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1,520 reference genomes from cultivated human gut bacteria enable functional microbiome analyses

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Reference genomes are essential for metagenomic analyses and functional characterization of the human gut microbiota. We present the Culturable Genome Reference (CGR), a collection of 1,520 nonredundant, high-quality draft genomes generated from >6,000 bacteria cultivated from fecal samples of healthy humans. Of the 1,520 genomes, which were chosen to cover all major bacterial phyla and genera in the human gut, 264 are not represented in existing reference genome catalogs. We show that this increase in the number of reference bacterial genomes improves the rate of mapping metagenomic sequencing reads from 50% to >70%, enabling higher-resolution descriptions of the human gut microbiome. We use the CGR genomes to annotate functions of 338 bacterial species, showing the utility of this resource for functional studies. We also carry out a pan-genome analysis of 38 important human gut species, which reveals the diversity and specificity of functional enrichment between their core and dispensable genomes.

The human gut microbiota refers to the all the microorganisms that inhabit the human gastrointestinal tract. Diverse roles of the gut microbiota in human health and disease have been recognized[1,2]. Metagenomic studies have transformed our understanding of the taxonomic and functional diversity of human microbiota, but more than half of the sequencing reads from a typical human fecal metagenome cannot be mapped to existing bacterial reference genomes[3,4]. The lack of high-quality reference genomes has become an obstacle for high-resolution analyses of the human gut microbiome.

Although the previously reported Integrated Gene Catalog (IGC) has enabled metagenomic, metatranscriptomic and metaproteomic analyses[3,5,6], the gap between compositional and functional analyses can only be filled by individual bacterial genomes. Genes that co-vary among samples can be clustered into metagenomic linkage groups[7], metagenomic clusters[8] and metagenomic species[9,10], whose annotation depends on alignment to the limited number of existing reference genomes. Other metagenomics-based analyses of the gut microbiome—for example, single nucleotide polymorphisms (SNPs), indels and copy number variations—rely on the coverage and quality of reference genomes[11–13].

Despite the rapid increase in the number of sequenced bacterial and archaeal genomes, reference genomes for gut bacteria are underrepresented. It is estimated that <4% of the bacterial genomes in the US National Center for Biotechnology Information (NCBI) database belong to the human gut microbiota. Rather, the focus has been on clinically relevant pathogenic bacteria, which are overrepresented in the microbial databases. The first catalog of 178 reference bacterial genomes for the human microbiota was reported by the Human Microbiome Project (HMP)[14] in 2010. To date, the HMP has sequenced >2,000 microbial genomes cultivated from human body sites, 437 of which are gut microbiota (data accessed 8 September 2017). However, the number of reference gut bacterial genomes is still far from saturated.

We present a reference catalog of genomes of cultivated human gut bacteria (named the CGR), established by culture-based isolation of >6,000 bacterial isolates from fecal samples of healthy individuals. The CGR comprises 1,520 nonredundant, high-quality draft bacterial genomes, contributing at least 264 new reference genomes to the gut microbiome. After inclusion of CGR genomes, the mapping rate of selected metagenomic datasets improved from around 50% to over 70%. In addition to improving metagenomic analyses, the CGR will improve functional characterization and pan-genomic analyses of the gut microbiota at high resolution.

Results

Expanded catalog of gut bacterial genomes. We obtained 6,487 bacterial isolates from fresh fecal samples donated by 155 healthy volunteers by using 11 different media under anaerobic

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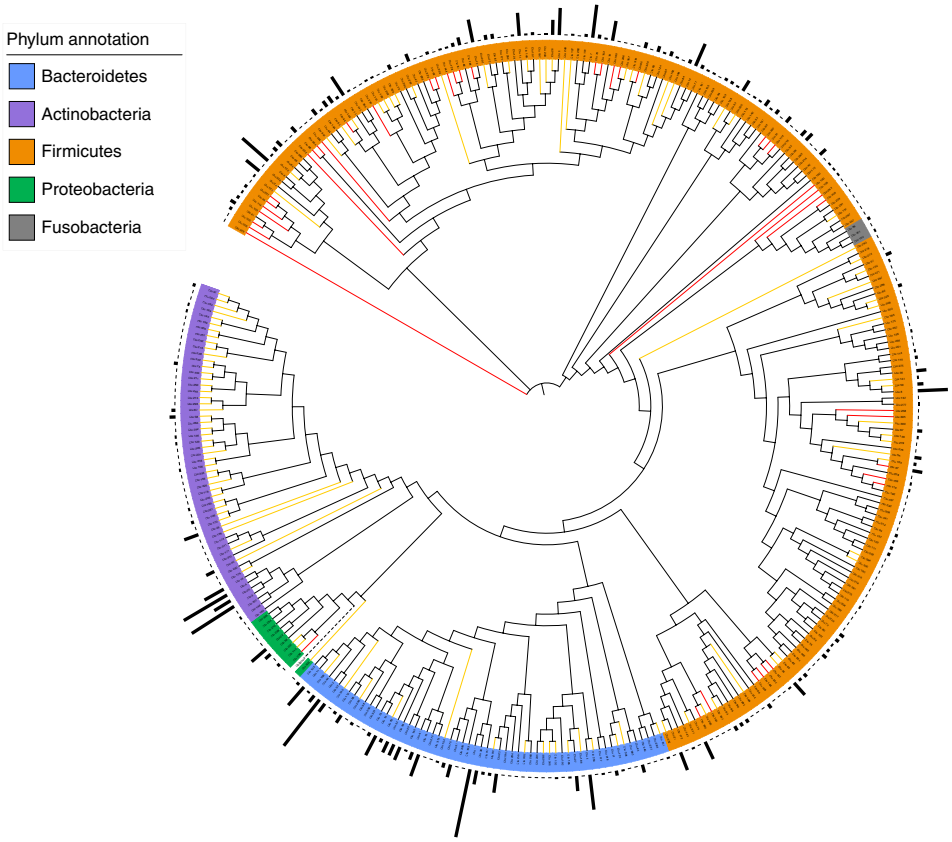

Fig. 1 | Phylogenetic tree of 1,520 isolated gut bacteria based on whole-genome sequences. The 1,520 high-quality genomes in CGR are classified into 338 species-level clusters (ANI ≥ 95%) based on their whole-genome sequences. Bacterial species from Firmicutes are colored in orange; Bacteroidetes, blue; Proteobacteria, green; Actinobacteria, violet; Fusobacteria, gray. Novel genera and species are highlighted by red and orange branches, respectively. The bar on the outermost layer indicates the number of genomes archived in each cluster. *Rhizobium selenitireducens* ATCC BAA 1503 was used as an outgroup for phylogenetic analysis.

