Microbial Cell Factories

RESEARCH

Open Access

Fecal microbiota fermenting simple organic carbon substrates in vitro as microbial factories capable of distinguishing Crohn's disease from healthy states

Anna Detman-Ignatowska[1†], Gabriele Schiro[2†], Rafał Filip[3,4], Emilia Samborowska[1], Jakub Karczmarski[1], Sara Jarmakiewicz-Czaja[5], Kinga Jakubowska[1], Anna Williams[2], Nabahi Ramos Hickman[2], Daniel Laubitz[2] and Anna Sikora[1*]

Abstract

Background Crohn's disease (CD) is characterized by low microbial richness and diversity of the gut microbiome, shifts in the abundance of specific taxa, reduced presence of C2-C6 organic acid producers, especially butyrate-forming bacteria, and alterations in gut metabolites. This study aimed to demonstrate differences in the dynamics and fermentation activity of the fecal microbiota of CD patients and healthy individuals (HIs) grown in vitro on glucose or a mixture of acetate and lactate (fecal microbiota batch cultures). Glucose was used as a substrate for glycolytic fermentation, whereas a mixture of acetate and lactate supported related pathways leading to the production of C2–C6 organic acids, particularly butyrate *via* the conversion of lactate and acetate.

Results HI fecal microbiota cultures produced butyrate mainly through lactate and acetate transformation rather than *via* glucose fermentation. This pathway was impaired in the CD fecal microbiota cultures, which exhibited reduced synthesis of butyrate, valerate, caproate and propionate, and excessive production of ethanol and certain amino acids. These distinct fermentation activities stemmed from differences in the original CD and HI fecal microbiota composition that were further accentuated in batch cultures. The number of beneficial commensal bacteria (e.g., *Coprococcus catus*, *Ruminococcus torques*, *Gemmiger formicilis*, *Eubacterium rectale*, *Fusicatenibacter saccharivoransi*, *Faecalibacterium prausnitzii*) were significantly lower in the CD fecal microbiota cultures and correlated with reduced butyrate, valerate and caproate levels. Conversely, an overabundance of the recognized CD dysbiosis-associated bacteria, such as *Escherichia coli*, was reflected in elevated ethanol and amino acid levels in post-fermentation liquids. Metabolic potential analysis further indicated an enrichment of genes encoding enzymes

†Anna Detman-Ignatowska and Gabriele Schiro have contributed equally to this work.

*Correspondence:
Anna Sikora
annaw@ibb.waw.pl

Full list of author information is available at the end of the article

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

involved in ethanol and amino acid biosynthesis in CD fecal microbiota cultures and highlighted the metabolic versatility of *E. coli*.

Conclusions Fermentation patterns of fecal microbiotas in batch cultures can distinguish CD-associated dysbiosis from a healthy microbiome, with particular emphasis on lactate and acetate conversion to butyrate as a key pathway of butyrate production. The differences are observed under standardized in vitro conditions without the need to reconstruct the intestinal environment. These findings, pending further validation, may offer novel diagnostic opportunities and have implications for strategies aimed at restoring a healthy gut microbiome.

Keywords Crohn's disease, Acetate and lactate, Fecal microbiota, Fecal cultures, Metagenomics, Fermentation activity, Dysbiosis, Butyrate

Background

As an integral part of the intestinal tract, the gut microbiome (approx. 10^{13} microbial cells) is vital to the health of the host, in part due to the production of C2-C6 organic acids, in the required proportions, from the fermentation of diet-derived complex polysaccharides. Bacteria are the most numerous group in this microbiome, with *Firmicutes* (currently *Bacillota*) and *Bacteroidetes* (currently *Bacteroidota*) comprising 90% of the total. *Proteobacteria* (currently *Pseudomonadota*) and *Actinobacteria* (currently *Actinomycetota*) are less prevalent phyla [1–7]. Imbalances in the microbial community composition (dysbiosis) of the gut have been linked to a range of ailments, including bowel disorders [6, 8–14]. Inflammatory bowel disease (IBD), one of the most common and prevalent gut diseases, is mainly attributed to a Westernized lifestyle [15]. IBD occurs in two main forms: ulcerative colitis (UC), restricted to the colon, and Leśniowski-Crohn's disease, commonly known as Crohn's disease (CD), localized in the ileum, cecum and colon. Untreated and late-diagnosed IBD is associated with a predisposition to colorectal cancer [1, 8, 16–18].

