *Bifidobacterium* species. Stress response protein YhaX, general stress proteins 13 and A and cold-shock protein 2, CspLA and CspD genes were present in *E. mundtii* PM10 only, while genes for groES and

groEL chaperonins were found exclusively in *Bifidobacterium* strains. Interestingly, *E. mundtii* PM10 harbours more survival, adhesion and stress resistance genes than bifidobacteria.

## DISCUSSION

The establishment of a healthy gut microbiota in early life plays an important role in maintaining immediate and long-term health. Alteration in microbiota composition in infancy, especially in LMIC communities, has been associated with several disease pathologies, such as diarrhoea, inflammatory bowel disease and necrotizing enterocolitis, thus increasing the risk of serious morbidity [11, 12]. Thus, interventions based on the use of probiotics, prebiotics or symbiotics provide promising therapeutic options in LMICs [15, 16]. However, it is important to consider screening geographically and population-relevant beneficial early-life gut microbiota members for next-stage development. In Cameroon, for instance, the prevalence of probiotic/symbiotic consumption is low, and even nil in some rural areas, with mainly dairy products containing probiotic strains or nutraceuticals, as well as probiotic infant formulas. Coupled with this, microbiome research has been largely limited to metagenomic studies investigating the effect of lifestyle and HIV infection on the gut microbiota composition [82–87]. Based on the scarcity of information on the health-promoting potential of regular members of the gut microbiota of Cameroonian infants, it is important to explore this ecological niche. We set out to fill this gap by isolating bifidobacteria and enterococci, sequencing their genomes and using *in silico* approaches with the aim of selecting local strains that may be used as probiotics in microbiota interventions in the future.

Upon isolation and whole-genome sequencing, we identified known infant-associated bifidobacteria and enterococci in our sample set. In terms of species diversity, our findings were consistent with previously published data from rural and semiurban areas of African LMICs, including Kenya [88], Ivory Coast, Malawi [89, 90] and Zimbabwe [91]. Surprisingly, we detected both *Bifidobacterium* and *Enterococcus* strains characterized by a low number of SNP differences (<30) in samples from different infants. The *B. animalis* subsp. *lactis* ATCC 27536<sup>T</sup> is a well-known probiotic used worldwide, and its presence in infants from our cohort can perhaps be explained by the consumption of probiotic products or nutraceuticals containing this strain by the mothers, while the presence of clonal *Enterococcus* strains in samples from different infants may be the consequence of an existing reservoir in the studied community.

Species and strains of *Bifidobacterium* are known to utilize HMOs, and this property likely contributes to their ability to function as a foundation genus within the wider context of the early-life microbiota [26, 67, 69, 70, 92, 93]. HMOs represent a key nutritional component of breast milk, and substantial variations in HMO concentrations and profiles have been identified in women around the world [94–97]. Previous studies reported high proportions of milk containing a low abundance of  $\alpha$ 1–2-fucosylated structures for West and South Africa (37% in South Africa, 36% in Gambia and 32% in Ghana) compared with values for other African countries, including Namibia (17%) and Malawi (25%) [96, 97]. To the best of our knowledge, no data exist on the oligosaccharide concentrations and profiles in breast milk from Cameroon; our analysis, however, identified *Bifidobacterium* strains, and *B. breve* and *B. bifidum* in particular, with genomic potential to metabolize a variety of HMOs, including fucosylated structures. Given that the presence of genes in the bacterial genomic sequence does not always result in a growth phenotype on the specified HMOs, experimental selection of robust HMO metabolizers as candidates for probiotic development will be a crucial next step.

Potentially, probiotic strains intended for commercial use should fulfil safety criteria, as per the European Food Safety Authority guidelines. Their genomes should be free from antibiotic-resistance genes or genes coding for known virulence factors such as toxins, invasion and adhesion factors [98]. AMR determinants located in the proximity of transposable elements or falling inside bacterial plasmids could contribute to the spread of AMR [99, 100]. In our study, the *in silico* analysis showed that *B. breve* LH-CAM-38, *B. breve* LH-CAM-63 and *E. mundtii* PM10 did not harbour plasmids, mobile genetic elements, ARG or virulence factors but contained prophage sequences. Several *Bifidobacterium* strains were found to possess resistance genes either alone or together with a number of IS, which is in line with previous findings [101–103]. While the presence of IS flanking ARG has been implicated in the promotion of AR gene transfer between micro-organisms [104, 105], previous studies have shown that the probability of gene transfer in *Bifidobacterium* possessing IS is extremely low [106, 107]. The analysed *E. faecalis* and *E. faecium* strains were all found to harbour plasmids, IS, ARG, virulence factors and prophage sequences, consistent with previous literature reports showing that members of the *Enterococcus* and *Lactobacillus* genera carry AR and virulence genes either on plasmids, within prophage fragments or in the proximity of conjugative transposons [108–110].