conditions (Supplementary Fig. 1a and Supplementary Table 1). Notably, more than half of the isolates were cultured from MPYG medium (Supplementary Fig. 1b). All the isolates were subjected to 16 S rRNA gene amplicon sequencing analysis, and 1,759 nonredundant isolates that provided broad coverage of the phylogenetic tree were selected for whole-genome sequencing (Supplementary Fig. 1c and Supplementary Table 2). After de novo assembly of the next-generation sequencing reads, we identified 104 isolates that contained more than one genome. These assembled sequences were then parsed into 212 genomes using our in-house pipeline (Supplementary Table 3). Briefly, multi-genomes were split at scaffold level on the basis of G + C content versus sequencing depth. The closest reference genomes for the spilt scaffolds were determined on the basis of average nucleotide identity (ANI), and the mis-split scaffolds were mapped back to their closest reference genome to get the final split genome (see Methods). In total, we obtained a collection of 1,867 newly assembled genomes, 1,520 (81.4%) of which fulfilled the HMP's criteria for high-quality draft genomes and exceeded 95% genome completeness and less than 5% contamination as evaluated by CheckM. The genome sizes and G + C contents of CGR ranged from 0.2 to 7.9 Mbp and 26.56% to 64.28%, respectively. A total of 5,749,641 genes were predicted from the annotation data (Supplementary Table 4).

Taxonomic annotation of CGR was carried out using a self-constructed, efficient ANI-based pipeline (Supplementary Fig. 2). The 1,520 high-quality genomes were classified into 338 species-level clusters (ANI ≥ 95%, a species delineation corresponding to 70% DNA–DNA hybridization), which covered all the major phyla of the human gut microbiota, including Firmicutes (211 clusters, 796 genomes), Bacteroidetes (60 clusters, 447 genomes), Actinobacteria (54 clusters, 235 genomes), Proteobacteria (10 clusters, 36 genomes) and Fusobacteria (3 clusters, 6 genomes) (Fig. 1a and Supplementary Table 5). Among these 338 clusters, 134 clusters (corresponding to 264 genomes) were not annotated to any present reference genomes in NCBI (Fig. 1a), and 50 clusters did not fall within any sequenced genera (Supplementary Table 5). To corroborate the presence of novel species in CGR, we carried out additional taxonomic identification using 16 S rRNA gene analysis. A species was recognized as novel if its 16 S rRNA gene sequence had < 98.7% similarity with known species in the EzBioCloud database (see Methods). Overall, we identified 350 distinct bacterial species (based on operational taxonomic units), including 149 candidate novel species, 42 of which represent candidate novel genera. These results underscore the value of the individual reference genomes provided by the CGR.

Despite the variation of individual microbiota at the genus level, the CGR identified bacterial populations with broad diversity, covering eight out of nine core genera in the Chinese gut microbiota[15]. More than 80 species were novel in comparison with the previously sequenced species from a reported 1,000 cultured bacterial species from the human gastrointestinal tract[16] (Supplementary Fig. 3a). Moreover, the CGR successfully identified 38 genera that were of low relative abundance (< 1%) according to the IGC[6], which is a large catalog of reference genes derived from a collection of ~1,250 metagenomic samples from individuals on three continents (Supplementary Fig. 3b). Among them, 7 genera were identified with more than 20 genomes (*Bifidobacterium*, *Collinsella*, *Coprobacillus*, *Dorea*, *Streptococcus*, *Prevotella* and *Parabacteroides*). The CGR also identified another 9 genera that were not detected

by IGC[6] (*Butyricicoccus*, *Butyricimonas*, *Catenibacterium*, *Dielma*, *Erysipelatoclostridium*, *Megamonas*, *Melissococcus*, *Peptoclostridium* and *Vagococcus*) (Supplementary Fig. 3b). These results underscore the contribution of the CGR to the existing database of gut bacterial whole genomes.

Improvement in metagenomic and SNP analyses. The existing reference genomes for metagenomic sequence mapping are far from saturated. For example, the genomes or draft genomes of bacteria and archaea used in a recent study allowed mapping of less than half of the sequences in the fecal metagenome[3,4]. To illustrate the value of the CGR to metagenomic analyses, we performed sequence mapping using previous metagenomic data[6] with or without CGR. For Chinese samples, the read mapping rate in the original study that used the IGCR dataset (3,449 reference genomes from IGC[6]) was 52.00%, which was significantly improved to 76.88% after the inclusion of the CGR dataset (Fig. 2a and Supplementary Table 6). Since all the samples in the CGR were from China, it is reasonable to assume that this genome dataset contributes substantially to the Chinese fecal metagenome. To evaluate the contribution of the CGR to the mapping of non-Chinese metagenomes, we carried out a similar analysis using metagenomic data from American, Spanish and Danish fecal samples. Notably, the metagenomic read mapping ratios of these samples all increased substantially (Fig. 2a), although to a lesser extent compared with that of Chinese samples (Supplementary Fig. 4a). The improvement of mapping rates in both Chinese and non-Chinese samples indicates that the CGR covers a considerable number of gut bacterial species shared by people between these countries. To reveal the improvement of gene and protein diversity enabled by the CGR, we compared the gene and protein cumulative curve based on genomes used in a previous IGC study and after addition of the CGR (Supplementary Fig. 4b,c). The number of gene and protein families increased with inclusion of the first 1,500 genomes, but more or less plateaued at around 3,000 genomes. The addition of our CGR genomes led to a substantial increase in the number of added gene and protein families as a function of genome number. A total of 373,555 gene clusters and 149,945 protein clusters were added by inclusion of the CGR, corresponding to a 22% and 16% increase in known gene and protein sequence diversity, respectively.