IBD-related dysbiosis is characterized by relatively low microbial richness and diversity, especially the increased contribution of *Proteobacteria* (including *Enterobacteriaceae*) and *Actinobacteria*; a disturbed *Bacteroidetes: Firmicutes* ratio due to a reduction in the latter phylum; and decreased representation of specific taxa, particularly butyrate producers. These variations result in microbiome instability, increased susceptibility to infection and alterations in microbial-produced metabolites [1, 8, 10, 19–29]. IBD dysbiosis leads to altered production of C2-C6 organic acids: critical metabolites that play pivotal roles in maintaining gut barrier integrity, regulating immune responses and modulating inflammation. The most common C2-C6 organic acids, acetate, butyrate and propionate, are primary nutrients that provide energy to colonocytes, play a key role in cell signaling and cell proliferation, regenerate the intestinal mucosa, strengthen the gut barrier, mitigate inflammation and maintain the physiological functions of the gut. Butyrate in particular has significant protective properties [29–33]. Other C2-C6 organic acids, such as caproate, formate and valerate are formed in the gut in small quantities [33]. Decreased levels of acetate, butyrate and propionate are observed in fecal samples of IBD patients, whereas lactate and pyruvate concentrations are increased [1, 29, 30, 34]. Metabolomic studies have also revealed changes in amino acid metabolism [1, 29, 30, 34–37].

Among the strategies for treating IBD are approaches aimed at restoring a healthy gut microbiome through the use of probiotics, prebiotics, postbiotics and paraprobiotics. The effects of these interventions are assessed by evaluating the patient's condition, clinical symptoms, endoscopic findings, metagenomic analysis of the fecal microbiota, and the measurement of selected metabolites in fecal samples [14, 38, 39]. Another way of assessing the effects of this type of treatment, and indirectly the state of the donor's microbiome, could involve examining specific functional capacities of the fecal microbiota in in vitro cultures on defined media. This approach may also support the selection of potential dietary supplements with a positive influence on the microbiome. Recently, in vitro approaches have been used to study the utilization of complex polysaccharides by fecal microbiota from HIs and UC patients [40, 41], and the effects of anthocyanins [42, 43]. Importantly, they do not require the precise recreation of intestinal conditions. Interestingly, despite the extensive IBD literature, there have been few studies investigating the use of simple organic carbon sources, such as glucose, acetate and lactate, in propagating fecal microbiota. While glucose serves as a substrate for glycolytic fermentations, lactate and acetate are intermediates in anaerobic fermentations that feed into other metabolic pathways, such as butyrate [32, 44, 45] or propionate synthesis [46]. Conversion of acetate to lactate is a relevant pathway of butyrate synthesis in the gut microbiome, yet it is often overlooked in metabolic pathways that lead to its production from dietary fiber [47, 48].

The main aim of this study was to examine the dynamics and fermentation activity of fecal microbiota from CD patients and HIs grown in batch cultures with glucose or acetate and lactate as carbon substrates. Special attention was paid to the capability of butyrate synthesis

from lactate and acetate in fecal microbiota cultures. We hypothesized that the selected metabolic properties observed in batch cultures in vitro, despite not fully replicating intestinal conditions, correspond to those of the gut microbiome in vivo. Our results reveal clear, consistent, and statistically significant trends involving distinct patterns of fermentation products in CD compared with HI fecal microbiota cultures. The primary pathway of butyrate synthesis in HI fecal microbiota cultures was the conversion of lactate and acetate, whereas this process was impaired in CD fecal microbiota cultures. With further validation, fecal microbiota batch cultures may serve as a supportive tool for monitoring microbiome status.