The production of antimicrobial compounds such as bacteriocins contributes to higher niche competitiveness and inhibition of intestinal pathogens, a property regarded as a probiotic trait. Especially during the early-life developmental window, the production of bacteriocins by beneficial microbiota members can facilitate efficient colonization and ecosystem structuring. The screen for the secondary metabolite biosynthesis clusters among bifidobacteria predicted the presence of class IV lanthipeptide biosynthetic cluster only in one isolate, *B. pseudocatenulatum* LH-CAM-02. This result is consistent with the findings of Yu *et al.* [111] and the general consensus that bifidobacteria are overall poor sources of bacteriocins

[112–115]. In contrast, enterococcal genomes encoded several diverse bacteriocin gene clusters, ranging from class I to III bacteriocins. This is in agreement with previous findings reporting that bacteriocin-encoding genes are widely disseminated among enterococci of different origins [116–118]. Notably, the *E. mundtii* PM10 strain, free from AR genes and virulence factors, showed genomic potential to produce T3PKS and terpene alongside the bacteriocins UViB and enterolysin A. Polyketide synthases have potential applications as anti-infective, anti-tumour and immunosuppressive agents. They have also been associated with bacterial viability and antibacterial activity of the producing bacteria in the intestinal environment [119–121]. Terpenes have been shown to play a significant role in treating various types of diseases through their anticancer, antimicrobial, anti-inflammatory, antioxidant, antiallergic, neuroprotective, anti-aggregator, anti-coagulation, sedative and analgesic effects [122, 123].

To be able to exert their health-promoting effects on the host, bacteria should survive gastrointestinal transit and transiently colonize the GIT, which is also a prerequisite for bacteria termed probiotics. Indeed, the harsh conditions of the stomach and small intestine, especially exposure to oxygen or other oxygen-derived free radicals, organic acids and bile, as well as osmotic stress, can have a negative impact on cell viability and hence on probiotic functionality [124]. Consistent with previous reports [28, 125], we detected a plethora of genes related to acid and bile salt tolerance, general response to stress and oxidative stress and temperature in our *Bifidobacterium* strains and *E. mundtii* PM10, indicating their high potential for survival in the GIT. These strains ought to be further characterized for their phenotypic probiotic and immune-modulatory properties, complemented with clinical trials.

## CONCLUSION

In this study, we isolated and genomically characterized a selection of bifidobacteria and enterococci from the intestinal microbiota of healthy Cameroonian infants. Screening genomes for beneficial as well as safety properties revealed that strains belonging to *B. bifidum*, *B. longum* subsp. *longum*, *B. breve*, *B. pseudocatenulatum*, *B. animalis* subsp. *lactis* and *E. mundtii* have genomic potential to be considered for further studies aiming at the development of customized probiotics from Cameroon in particular and LMICs in general. This is important for the creation of locally sourced microbiota-based therapies to improve infants' health in specific regions. As a next step, it is important to define the applicability of these promising strains as therapeutics through phenotypic characterization and clinical trials. Moreover, the results of this study will significantly complement the information available on the intestinal microbiota composition of infant cohorts from underrepresented geographical areas of the world.

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### Author contributions

P.M.K., F.Z.N. and L.J.H. conceived the overall study. E.M.F.K., L.T.P. and M.L.T.T. participated in obtaining the ethical clearance, collected the faecal samples and provided metadata. P.M.K., J.M., A.F. and B.-A.T. processed the samples and isolated strains. P.M.K., J.M. and A.F. prepared the DNA for whole-genome sequencing, and P.M.K. and M.K. performed genomic analysis and visualization, and P.M.K., M.K. and L.J.H. analysed the data. P.M.K., M.K., F.Z.N. and E.M.F.K. drafted the manuscript, and L.J.H. provided further edits and co-writing of the final version. All authors read and approved the final manuscript.