To further illustrate the utility of the CGR, we used it to analyze gut microbiome SNPs in a cohort of 250 samples from the TwinsUK registry[17]. We generated a new set of 282 nonredundant representative genomes from the CGR (see Methods, Supplementary Fig. 5 and Supplementary Table 7), which number nearly doubled the 152 reference genomes used in the original TwinsUK analysis[17]. To highlight the new reference genomes identified by analysis with the existing genomes and the CGR genomes, we performed an ANI-based alignment of the 282 genomes with the previously reported 152 genomes. Among the 192 newly added reference genomes, 85 were classified species while 107 were unclassified species (Fig. 2b). A high SNP density was found in *Ruminococcus* sp. CAG:108 (Clu 21), unclassified Firmicutes (Clu 157), *Eubacterium rectale* (Clu 6), *Escherichia coli* (Clu 22), and *Ruminococcus* sp. CAG:57 (Clu 19), suggesting a high degree of variations in the genomes of these species, while *Lactobacillus gasseri* (Clu 241), *Enterococcus fecalis* (Clu 316), *Enterococcus durans* (Clu 274) and *Streptococcus mutans* (Clu 217) showed lower SNP density. A total of 9.14 million SNPs were identified. The number of SNPs was increased for some species due to the newly added high-quality reference genomes in the CGR. We conclude that the CGR is a valuable resource for metagenomic studies because of the significant improvement in metagenomic resolution it enables.

Functions of gut microbiome bacteria. To better elucidate functions of the gut microbiota, we annotated gene functions in 1,520

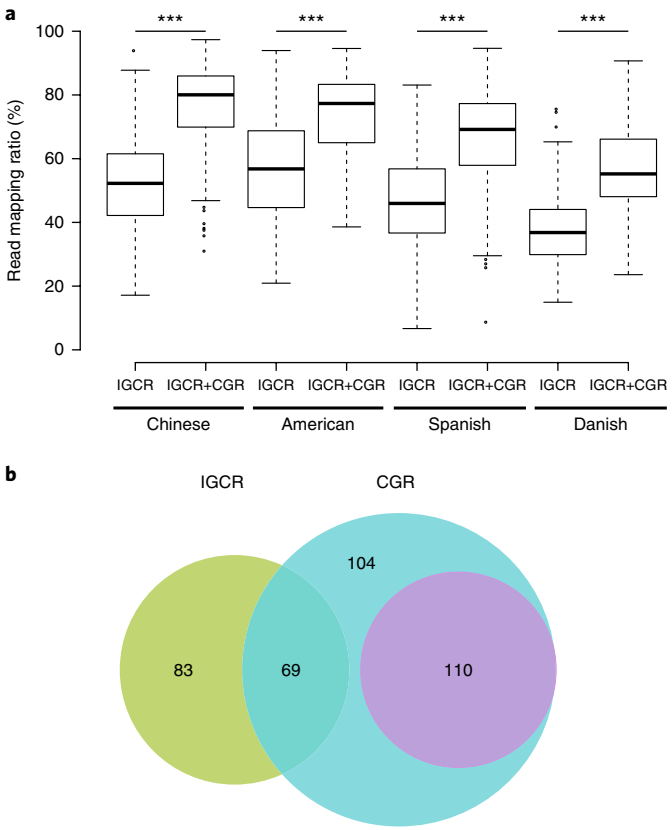

Fig. 2 | Contribution of CGR to metagenomic and SNP analyses. a, The read mapping ratio of a previous metagenomic analysis (IGCR) was significantly improved by CGR (IGCR + CGR) in fecal samples from Chinese ($n = 368$, $P = 6 \times 10^{-78}$), American ($n = 139$, $P = 2 \times 10^{-17}$), Spanish ($n = 320$, $P = 4 \times 10^{-50}$) and Danish ($n = 109$, $P = 4 \times 10^{-17}$) individuals. The significance of improvement was determined by two-side Wilcoxon rank-sum test. IGCR, 3,449 reference genomes used in the IGC study[6]; CGR, 1,520 reference genomes generated in this study. Each box plot illustrates the estimated median (center line), upper and lower quartiles (box limits), 1.5 × interquartile range (whiskers), and outliers (points) of the read mapping ratio. **b**, Reference genomes for SNP analysis generated in previous study[17] (IGCR, green) and current study (CGR, blue). The unclassified species of reference genomes in CGR are highlighted in violet.

CGR genomes using KEGG (the Kyoto Encyclopedia of Genes and Genomes)[18]. Functional pathways at KEGG level 2 showed that pathways involved in carbohydrate and amino acid metabolism are abundant in all isolated strains, suggesting that these are core functions of the gut microbiota (Supplementary Fig. 6). We also analyzed KEGG level 3 pathways and focused on those enriched at the phylum or genus level (Fig. 3a). We found that lipopolysaccharide biosynthesis (ko00540) genes were widely distributed in the phyla Fusobacteria, Bacteroidetes and Proteobacteria, the main phyla of gram-negative bacteria. Genes involved in glycan degradation (ko00531 and ko00511) were abundant in the genomes of the Bacteroidetes phylum. This observation is consistent with the notion that members of Bacteroidetes are prominent human gut symbionts that help degrade glycans in the diet and the gut mucosa[19]. The members of the Bacteroidetes also possess a high proportion of genes involved in sphingolipid metabolism (ko00600), glycosphingolipid biosynthesis (ko00601, ko00603 and ko00604) and steroid hormone biosynthesis (ko00140). Sphingolipids and hormone biosynthesis are ubiquitous in eukaryotic cells but not present in most bacteria. These results suggest that members of the Bacteroidetes