Methods

Patients

Fecal samples were collected from CD patients and HIs at the Centre for Comprehensive Treatment of Inflammatory Bowel Diseases, Department of Gastroenterology, Clinical Regional Hospital No. 2 in Rzeszow (Poland). The CD patient group ($n=7$; CD1-CD7) comprised four males and three females between the ages of 23 and 50. All CD patients were within the normal range with respect to their height, body weight, mean hemoglobin, AST, ALT, GRF, and creatinine levels. Their clinical characteristics and treatment are summarised in Supplementary Table S1. The level of acute phase protein (CRP) was >5 mg/L for all patients apart from one (4 mg/L). This outlier was negative for calprotectin in the fecal sample (measured with the Simple Calprotectin one-step immunochromatographic test kit, Operon S.A., Spain), whereas all the other CD patients were positive for fecal calprotectin.

The group of HIs ($n=6$, H1-H6) comprised four males and two females aged 18–40. All HIs were negative for fecal calprotectin. Inclusion criteria for both CD patients and HIs are provided in Supplementary Table S1.

The proposed study was rigorously reviewed and approved by the Bioethics Committee of the University of Rzeszow (No. 2022/067), and it adheres to the highest ethical standards in medical research.

Fecal microbiota batch cultures

In vitro tests of the metabolic activity of fecal microbiotas were conducted in static batch cultures under anaerobic conditions, as shown in Fig. 1. The liquid growth medium was a modified M9 medium [49] without $CaCl_2$ and $MgSO_4$ supplemented with 2 g/L yeast extract, 10 g/L (55.5 mM) glucose (Difco™ Becton, Dickinson and Company Sparks, USA), 3.5 g/L (43 mM) sodium acetate (Chempur, Poland) and 7.41 g/L (66 mM) sodium lactate (VWR International), as specified for each medium