### Conflicts of interest

The authors declare that there are no conflicts of interest.

### Ethical statement

This study was approved by the Cameroon Bioethics Initiative (CAMBIN) Ethics Review and Consultancy Committee (Reference No. CBI/483/ERCC/CAMBIN; Protocol No. 1163) and conducted according to the approved protocol guidelines.

### References

1. Kaktcham PM, Kujawska M, Kouam EMF, Piame LT, Tientcheu MLT, et al. Genomic insights into the beneficial potential of bifidobacterium and enterococcus strains isolated from cameroonian infants. *Figshare* 2025. DOI: 10.6084/m9.figshare.28278884.v1.
2. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. *BMJ* 2018;361:k2179.
3. Hou K, Wu Z-X, Chen X-Y, Wang J-Q, Zhang D, et al. Microbiota in health and diseases. *Signal Transduct Target Ther* 2022;7:135.

4. Turrone F, Milani C, Duranti S, Lugli GA, Bernasconi S, et al. The infant gut microbiome as a microbial organ influencing host well-being. *Ital J Pediatr* 2020;46:16.
5. Yao Y, Cai X, Ye Y, Wang F, Chen F, et al. The role of microbiota in infant health: from early life to adulthood. *Front Immunol* 2021;12:708472.
6. Wernroth M-L, Peura S, Hedman AM, Hetty S, Vicenzi S, et al. Development of gut microbiota during the first 2 years of life. *Sci Rep* 2022;12:9080.
7. Alcon-Giner C, Dalby MJ, Caim S, Ketskemety J, Shaw A, et al. Microbiota supplementation with *Bifidobacterium* and *Lactobacillus* modifies the preterm infant gut microbiota and metabolome: an observational study. *Cell Rep Med* 2020;1:100077.
8. Lordan C, Roche AK, Delsing D, Nauta A, Groeneveld A, et al. Linking human milk oligosaccharide metabolism and early life gut microbiota: bifidobacteria and beyond. *Microbiol Mol Biol Rev* 2024;88:e0009423.
9. Wiciński M, Sawicka E, Gębalski J, Kubiak K, Malinowski B. Human milk oligosaccharides: health benefits, potential applications in infant formulas, and pharmacology. *Nutrients* 2020;12:266.
10. Liu P, Wang Y, Yang G, Zhang Q, Meng L, et al. The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis. *Pharmacol Res* 2021;165:105420.
11. Hrnčir T. Gut microbiota dysbiosis: triggers, consequences, diagnostic and therapeutic options. *Microorganisms* 2022;10.
12. Fassarella M, Blaak EE, Penders J, Nauta A, Smidt H, et al. Gut microbiome stability and resilience: elucidating the response to perturbations in order to modulate gut health. *Gut* 2021;70:595–605.
13. Ayeni KI, Berry D, Wisgrill L, Warth B, Ezekiel CN. Early-life chemical exposome and gut microbiome development: african research perspectives within a global environmental health context. *Trends Microbiol* 2022;30:1084–1100.
14. Oyedemi OT, Shaw S, Martin JC, Ayeni FA, Scott KP. Changes in the gut microbiota of nigerian infants within the first year of life. *PLoS One* 2022;17:e0265123.
15. Catania J, Pandit NG, Ehrlich JM, Zaman M, Stone E, et al. Probiotic supplementation for promotion of growth in children: a systematic review and meta-analysis. *Nutrients* 2021;14.
16. Heuven LA, Pyle S, Greyling A, Melse-Boonstra A, Eilander A. Gut microbiota-targeted nutritional interventions improving child growth in low- and middle-income countries: a systematic review. *Curr Dev Nutr* 2021;5:nzab124.
17. WHO/FAO. Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria; 2001. <http://www.fao.org/publications/card/en/c/7c102d95-2fd5-5b22-8faf-f0b2e68dfbb6>
18. Saturio S, Nogacka AM, Suarez M, Fernandez N, Mantecon L, et al. Early-life development of the bifidobacterial community in the infant gut. *Int J Mol Sci* 2021;22.
19. Korpela K. Impact of delivery mode on infant gut microbiota. *Ann Nutr Metab* 2021;1–9.
20. Im EJ, Lee HH-Y, Kim M, Kim M-K. Evaluation of enterococcal probiotic usage and review of potential health benefits, safety, and risk of antibiotic-resistant strain emergence. *Antibiotics* 2023;12:1327.
21. Püngel D, Treveil A, Dalby MJ, Caim S, Colquhoun IJ, et al. *Bifidobacterium breve* UCC2003 exopolysaccharide modulates the early life microbiota by acting as a potential dietary substrate. *Nutrients* 2020;12:948.
22. Liu Y, Wang J, Wu C. Modulation of gut microbiota and immune system by probiotics, pre-biotics, and post-biotics. *Front Nutr* 2021;8:634897.
23. Fanning S, Hall LJ, Cronin M, Zomer A, MacSharry J, et al. Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proc Natl Acad Sci U S A* 2012;109:2108–2113.
24. Liu Q, Yu Z, Tian F, Zhao J, Zhang H, et al. Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. *Microb Cell Fact* 2020;19:23.
25. Paone P, Cani PD. Mucus barrier, mucins and gut microbiota: the expected slimy partners? *Gut* 2020;69:2232–2243.
26. Lawson MAE, O'Neill IJ, Kujawska M, Gowrinadh Javvadi S, Wijeyesekera A, et al. Breast milk-derived human milk oligosaccharides promote *Bifidobacterium* interactions within a single ecosystem. *ISME J* 2020;14:635–648.
27. Castro-López C, García HS, Guadalupe Martínez-Ávila GC, González-Córdova AF, Vallejo-Córdoba B, et al. Genomics-based approaches to identify and predict the health-promoting and safety activities of promising probiotic strains – a probiogenomics review. *TFST* 2021;108:148–163.
28. Valdez-Baez JL, Jesus L, Marques PH, LCdS P, Felice AG, et al. Comparative genomics in probiotic bacteria. In: Ray RC, Paramithiotis S, VAdC A and Montet D (eds). *Lactic Acid Bacteria in Food Biotechnology: Innovations and Functional Aspects*. Elsevier; 2022. pp. 245–278.
29. Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
30. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 2018;34:i884–i890.
31. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
32. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017;13:e1005595.
33. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
34. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. *Bioinformatics* 2022;38:5315–5316.
35. Pritchard L, Glover RH, Humphris S, Elphinstone JG, Toth IK. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. *Anal Methods* 2016;8:12–24.
36. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
37. Olm MR, Brown CT, Brooks B, Firek B, Baker R, et al. Identical bacterial populations colonize premature infant gut, skin, and oral microbiomes and exhibit different in situ growth rates. *Genome Res* 2017;27:601–612.
38. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
39. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* 2020;37:1530–1534.
40. Seemann T. Snippy: fast bacterial variant calling from NGS reads; 2015. <https://github.com/tseemann/snippy>
41. Zheng J, Ge Q, Yan Y, Zhang X, Huang L, et al. dbCAN3: automated carbohydrate-active enzyme and substrate annotation. *Nucleic Acids Res* 2023;51:W115–W121.
42. Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, et al. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Res* 2023;51:W46–W50.