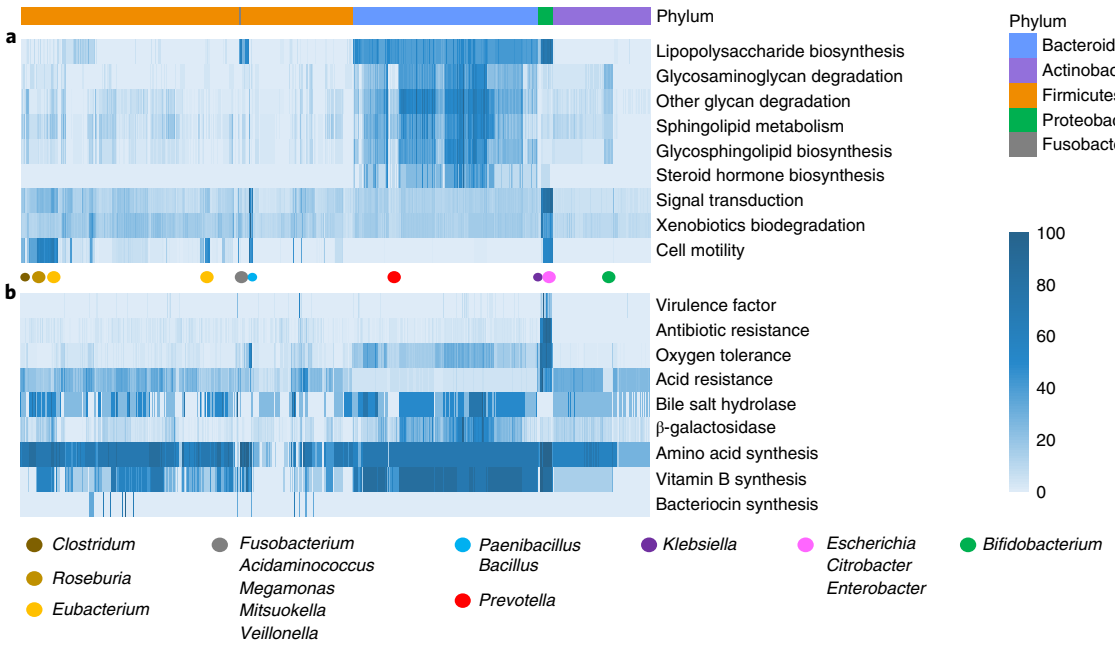

Fig. 3 | Functional landscape of gut microbiota. The gene abundance of listed functions in 1,520 genomes of CGR is indicated by the color depth in the heat map. The listed functions are enriched in specific phyla or genera (**a**) or might have deleterious or beneficial effects on human health (**b**). The bacterial species are ordered according to the phylogenetic tree in Fig. 1. The relative positions of phyla and genera in the phylogenetic tree are indicated by the colored ribbons and dots, respectively.

not only participate in energy metabolism in the gut, but may also act in sphingolipid and hormone signaling in mammalian cells. The Proteobacteria showed relatively high abundance in genes involved in degradation of xenobiotics (ko01220), possibly contributing to the degradation of environmental chemicals and pharmaceuticals in the gut.

The signal transduction system (two-component system, ko02020) and xenobiotics degradation (KEGG level 2 pathway) were ubiquitous in the genera *Paenibacillus*, *Bacillus*, *Klebsiella*, *Escherichia*, *Citrobacter* and *Enterobacter*, which are also presented in environmental niches, such as soil and water. The abundant signal transduction and xenobiotics degradation systems allow these genera to sense and respond to various stresses and toxic substance presented in natural environments. Cell motility (chemotaxis, ko02030; flagellar assembly, ko02040) was conserved in the genera *Roseburia*, *Paenibacillus*, *Bacillus*, *Escherichia*, *Citrobacter* and *Enterobacter*, but varied within the genera *Clostridium* and *Eubacterium*.

Next we investigated functions and pathways that are annotated in the KEGG database, but not categorized as KEGG pathways (Fig. 3b and Supplementary Table 9). Virulence factors and antibiotic resistance genes were annotated using the Virulence Factors Database (VFDB)[20] and Comprehensive Antibiotic Resistance Database (CARD)[21], respectively. Virulence factors and antibiotic resistance are clinically relevant and are abundant in the Proteobacteria phylum, suggesting that this phylum may be a reservoir for opportunistic pathogens. We examined the distribution of genes involved in responses to stresses frequently encountered by gut bacteria: oxygen tolerance and acid resistance. Oxygen tolerance was reflected by the number of genes encoding catalase and superoxide dismutase, two detoxification enzymes that scavenge reactive oxygen species produced during aerobic respiration. As expected, the facultative anaerobic bacteria in the genera *Paenibacillus*, *Bacillus*, *Klebsiella*, *Escherichia*, *Citrobacter* and *Enterobacter* were more oxygen tolerant. In addition to the previously reported *Bacteroides fragilis*[22], other members of Bacteroidetes also showed moderate oxygen tolerance. Notably, bacteria in the Bacteroidetes phylum and the *Bifidobacterium* genus generally lacked acid resistance genes, suggesting that potential probiotics based on these organisms may suffer impaired survival in the acidic stomach environment after oral administration. Finally, we examined the distribution of six bacterial functions in the CGR that might have beneficial effects on human health. Amino acid and vitamin B synthesis genes were widely present in various gut bacteria, suggesting that gut microbiota might be an alternative source for nutrients that are sparse in vegetarian diets. Genes encoding bacterial bile salt hydrolases, which transform primary bile acids into secondary bile acids in the human intestine, were also ubiquitous in most gut bacteria. Genes encoding β-galactosidases, which might attenuate problems associated with lactose intolerance, were relatively abundant in the phylum Bacteroidetes. Genes involved in bacteriocin synthesis in gut bacteria were relatively rare and did not show phylum- or genus-specific distribution.

Core and pan-genomes of underrepresented gut bacteria. We carried out a pan-genome analysis of 36 species or clusters that contain more than ten genomes, as well as two other species enriched in healthy controls compared with patients with type 2 diabetes in previous studies[7,23,24], *Fecalibacterium prausnitzii* (cluster 63, seven genomes) and butyrate-producing bacterium SS3_4 (cluster 45, nine genomes). These clusters covered the phyla Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (Supplementary Fig. 7a and Supplementary Table 8a). The pan-genome of a cluster can be defined as the sum of the core genes and dispensable genes (including unique genes and accessory genes) of all the members within that cluster[25]. Our pan-genome analysis showed that *Eubacterium rectale* (cluster 37) contained the lowest proportion of core genes (12%); the remaining genes fell into accessory and unique genomes (38% and 40%, respectively). In contrast, *Eubacterium* 3_1 (cluster 6) contained the largest proportion of core genes (53%) (Supplementary Fig. 7b). The pan-genome fitting curves showed that most clusters in Bacteroidetes displayed an 'open' pan-genome and had a relatively large pan-genome size, with *Bacteroides vulgatus*