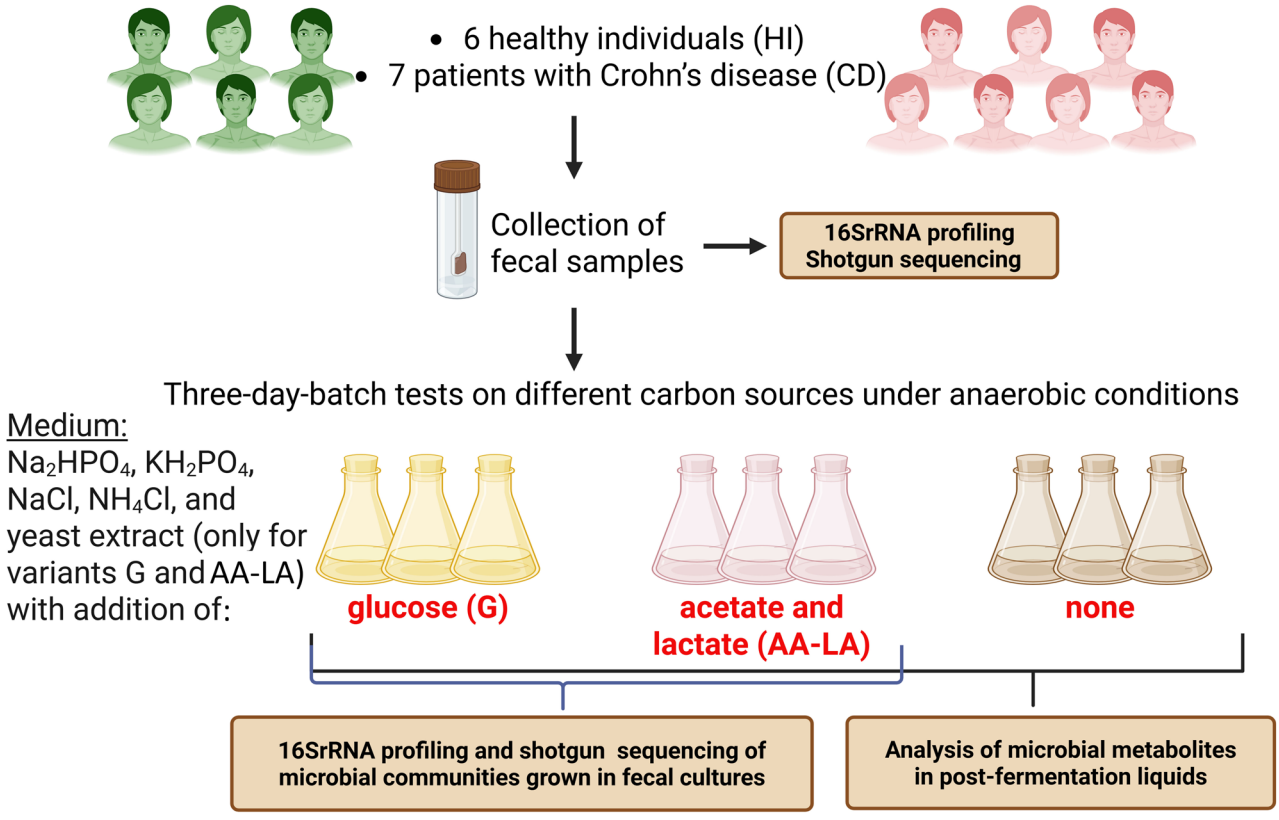


Fig. 1 Scheme of the experimental setup

variant in Table 1. Yeast extract serves as a source of nitrogen, including amino acids and peptides, as well as vitamins (mainly from the B group) and minerals such as Na, Mg, Ca, K, P, Ni, Se, Mn, Zn, Mo, Cu, Co and Al [50]. The composition of the growth media was based on our previous work [51, 52] and confirmed by preliminary data (Supplementary Table S2).

After collection from CD patients and HIs, fecal samples were immediately frozen at – 80 °C until use, as previously described [53]. No cryoprotectant was added to avoid introducing an extra carbon source that could be metabolized by the fecal microbiota in batch cultures, in accordance with the literature [54]. Such a storage procedure for collected fecal samples was used in Fecal Microbiota Transplantation (FMT) from humans to mice [53, 55]. To initiate each culture, 1 g of fecal samples (after one freeze-thaw cycle, thawed on ice) were added to 100 mL of the respective sterile media in 250-mL Erlenmeyer flasks and these were incubated for three days without shaking at 32 °C in a Vinyl Anaerobic Chamber (Coy Laboratory Products, Inc., USA). A comment on the incubation temperature can be found in Results in the section "*Shotgun metagenomic analysis of the fecal samples and their corresponding batch cultures*". As a control, the same volume of the M9 medium without added organic substrates was also inoculated with each fecal sample to characterize metabolites not resulting from the fermentation of glucose or acetate and lactate. In all cases the starting pH was 7.0 and no additional means of pH control were used. After three days of incubation, the cultures were centrifuged (10,000 × g for 10 min, 4 °C) to remove microbial cells and debris. The supernatants were collected for chemical analysis of the post-fermentation liquids, while the pellets were frozen at – 80 °C and used for total microbial DNA isolation. Three independent cultures were performed for each fecal sample in the three media: M9-based, M9-based plus glucose, M9-based plus acetate and lactate. The 3-day (72-hour) incubation period and endpoint measurement were intended to allow the bacteria to reach the stationary phase and achieve maximal accumulation of metabolic end products [51, 52, 56–58].

In the following sections, the terms "HI feces" and "CD feces" are used to refer to fecal samples (fecal microbiota) from HIs and CD patients, respectively. Similarly, "HI fecal culture" and "CD fecal culture" denote fecal microbiota cultures derived from HIs and CD patients, respectively, grown on glucose or acetate and lactate.

Analytical methods

A standard pH meter (ELMETRON model CP-502, Poland) was used to measure the pH of the media and post-fermentation liquids. Analysis of C2-C6 organic acids (acetic acid, lactic acid, propionic acid, butyric acid, 2-methyl butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid and isocaproic acid) was performed as described previously [59]. Ethanol was quantified using a gas chromatography/mass spectrometry (GC/MS) method based on the protocol of Pinu and co-workers [60]. Concentrations of γ-aminobutyrate

Table 1 Composition of the media and fermentation liquids (median concentrations shown in Fig. 2a)