43. van Heel AJ, de Jong A, Song C, Viel JH, Kok J, et al. BAGEL4: a user-friendly web server to thoroughly mine RiPPs and bacteriocins. *Nucleic Acids Res* 2018;46:W278–W281.
44. Johansson MHK, Bortolaia V, Tansirichaiya S, Aarestrup FM, Roberts AP, et al. Detection of mobile genetic elements associated with antibiotic resistance in *Salmonella enterica* using a newly developed web tool: MobileElementFinder. *J Antimicrob Chemother* 2021;76:101–109.
45. Malberg Tetzschner AM, Johnson JR, Johnston BD, Lund O, Scheutz F. *In silico* genotyping of *Escherichia coli* isolates for extraintestinal virulence Genes by use of whole-genome sequencing data. *J Clin Microbiol* 2020;58.
46. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, et al. The comprehensive antibiotic resistance database. *Antimicrob Agents Chemother* 2013;57:3348–3357.
47. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res* 2020;48:D517–D525.
48. Florensa AF, Kaas RS, Clausen PTLC, Aytan-Aktug D, Aarestrup FM. ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. *Microb Genom* 2022;8:000748.
49. Wishart DS, Han S, Saha S, Oler E, Peters H, et al. PHASTEST: faster than PHASTER, better than PHAST. *Nucleic Acids Res* 2023;51:W443–W450.
50. Merkel D. Docker: lightweight linux containers for consistent development and deployment. *Linux J* 2014;2014.
51. Nayfach S, Camargo AP, Schulz F, Eloë-Fadrosch E, Roux S, et al. CheckV assesses the quality and completeness of metagenome-assembled viral genomes. *Nat Biotechnol* 2021;39:578–585.
52. Team RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2017. <http://www.r-project.org/index.html>
53. Kassambara A, Mundt F. factoextra: Extract and Visualize the Results of Multivariate Data Analyses; 2020. <http://www.sthda.com/english/rpkgs/factoextra>
54. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016;32:2847–2849.
55. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res* 2021;49:W293–W296.
56. Milani C, Lugli GA, Duranti S, Turrone F, Bottacini F, et al. Genomic encyclopedia of type strains of the genus *Bifidobacterium*. *Appl Environ Microbiol* 2014;80:6290–6302.
57. Ramsey M, Hartke A, Huycke M. The Physiology and Metabolism of Enterococci. In: Gilmore MS, Clewell DB, Ike Y and Shankar N (eds). *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*. Boston, 2014.
58. Lugli GA, Milani C, Turrone F, Duranti S, Mancabelli L, et al. Comparative genomic and phylogenomic analyses of the *Bifidobacteriaceae* family. *BMC Genomics* 2017;18:568.
59. Wu G, Zhang C, Wu H, Wang R, Shen J, et al. Genomic microdiversity of *Bifidobacterium pseudocatenulatum* underlying differential strain-level responses to dietary carbohydrate intervention. *mBio* 2017;8:e02348-16.
60. Arbolea S, Bottacini F, O'Connell-Motherway M, Ryan CA, Ross RP, et al. Gene-trait matching across the *Bifidobacterium longum* pan-genome reveals considerable diversity in carbohydrate catabolism among human infant strains. *BMC Genomics* 2018;19:33.
61. Milani C, Lugli GA, Duranti S, Turrone F, Mancabelli L, et al. Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut. *Sci Rep* 2015;5:15782.
62. Stam MR, Danchin EGJ, Rancurel C, Coutinho PM, Henrissat B. Dividing the large glycoside hydrolase family 13 into subfamilies: towards improved functional annotations of alpha-amylase-related proteins. *Protein Eng Des Sel* 2006;19:555–562.