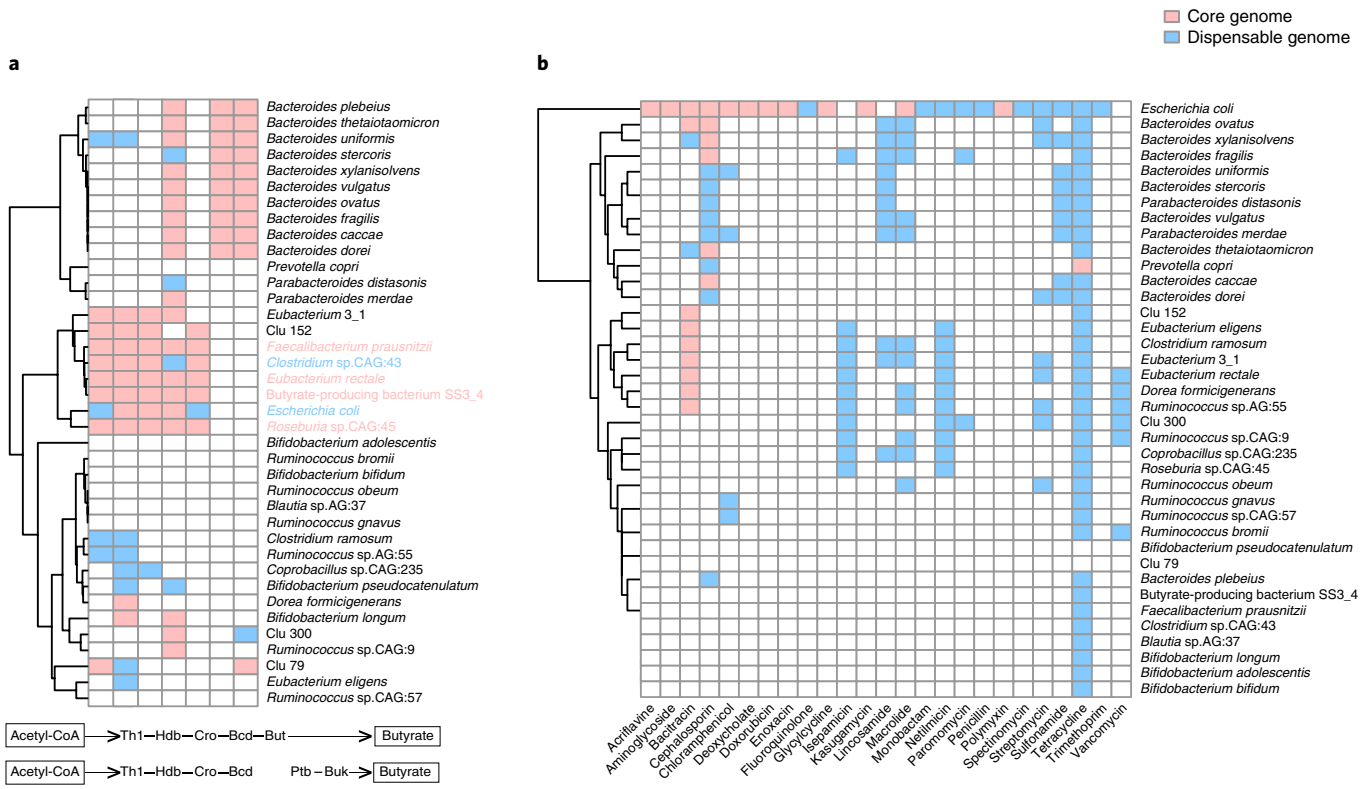

Fig. 4 | Pan-genome analysis of 38 representative clusters. a, The distribution of genes involved in butyrate biosynthesis pathway in the core genomes (pink) and dispensable genomes (cyan). The two pathways for butyrate biosynthesis from acetyl-CoA are shown below. The species with a complete butyrate biosynthesis pathway in the core genome and pan-genome are highlighted in pink and cyan, respectively. Thl, thiolase; Hdb, β-hydroxybutyryl-CoA dehydrogenase; Cro, crotonase; Bcd, butyryl-CoA dehydrogenase (including electron transfer protein α and β subunits); But, butyryl-CoA:acetate CoA transferase; Ptb, phosphate butyryltransferase; Buk, butyrate kinase. **b**, The distribution of ARGs in in the core genomes (pink) and dispensable genomes (cyan).

having the largest pan-genome size at 14,970 genes (Supplementary Figs. 8 and 9 and Supplementary Table 8b). In contrast, members in the phylum Actinobacteria tend to represent a relatively 'closed' pan-genome, which was only slightly expanded by the addition of CGR genomes. These results suggest that gut bacterial genomes are variable in the Bacteroidetes phylum, less variable in the Firmicutes and Proteobacteria, and fairly conserved in the Actinobacteria.

We also explored the distribution of genes involved in butyrate synthesis and antibiotic resistance in the pan-genomes of gut bacteria. Functional annotation showed that six clusters contained the complete acetyl-CoA to butyrate biosynthesis pathway (Fig. 4a). Among them, *F. prausnitzii*, *E. rectale*, butyrate-producing bacterium SS3_4 and *Roseburia* sp. CAG:45 harbored the complete pathway in their core genome, suggesting that the butyrate-producing function was highly conserved in these species. This result is consistent with the reported butyrate-producing capacity of these species[26–28]. To explore the distribution of antibiotic resistance within the established pan-genomes, we annotated 25 antibiotic resistance genes (ARGs) in each pan-genome. Consistent with a previous report[29], the tetracycline resistance gene was widely present in the dispensable genome of these clusters (Fig. 4b). Notably, *Escherichia coli* contained almost all ARGs (23 of 25) in its pan-genome, with half of these present in the core genome (Fig. 4b). In contrast, *Bifidobacterium* species, including *B. bifibium*, *B. adolescentis*, *B. longum* and *B. pseudocatenulatum*, rarely contained ARGs in their pan-genomes.

To obtain a better understanding of the distribution of bacterial functions in the core and dispensable genomes, we annotated the genomes using the Clusters of Orthologous Groups (COG) database[30]. This revealed that several housekeeping functions were significantly enriched in the core genome, including post-translational modification, protein turnover and chaperones (O, $P=7.28\times10^{-12}$); translation, ribosomal structure and biogenesis (J, $P=7.28\times10^{-12}$); energy production and conversion (C, $P=7.28\times10^{-12}$); amino acid transport and metabolism (E, $P=7.28\times10^{-12}$); nucleotide transport and metabolism (F, $P=7.28\times10^{-12}$); coenzyme transport and metabolism (H, $P=1.46\times10^{-11}$); lipid transport and metabolism (I, $P=2.40\times10^{-10}$); and inorganic ion transport and metabolism (P, $P=2.40\times10^{-10}$) (Supplementary Fig. 10). By contrast, COG categories enriched in the dispensable genome included cell wall-membrane-envelope biogenesis (M, $P=2.70\times10^{-9}$); cell motility (N, $P=3.11\times10^{-5}$); signal transduction mechanisms (T, $P=0.00039$); intracellular trafficking secretion and vesicular transport (U, $P=1.22\times10^{-7}$); defense mechanisms (V, $P=7.28\times10^{-12}$); transcription (K, $P=3.64\times10^{-11}$); replication recombination and repair (L, $P=7.28\times10^{-12}$); and function unknown (S, $P=0.03111$). The remaining COG categories showed no significant differences in core and dispensable genome.