Medium variant or post-fermentation liquid Component	M9 with no additives	Feces incubated in M9 with no additives		Acetate and lactate medium (AA-LA)*	Fecal culture on acetate and lactate		Glucose medium (G)*	Fecal culture on glucose	
		healthy	CD		healthy	CD		healthy	CD
Glucose (g/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	10	0.0038	0.3032
AA (g/L)	< LOQ	0.0766	0.1212	2.5200	3.0171	3.4549	0.0200	2.7124	1.0341
LA (g/L)	< LOQ	0.0001	0.0018	6.0400	0.0021	0.0074	0.0400	6.9165	6.5244
BA (g/L)	< LOQ	0.0360	0.0171	0.0014	1.3571	0.1078	0.0014	0.2549	0.0726
IBA (g/L)	< LOQ	0.0071	0.0024	0.0016	0.0582	0.0055	0.0016	0.0053	0.0015
2MeB (g/L)	< LOQ	0.0056	0.0016	0.0003	0.0471	0.0072	0.0003	0.0015	0.0001
GABA (mg/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	32.2	53.7
Ethanol (g/L)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.0640	< LOQ	0.5655	0.7140
PA (g/L)	< LOQ	0.0548	0.0504	0.0010	2.0696	2.4677	0.0010	0.5086	0.0188
VA (g/L)	< LOQ	0.0076	0.0012	0.0002	0.0850	0.0056	0.0002	0.0142	0.0005
IVA (g/L)	< LOQ	0.0074	0.0026	0.0006	0.0545	0.0046	0.0006	0.0047	0.0008
CA (g/L)	< LOQ	0.0005	0.0003	0.0002	0.0011	0.0001	0.0002	0.0004	< LOQ
ICA (g/L)	< LOQ	0.0001	0.0001	0.0002	0.0102	0.0081	0.0002	0.0004	< LOQ
L-alanine (mg/L)	< LOQ	< LOQ	< LOQ	44.2	< LOQ	22.1	44.2	43.8	35.8
Leucine (mg/L)	< LOQ	< LOQ	< LOQ	62.7	< LOQ	36.7	62.7	26.3	38.9
Isoleucine (mg/L)	< LOQ	< LOQ	< LOQ	34.3	< LOQ	22.3	34.3	17.1	25.1
Glycine (mg/L)	< LOQ	< LOQ	0.005	17.1	< LOQ	6.6	17.1	6.3	12.2

* Yeast extract served as the source of L-alanine, leucine, isoleucine, glycine, BA, IBA, 2MeB, GABA, PA, VA, IVA, CA, ICA, as well as AA and LA. < LOQ – below Limit of Quantification

(GABA), L-alanine, L-leucine, L-isoleucine and glycine were determined using a similar GC/MS approach. DB-1701 and TG-5SILMS columns were used for chromatographic separations and the metabolites were quantified using GC-MS with a Trace 1310 GC system coupled to a TSQ9000 Triple Quadrupole mass spectrometer (Thermo Scientific). Chromeleon 7 software was used for instrument control and data acquisition. MS data were collected in Selected Reaction Monitoring mode. The sample preparation method for GABA, L-alanine, L-leucine, L-isoleucine and glycine analysis was adapted from that of [61]. Calibration curves and internal standards were utilized for all quantified compounds.

Microbial DNA extraction

DNA was isolated from 0.2 g fecal samples and 0.2 g samples of material pelleted from fecal cultures by centrifugation (after one freeze-thaw cycle, thawed on ice) using a QIAamp PowerFecal Pro DNA Kit (Qiagen, Cat. No. 51804) according to the manufacturer's protocol. Cell lysis was achieved using a Vortex-Genie 2 equipped with a Vortex Adapter for 1.5-2 mL tubes (Scientific Industries, Inc., USA). DNA was eluted in 10 mM Tris-HCl (pH 8.5) and stored at − 20 °C until required. Extraction blanks were included for contamination control.