63. Mewis K, Lenfant N, Lombard V, Henrissat B. Dividing the large glycoside hydrolase family 43 into subfamilies: a motivation for detailed enzyme characterization. *Appl Environ Microbiol* 2016;82:1686–1692.
64. Juge N, Tailford L, Owen CD. Sialidases from gut bacteria: a mini-review. *Biochem Soc Trans* 2016;44:166–175.
65. Luo C, Li Y, Chen Y, Fu C, Long W, et al. Bamboo lignocellulose degradation by gut symbiotic microbiota of the bamboo snout beetle *Cyrtotrachelus buqueti*. *Biotechnol Biofuels* 2019;12:70.
66. Salamaga B, Turner RD, Elsarmane F, Galley NF, Kulakauskas S, et al. A moonlighting role for LysM peptidoglycan binding domains underpins *Enterococcus faecalis* daughter cell separation. *Commun Biol* 2023;6:428.
67. Sela DA, Chapman J, Adeuya A, Kim JH, Chen F, et al. The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. *Proc Natl Acad Sci U S A* 2008;105:18964–18969.
68. Garrido D, Ruiz-Moyano S, Lemay DG, Sela DA, German JB, et al. Comparative transcriptomics reveals key differences in the response to milk oligosaccharides of infant gut-associated bifidobacteria. *Sci Rep* 2015;5:13517.
69. James K, Motherway MO, Bottacini F, van Sinderen D. *Bifidobacterium breve* UCC2003 metabolises the human milk oligosaccharides lacto-N-tetraose and lacto-N-neo-tetraose through overlapping, yet distinct pathways. *Sci Rep* 2016;6:38560.
70. Garrido D, Ruiz-Moyano S, Kirmiz N, Davis JC, Totten SM, et al. A novel gene cluster allows preferential utilization of fucosylated milk oligosaccharides in *Bifidobacterium longum* subsp. *longum* SC596. *Sci Rep* 2016;6:35045.
71. Laursen MF, Sakanaka M, von Burg N, Mörbe U, Andersen D, et al. *Bifidobacterium* species associated with breast-feeding produce aromatic lactic acids in the infant gut. *Nat Microbiol* 2021;6:1367–1382.
72. Milani C, Turrone F, Duranti S, Lugli GA, Mancabelli L, et al. Genomics of the genus *Bifidobacterium* reveals species-specific adaptation to the glycan-rich gut environment. *Appl Environ Microbiol* 2016;82:980–991.
73. Arzamasov AA, Nakajima A, Sakanaka M, Ojima MN, Katayama T, et al. Human milk oligosaccharide utilization in intestinal bifidobacteria is governed by global transcriptional regulator NagR. *mSystems* 2022;7:e0034322.
74. Gotoh A, Katoh T, Sakanaka M, Ling Y, Yamada C, et al. Sharing of human milk oligosaccharides degradants within bifidobacterial communities in faecal cultures supplemented with *Bifidobacterium bifidum*. *Sci Rep* 2018;8:13958.
75. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. *Antonie van Leeuwenhoek* 2002;82:279–289.
76. Westerlund B, Korhonen TK. Bacterial proteins binding to the mammalian extracellular matrix. *Mol Microbiol* 1993;9:687–694.
77. Styriak I, Nemcová R, Chang Y-H, Ljungh A. Binding of extracellular matrix molecules by probiotic bacteria. *Lett Appl Microbiol* 2003;37:329–333.
78. Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: a probiotic trait? *Appl Environ Microbiol* 2012;78:1–6.
79. Hegemann JD, Süßmuth RD. Matters of class: coming of age of class III and IV lanthipeptides. *RSC Chem Biol* 2020;1:110–127.
80. Rozman V, Mohar Lorbeg P, Accetto T, Bogovič Matijašić B. Characterization of antimicrobial resistance in lactobacilli and bifidobacteria used as probiotics or starter cultures based on integration of phenotypic and *in silico* data. *Int J Food Microbiol* 2020;314:108388.
81. Schell MA, Karmirantzou M, Snel B, Vilanova D, Berger B, et al. The genome sequence of *Bifidobacterium longum* reflects its