Discussion

We used 11 culturing conditions for isolation of gut bacteria and archived more than 6,000 isolates. From this collection of isolates, we generated 1,520 high-quality draft reference genomes. The high coverage of the resulting CGR at the genus and species levels (including low-abundance species) demonstrates the value of culture-based methods for strain isolation from the gut microbiota. In line with this, a large number of gut bacterial species that were previously considered as unculturable have been successfully cultivated

in two recent studies[31,32]. Although there was some overlap between the novel species archived by CGR and in these two studies, the CGR contains 659 additional genomes (representing 209 clusters or species). Our cultivation methods can be applied to expand the CGR until it is saturated with the genomes of culturable gut bacteria. After that, single-cell sequencing can be used to investigate genomes of unculturable bacteria, with an overall aim of defining a saturated set of reference genomes of gut microbiota to underpin a better understanding of gut microbiome biology.

We applied out CGR genome dataset to assign functions to gut bacteria. For example, we found that virulence factors and antibiotic resistance genes are enriched in *Klebsiella*, *Escherichia*, *Citrobacter* and *Enterobacter*, which are opportunistic pathogens frequently isolated in clinical samples[33]. The abundance of signal transduction and cell motility genes in these bacteria could further contribute to their pathogenicity[34,35]. Notably, the Proteobacteria also possess abundant genes for degradation of xenobiotics, which might affect drug metabolism of patients in drug therapy. In line with this, a recent study reported that intratumor Proteobacteria can metabolize chemotherapeutic drugs into inactive forms and thus attenuate the efficacy of cancer therapies[36]. The genes involved in beneficial functions such as glycan degradation and vitamin B synthesis are enriched in the *Bacteroides* genus, consistent with its mutualistic role in the human gut. Notably, we found that *Bacteroides* species contain a considerable number of genes involved in sphingolipid and steroid hormone synthesis, suggesting their potential for modulating signaling in mammalian cells. In support of this, a recent study reported that *Bacteroides fragilis* can take advantage of sphingolipid signaling to enable symbiosis in the intestine[37]. It is noteworthy that genes involved in glycan degradation and sphingolipid metabolism were also enriched in the genus *Bifidobacterium*, another well-known gut commensal microbe. However, genes involved in both pathways were not abundant in the *Prevotella* genus, suggesting a distinct function of *Prevotella* compared with other members of the Bacteroidetes phylum. This might account for observed negative correlations between the relative abundances of *Prevotella* and *Bacteroides* in the gut microbiota[38]. The potential role of gut bacteria in metabolism of estrogens has long been recognized[39], but detailed mechanistic studies are still lacking. It will be interesting to explore the implication of this unique function of gut bacteria in hormone-related health or disease. The CGR also enabled the identification of several potential bacteriocin-producing bacteria strains, which merit further verification.

The CGR will improve metagenomic analyses, genome variation analyses, functional characterization and pan-genome analyses. The isolated gut bacteria strains have been deposited in the China National GeneBank (CNGB) and may be useful for studies that aim to alter microbiota functions, as novel probiotics, or for verification of disease-associated bacterial markers.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability, and associated accession codes are available at https://doi.org/10.1038/s41587-018-0008-8.

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Author contributions

H.J., J.L., L.X., Y.Z. and W.X. conceived and designed the project. H.J, J.L. and L.X monitored the project. Y.Z., W.X., Y.D., D.W. and R.J. collected samples and performed experiments. Y.Z., W.X., G.L., P.Q., Z.D., R.G., H.S., Y.X., S.L., Q.F., Z.J., L.S., T.G., X.X., Y.H., X.L., J.W., H.Y., Y.L., S.T., G.H., C.L., Z.X., J.Y., K.K., L.X., J.L. and H.J. analyzed and interpreted the data. Y.Z. and Z.D. wrote the paper. K.K., H.J. and L.X. revised the paper. All authors commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Anaerobic cultivation of fecal bacteria. Fecal samples were collected from 155 healthy human donors not taking any drugs during the last month before sampling. Detailed information is given in Supplementary Table 2. The samples were immediately transferred to an anaerobic chamber (Bactron Anaerobic Chamber, Bactron IV-2, Shellab, USA), homogenized in pre-reduced phosphate buffered saline (PBS) supplemented with 0.1% cysteine, and then diluted and spread on agar plates with different growth media (Supplementary Table 1). Plates were incubated under anaerobic condition in an atmosphere of 90% N_2, 5% CO_2 and 5% H_2 at 37 °C for 2–3 d. Single colonies were picked and streaked onto new plates to obtain single clones. All the strains were stored in a glycerol suspension (20%, v/v) containing 0.1% cysteine at –80 °C. The collection of the 155 samples was approved by the Institutional Review Board on Bioethics and Biosafety of BGI under number BGI-IRB17005-T1. All protocols were in compliance with the Declaration of Helsinki and explicit informed consent was obtained from all participants. Bacteria in the CGR (Culturable Genome Reference) are deposited in and are available from the E-BioBank (EBB) of the China National GeneBank (http://ebiobank.cngb.org/index.php?g=Content&m=Hql&a=sample_5&id=393).

Whole-genome sequencing and de novo assembly. *DNA extraction.* Isolates cultivated to stationary phase were centrifuged at 7,227*g* at 4 °C for 10 min, and the resulting pellets were resuspended in 1 ml of Tris-EDTA. For bacterial cell lysis, 50 µl of 10% SDS and 10 µl of proteinase K (20 mg/ml) were added, and the solution was incubated at 55 °C in a water bath for 2 h. The released genomic DNA was extracted using the phenol-chloroform method[40].

Genome sequencing and assembly. Paired-end libraries with an insert size of 500 bp were constructed and sequenced on Illumina Hiseq 2000 platform to obtain about 100× clean data for each sample. The reads were assembled using SOAPdenovo 2.04[41] to form scaffolds from which the rRNA genes were extracted by RNAMMer 1.2[42]. An in-house pipeline was used to obtain the best assembly containing an orthogonal design to investigate L,M,d,D,L,u,G (arguments of SOAPdenovo) and a single-factor design to investigate K (argument of SOAPdenovo) by comprehensively considering contig average length, longest scaffold and rRNA score. Libraries with an insert size of 240 bp were constructed and sequenced on the ionProton platform, which produced about 100× clean data for each sample. The reads were assembled through SPAdes (version 3.1.0)[43] to form scaffolds.

Assessment of genome quality. Six high-quality draft assembly criteria from the Human Microbiome Project (HMP)[14] and rRNA (5 s, 16 s and 23 s) completeness were adopted to ensure the assembly quality. The criteria are (i) 90% of the genome assembly must be included in contigs > 500 bp, (ii) 90% of the assembled bases must be at > 5× read coverage, (iii) the contig N50 must be > 5 kb, (iv) scaffold N50 must be > 20 kb, (v) average contig length must be > 5 kb, and (vi) > 90% of the core genes[44,45] must be present in the assembly.

Splitting for multi-genome isolates. The multi-genomes in isolates were initially identified using CheckM[46] (contamination > 5%) and confirmed by manual inspection of the plot of G + C percentage vs. sequencing depth. An in-house pipeline was developed to split the scaffolds of multi-genomes into single genomes. Briefly, scaffolds in multi-genomes were first split on the basis of G + C percentage vs. sequencing depth values using the dbscan function of R (package "fpc"). The "complete" and "contamination" of split genomes were checked using CheckM. For split genomes with "complete" > 100% or "contamination" > 15%, an additional species-designating pipeline was used to obtain their closest reference (with ANI value > 90%). Finally, the mis-split scaffolds in each split genome were mapping back to the closest reference genome using BLASTn (-e 1e-5 -F F -m 8, blastn hits' length > 90 nt, query scaffold coverage ≥ 50%) to obtain the final split genomes.

Massive species and genus assignment process. *NCBI-retrieved prokaryotic genomes.* All complete genomes (update time 19 November 2014) and draft genomes (update time 8 August 2014) on the NCBI ftp site were downloaded to a local server. Items with more than one NCBI taxonomy identifier (taxid) or genome sequence not available or of non-prokaryotic source were removed, and of redundant items, only one was kept. As a result, 24,552 genomes, 19,116 genome-scale amino acid sequences, and their taxonomic information were obtained.

Average nucleotide identity (ANI)[47] for species level taxonomic assignment. The taxonomic assignment of each query genome was determined by the taxonomic information of all the NCBI-available prokaryotic genomes. The tetra-base signature profiles of all the genomes and each query genome were acquired. A Pearson correlation test was performed between each query genome and all the genomes, resulting in a reference list sorted by decreasing correlation coefficient for each query genome. Then pairwise ANI alignment was performed between query and reference genomes one by one according to the reference list (tetra-base profile's Pearson correlation test: correlation coefficient > 0 and $P < 0.001$) until the ANI value was larger than 95% in the top 500 items (defined as assigned in this case) or reference item number exceeded 500 without any ANI value being larger than 95% (defined as not assigned in this case).

Percentage of conserved proteins (POCP)[48] for genus-level taxonomic assignment. The taxonomic assignment of each query genome was determined by the taxonomic information of all the NCBI-available prokaryotic genomes. The tetra-base signature profiles of all the reference genomes and the query genomes with no species assignment based on ANI were acquired. A Pearson correlation test was performed between each query genome and all the reference genomes, resulting in a reference list sorted by decreasing correlation coefficient for each query genome. Then the POCP calculation was performed between query and reference genomes one by one according to the reference list until the POCP value was larger than 50% in the top 500 items (defined as "assigned" in this case) or reference items number exceeded 500 without any POCP value being larger than 50% (defined as "not assigned" in this case).

16S rRNA sequence analysis and novel species determination. 16 S rRNA gene sequences were extracted from the isolate genomes using RNAmmer[42], except for 16 genomes where extraction failed. The sequences were quality-control processed in EzBioCloud (http://www.ezbiocloud.net)[49]. The species-level operational taxonomic units (OTUs) were classified using mothur[50] with an identity of 98.7% as a species-level cut-off, and cut-offs of 94.5% and 86.5% were used for genera and families[51], respectively.

Comparison of CGR with genome datasets from other studies. To compare the new genomes and novel species archived in CGR with those identified in two recent studies, we downloaded 215 genomes reported by Browne et al.[32] and 169 genomes reported by Lagier et al.[31]. We adopted a similar ANI pipeline as described above for species-level comparison by replacing the NCBI references with these newly downloaded genomes. "Map" was defined if the pairwise ANI value between a query genome in our 1,520 high-quality genomes and any one of references genomes (tetra-base profile's Pearson correlation test: correlation coefficient > 0 and $P < 0.001$) was larger than 95%; if not, the species was defined as "unmap."

Construction of species clusters. Pairwise ANI alignment was performed among the 1,520 high-quality genomes, and then hclust from the R package was used for hierarchical clustering with distance of 0.05 (equivalent to 95% ANI). A set of 40 universally conserved single-copy genes encoding proteins in bacteria and archaea was used for construction of a phylogenetic tree. Marker genes were detected and aligned using specI[52] and prank[53]. Alignments were trimmed by trimal[54] and concatenated with in-house scripts. A phylogenetic tree was inferred using the maximum likelihood method with RAxML (version 8.2.8)[55] for the clusters' representative genomes (N50 longest among cluster) with *Rhizobium selenitireducens* ATCC BAA 1503 (taxoid:1336235) as an outgroup, and was visualized in iTOL (http://itol.embl.de/)[56] online.

Genome function annotation. The 1,520 high-quality genomes were functionally annotated. Genes were identified using Genemark[57]. The translated amino acid sequences of coding genes were aligned with RAPSearch (-s f -e 1e-2 -v 100 -u 2)[58] against the Kyoto Encyclopedia of Genes and Genomes (KEGG version 76)[18,59] (query match length higher than 50%) or with BLASTp (-e 1e-2 -F T -b 100 -K 1 -a 1 -m 8) against the Antibiotic Resistance Genes Database (ARDB) (both query and subject match length higher than 40%, with identity higher than the ARDB-recommended thresholds)[60], the Virulence Factor Database (VFDB)[20,61] (query match length higher than 50%, with identity higher than 60%), and the bacteriocin database (downloaded from BAGEL3[62], with identity higher than 60%). Annotation of genes against the Comprehensive Antibiotic Resistance Database (CARD)[21] was performed using Resistance Gene Identifier available as a downloadable command-line tool in the download section of the CARD website using default parameters.