- adaptation to the human gastrointestinal tract. *Proc Natl Acad Sci USA* 2002;99:14422–14427.
82. Morton ER, Lynch J, Froment A, Lafosse S, Heyer E, et al. Variation in rural African gut microbiota is strongly correlated with colonization by *Entamoeba* and subsistence. *PLoS Genet* 2015;11:e1005658.
  83. Gomez A, Petrzekova KJ, Burns MB, Yeoman CJ, Amato KR, et al. Gut microbiome of coexisting BaAka pygmies and Bantu reflects gradients of traditional subsistence patterns. *Cell Rep* 2016;14:2142–2153.
  84. Lokmer A, Aflalo S, Amougou N, Lafosse S, Froment A, et al. Response of the human gut and saliva microbiome to urbanization in Cameroon. *Sci Rep* 2020;10:2856.
  85. Rubel MA, Abbas A, Taylor LJ, Connell A, Tanes C, et al. Lifestyle and the presence of helminths is associated with gut microbiome composition in Cameroonians. *Genome Biol* 2020;21:122.
  86. Abange WB, Martin C, Nanfack AJ, Yatchou LG, Nusbacher N, et al. Alteration of the gut fecal microbiome in children living with HIV on antiretroviral therapy in Yaounde, Cameroon. *Sci Rep* 2021;11:7666.
  87. Even G, Lokmer A, Rodrigues J, Audebert C, Viscogliosi E, et al. Changes in the human gut microbiota associated with colonization by *Blastocystis* sp. and *Entamoeba* spp. in non-industrialized populations. *Front Cell Infect Microbiol* 2021;11:533528.
  88. Derrien M, Mikulic N, Uyoga MA, Chenoll E, Climent E, et al. Gut microbiome function and composition in infants from rural Kenya and association with human milk oligosaccharides. *Gut Microbes* 2023;15:2178793.
  89. Grześkowiak Ł, Collado MC, Mangani C, Maleta K, Laitinen K, et al. Distinct gut microbiota in southeastern African and northern European infants. *J Pediatr Gastroenterol Nutr* 2012;54:812–816.
  90. Fontana F, Mancabelli L, Lugli GA, Taracchini C, Alessandri G, et al. Investigating the infant gut microbiota in developing countries: worldwide metagenomic meta-analysis involving infants living in sub-urban areas of Côte d'Ivoire. *Environ Microbiol Rep* 2021;13:626–636.
  91. Robertson RC, Church JA, Edens TJ, Mutasa K, Min Geum H, et al. The fecal microbiome and rotavirus vaccine immunogenicity in rural Zimbabwean infants. *Vaccine* 2021;39:5391–5400.
  92. Sela DA, Garrido D, Lerno L, Wu S, Tan K, et al. *Bifidobacterium longum* subsp. *infantis* ATCC 15697  $\alpha$ -fucosidases are active on fucosylated human milk oligosaccharides. *Appl Environ Microbiol* 2012;78:795–803.
  93. Kujawska M, La Rosa SL, Roger LC, Pope PB, Hoyles L, et al. Succession of *Bifidobacterium longum* strains in response to a changing early life nutritional environment reveals dietary substrate adaptations. *iScience* 2020;23:101368.
  94. Erney RM, Malone WT, Skelding MB, Marcon AA, Kleman-Leyer KM, et al. Variability of human milk neutral oligosaccharides in a diverse population. *J Pediatr Gastroenterol Nutr* 2000;30:181–192.
  95. Totten SM, Zivkovic AM, Wu S, Ngyuen U, Freeman SL, et al. Comprehensive profiles of human milk oligosaccharides yield highly sensitive and specific markers for determining secretor status in lactating mothers. *J Proteome Res* 2012;11:6124–6133.
  96. McGuire MK, Meehan CL, McGuire MA, Williams JE, Foster J, et al. What's normal? oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically. *Am J Clin Nutr* 2017;105:1086–1100.
  97. Vinjamuri A, Davis JCC, Totten SM, Wu LD, Klein LD, et al. Human milk oligosaccharide compositions illustrate global variations in early nutrition. *J Nutr* 2022;152:1239–1253.
  98. Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, et al. Update of the list of Qualified Presumption of Safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 19: suitability of taxonomic units notified to EFSA until September 2023. *EFSA J* 2024;22:e8517.
  99. Lermieux NA, Cameron ADS. Horizontal transfer of antibiotic resistance genes in clinical environments. *Can J Microbiol* 2019;65:34–44.
  100. Jian Z, Zeng L, Xu T, Sun S, Yan S, et al. Antibiotic resistance genes in bacteria: occurrence, spread, and control. *J Basic Microbiol* 2021;61:1049–1070.
  101. Gueimonde M, Sánchez B, G de Los Reyes-Gavilán C, Margolles A. Antibiotic resistance in probiotic bacteria. *Front Microbiol* 2013;4:202.
  102. Chung The H, Nguyen Ngoc Minh C, Tran Thi Hong C, Nguyen Thi Nguyen T, Pike LJ, et al. Exploring the genomic diversity and antimicrobial susceptibility of *Bifidobacterium pseudocatenuatum* in a Vietnamese population. *Microbiol Spectr* 2021;9:e0052621.
  103. Tarracchini C, Viglioli M, Lugli GA, Mancabelli L, Fontana F, et al. The integrated probiotic database: a genomic compendium of bifidobacterial health-promoting strains. *Microbiome Res Rep* 2022;1:9.
  104. Razavi M, Kristiansson E, Flach C-F, Larsson DGJ. The association between insertion sequences and antibiotic resistance genes. *mSphere* 2020;5:e00418–20.
  105. Che Y, Yang Y, Xu X, Břinda K, Polz MF, et al. Conjugative plasmids interact with insertion sequences to shape the horizontal transfer of antimicrobial resistance genes. *Proc Natl Acad Sci U S A* 2021;118:e2008731118.
  106. Wang N, Hang X, Zhang M, Liu X, Yang H. Analysis of newly detected tetracycline resistance genes and their flanking sequences in human intestinal bifidobacteria. *Sci Rep* 2017;7:6267.
  107. Duranti S, Lugli GA, Mancabelli L, Turroni F, Milani C, et al. Prevalence of antibiotic resistance genes among human gut-derived bifidobacteria. *Appl Environ Microbiol* 2017;83:e02894–16.
  108. Miller WR, Munita JM, Arias CA. Mechanisms of antibiotic resistance in enterococci. *Expert Rev Anti Infect Ther* 2014;12:1221–1236.
  109. Rozman V, Mohar Lorbeg P, Treven P, Accetto T, Janežič S, et al. Genomic insights into antibiotic resistance and mobilome of lactic acid bacteria and bifidobacteria. *Life Sci Alliance* 2023;6:e202201637.
  110. Pei Z, Liu Y, Chen Y, Pan T, Sun X, et al. A universe of human gut-derived bacterial prophages: unveiling the hidden viral players in intestinal microecology. *Gut Microbes* 2024;16:2309684.
  111. Yu D, Pei Z, Chen Y, Wang H, Xiao Y, et al. *Bifidobacterium longum* subsp. *infantis* as widespread bacteriocin gene clusters carrier stands out among the *Bifidobacterium*. *Appl Environ Microbiol* 2023;89:e0097923.
  112. Yildirim Z, Johnson MG. Characterization and antimicrobial spectrum of bifidocin B, a bacteriocin produced by *Bifidobacterium bifidum* NCFB 1454. *J Food Prot* 1998;61:47–51.
  113. Cheikhoussef A, Cheikhoussef N, Chen H, Zhao J, Tang J, et al. Bifidin I – a new bacteriocin produced by *Bifidobacterium infantis* BCRC 14602: purification and partial amino acid sequence. *Food Control* 2010;21:746–753.
  114. Lee JH, Li X, O'Sullivan DJ. Transcription analysis of a lantibiotic gene cluster from *Bifidobacterium longum* DJO10A. *Appl Environ Microbiol* 2011;77:5879–5887.
  115. Martínez FAC, Domínguez JM, Converti A, Oliveira RP de S. Production of bacteriocin-like inhibitory substance by *Bifidobacterium lactis* in skim milk supplemented with additives. *J Dairy Res* 2015;82:350–355.
  116. Bonacina J, Suárez N, Hormigo R, Fadda S, Lechner M, et al. A genomic view of food-related and probiotic *Enterococcus* strains. *DNA Res* 2017;24:dsw043.
  117. Prichula J, Primon-Barros M, Luz RCZ, Castro ÍMS, Paim TGS, et al. Genome mining for antimicrobial compounds in wild marine animals-associated enterococci. *Mar Drugs* 2021;19:328.
  118. Okoye CO, Dong K, Wang Y, Gao L, Li X, et al. Comparative genomics reveals the organic acid biosynthesis metabolic pathways among five lactic acid bacterial species isolated from fermented vegetables. *N Biotechnol* 2022;70:73–83.