Mapping ratio of metagenomic samples. The metagenomic reads[6] were first aligned to the reference genomes of IGCR (3,449 sequenced prokaryotic genomes from IGC[6]) using SOAP2[63] (default parameters, except -m 100 -x 1000 -r 1 -l 30 -v 5 -c 0.95 -u). The unmapped reads were then aligned to the newly sequenced genomes of CGR. The read mapping ratio of different samples was calculated, and the difference between samples was determined by Wilcoxon test in R.

Analysis of gene and protein diversity. *Gene clusters.* 5,749,641 genes in the 1,759 CGR genomes and 11,330,042 genes in 3,449 IGCR genomes were clustered using CD-HIT[64] with default parameters, except -G 0 -aS 0.9 -c 0.95 -M 0 -d 0 -r 1 -g 1, which amounts to 95% local sequence identity over 90% alignment coverage for the shorter sequence. A cluster is composed of two or more genes. An accumulative curve of gene clusters was drawn according to the sample name alphabetically with IGCR at the front part and CGR at the latter part.

Protein clusters. 5,749,641 protein sequences translated from genes in the 1,759 CGR genomes and 11,330,042 protein sequences translated from genes in 3,449 IGCR genomes were clustered using the kClust algorithm[65] with default parameters, which amounts to 20–30% maximum pairwise sequence identity over 80% alignment length with the longest sequence or seed of the cluster. A cluster is composed of two or more protein sequences. An accumulative curve of protein

clusters was drawn according to the sample name alphabetically with IGCR at the front part and CGR at the latter part.

SNP identification and similarity score. 1,520 genomes from the CGR were aligned with the sequenced reads from the 250 TwinsUK samples using SOAP2 with identity ≥90%. Representative genomes used for SNP analysis were identified according to three criteria described previously[17]. The resulting 282 genomes (Supplementary Table 7) that fulfilled these criteria were used as references for SNP calling using SAMtools (frequency > 1% and supported by ≥4 reads) as previously described[10,17,20]. The reference genomes used in a previous study[18] (152 genomes) were compared with that from CGR of this study (282 genomes) to identify shared and new reference genomes using ANI ≥ 95% as a threshold (species level).

Pan genome analysis for 38 cluster. Clusters containing more than ten genomes (from CGR and NCBI), as well as *Fecalibacterium prausnitzii* (seven genomes) and butyrate-producing bacterium SS3_4 (nine genomes), were used for pan-genome analysis using the Bacterial Pan Genome Analysis tool (BPGA) pipeline[66]. The set of genes shared by all the members of cluster was defined as core genes, while genes partially shared in members (accessory genes) and unique to single members (unique genes) in a cluster were defined as dispensable gene[67]. The pan-genome fitting curves of 38 clusters were generated by the BPGA workflow and plotted in R (v.3.3.3). The functions of genes in the pan-genomes of 38 clusters were annotated by KEGG and ARDB, using arguments identical to those used for functional annotation of genomes. The acetyl-CoA-to-butyrate biosynthesis pathway was generated according to a previous study[68], and the associated enzymes were identified according to the functional annotation and BLAST to the NCBI protein database (cut-off 1e−5, identity ≥70%, coverage ≥70%). The COG database[30] was also used to identify the functional distribution in the core and dispensable sections via the BPGA pipeline. The significance of the difference between COG distribution in core and dispensable genomes was examined using Wilcoxon test as implemented in R (v.3.3.3).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The assembly draft genomes and annotation information for the 1,520 CGR strains are deposited in the NCBI under accession code PRJNA482748, and these data are also available in the China National GeneBank (CNGB) Nucleotide Sequence Archive (CNSA; accession code CNP0000126). All bacterial strains in the CGR have been deposited in the CNGB, a nonprofit, public-service-oriented organization in China. The accession code for each strain is given in Supplementary Table 5 (Genebank_id). Researchers can explore strain information and request strains via http://ebiobank.cngb.org/index.php?g=Content&m=Hql&a=sample_5&id=393#.

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Software and code

Policy information about availability of computer code

Data collection	NCBI (19 Nov, 2014) , Ezbiocloud (23 Oct, 2017),KEGG (version 76), ARDB (Version 1.1), VFDB (2012),bacteriocin database (2013), CARD (2013)
Data analysis	SOAPdenovo2(v r240), RNAMMer (v1.2) , SPAdes (v3.1.0), CheckM (v1.0.12), specI (v1.0), prank (v.150803), trimal (v1.4.1), RAxML (v8.2.9), Genemark (v2.6r), RAPSearch (v2.23), BLASTp (v2.2.26), BPGA (v.1.3), RStudio (v1.0.153), R (v.3.3.3).

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The assembly draft genome and annotation information of the 1,520 strains have been deposited into the NCBI under accession number PRJNA482748 and the data are also available in the CNGB Nucleotide Sequence Archive (CNSA: https://db.cngb.org/cnsa; accession number CNP0000126).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	This study collected 155 feces, isolated 6,487 bacterial clones, and generated 1520 high quality genomes, which is the largest dataset to date for gut bacterial reference genomes.
Data exclusions	No data were excluded from analyses.
Replication	not applicable; no biological experiments were involved.
Randomization	not applicable; no biological experiments were involved.
Blinding	not applicable; no biological experiments were involved.

Behavioural & social sciences study design

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Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access and import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	*Describe any restrictions on the availability of unique materials OR confirm that all unique materials used are readily available from the authors or from standard commercial sources (and specify these sources).*

Antibodies

Antibodies used	*Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Validation	*Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	*State the source of each cell line used.*
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology

Specimen provenance	*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).*
Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	*For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.*
Wild animals	*Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*

Human research participants

Policy information about studies involving human research participants

Population characteristics	This study recruited 155 participants from China, including 79 male and 76 females; Among them 2 were age 1-10, 21 were age 11-20, 109 were age 21-50, and 23 were age >50; Please also see population characteristics in Supplementary Figure 1a.
Recruitment	All participants were recruited in ShenZhen, China; Fecal samples were collected from 155 healthy donors not taking any drugs during the last months prior to sampling;

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference
(See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.