119. Miyanaga A. Structure and function of polyketide biosynthetic enzymes: various strategies for production of structurally diverse polyketides. *Biosci Biotechnol Biochem* 2017;81:2227–2236.
120. Hou L, Huang H, Li H, Wang S, Ju J, et al. Overexpression of A type III PKS gene affording novel violapyrones with enhanced anti-influenza A virus activity. *Microb Cell Fact* 2018;17:61.
121. Zhu WZ, Wang SH, Gao HM, Ge YM, Dai J, et al. Characterization of bioactivities and biosynthesis of angucycline/angucyclinone derivatives derived from *gephyromycinifex aptenodytis* gen. nov., sp. nov. *Mar Drugs* 2021;20:sp.
122. Helfrich EJN, Lin GM, Voigt CA, Clardy J. Bacterial terpene biosynthesis: challenges and opportunities for pathway engineering. *Beilstein J Org Chem* 2019;15:2889–2906.
123. Masyita A, Mustika Sari R, Dwi Astuti A, Yasir B, Rahma Rumata N, et al. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chem X* 2022;13:100217.
124. Bottacini F, Ventura M, van Sinderen D, O'Connell Motherway M. Diversity, ecology and intestinal function of bifidobacteria. *Microb Cell Fact* 2014;13 Suppl 1:S4.
125. Alessandri G, van Sinderen D, Ventura M. The genus *Bifidobacterium*: from genomics to functionality of an important component of the mammalian gut microbiota running title: bifidobacterial adaptation to and interaction with the host. *Comput Struct Biotechnol J* 2021;19:1472–1487.

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