16 S rRNA gene sequencing and bioinformatics

The V4 hypervariable region of the 16S rRNA gene was PCR (polymerase chain reaction)-amplified from the prepared microbial DNA samples using the primer pair 515-F and 806-R [62], which carried Illumina adapters and a unique 12-base pair (bp) barcode identifying each sample. The PCR products were quantified with a Quant-iT PicoGreen dsDNA assay Kit (Invitrogen). The DNA fragments were then pooled in equimolar concentrations, and products longer than 200 bp were selected using QIAseq beads and sequenced on a 2 × 150 bp Illumina MiSeq platform (Illumina) at the PANDA core for genomics and microbiome research, University of Arizona, USA. Demultiplexing was performed using idemp (https://github.com/yhwu/idemp). The DADA2 pipeline was used to infer amplicon sequence variants (ASVs). The reads were trimmed to 145 bp, low-quality reads exceeding a maximum expected error of 2 bp were removed, and the remaining reads were used for error model training and correction. Reads were then merged, chimera sequences eliminated, and taxonomic identities assigned using the Ribosomal Database Project (RDP) classifier [63] with the SILVA nr version 138.1 rRNA database [64]. The number of reads per sample after quality filtering, error correction, chimera removal, and taxonomy cleaning ranged from 50,154 to 97,882. The raw 16 S rRNA DNA sequences have been deposited in NCBI databases with the accession number PRJNA1160194.

Shotgun metagenomics sequencing and bioinformatics

DNAs prepared from fecal culture samples from ten subjects (five HIs and five CD patients) were randomly selected for shotgun metagenomic analysis. From the set of three biological replicates per substrate from each individual, the one closest to the group centroid calculated with Bray Curtis (BC) dissimilarities was sequenced. Additionally, DNA from an initial fecal sample was sequenced for each individual patient. Quality filtering and adapter removal were performed with trimmomatic v 0.38 (LEADING:20 TRAILING:20 SLIDINGWINDOW:4:15 MINLEN:50) [65]. Host contamination was removed following alignment to the human reference genome *GRCh38* using bowtie2 [66] with standard parameters, leaving between 23,651,272 to 62,620,933 read pairs per sample. Reads were taxonomically profiled using MetaPhlAn4 with the "mpa_vOct22_CHOCOPhlAnSGB_202212" database [67]. The results were then imported into HUMAnN3 for functional profiling [68]. The raw shotgun sequencing data have been deposited in NCBI databases with the accession number PRJNA1160072.

Statistical analysis

Statistical analyses were performed in R 4.2.1 [69] with the aid of the vegan package [70]. Technical replicates were merged and considered as one sample. Alpha (ASV richness and Shannon's diversity index) and beta diversity metrics were calculated on rarefied data (50,000 reads). Differences in alpha diversity were calculated with a linear mixed effects model with HIs fecal samples as reference, subjects as random effects, and interactions between substrate and diagnosis. For beta diversity, BC dissimilarities were calculated on rarefied data [71]. The effect of different groups on community dissimilarities was first visualized with non-metric multidimensional scaling (NMDS) plots and quantified through a Permutational multivariate analysis of variance (PERMANOVA) [72]. Pairwise differences in BC dissimilarities between groups were further assessed using the Wilcoxon rank-sum test. Differential abundance of species and metabolites between combinations was also calculated with linear mixed effects models on log2 transformed relative abundances using the subject as a random effect, as implemented in the package maaslin2 [73].

Graphical elements were prepared using BioRender.

Results

Fermentation products as a reflection of the metabolic activity in fecal microbiota cultures

To evaluate whether the fermentation activity of the fecal microbiota on simple carbon substrates in batch cultures can distinguish CD patients from HIs, we examined the concentrations of microbial fermentation products

in post-fermentation liquids, with particular emphasis on butyrate, a metabolite characteristic of a healthy gut microbiome. Our metabolomic analysis revealed distinct fermentation patterns associated with the health status of the fecal donor with respect to the major fermentation products, including butyrate, lactate, acetate, propionate, and ethanol, as well as minor products such as isobutyrate, 2-methyl butyrate, caproate, isocaproate, valerate, isovalerate, GABA, and selected amino acids.

The controls included (i) post-incubation liquids obtained from fecal microbiota cultured in M9 medium without added substrates, allowing us to exclude metabolites originating from the organic compounds present in the fecal inocula (Fig. 2a, left panel; Table 1; Supplementary Table S3), and (ii) the composition of the fermentation medium containing yeast extract, which was analyzed to determine the concentrations of amino acids and minor C2–C6 organic acids originating from the medium itself (Table 1). The concentrations of all identified metabolites in post-fermentation liquids are presented in Fig. 2a (right panel: fecal microbiota cultures grown on acetate/lactate or glucose), Table 1 (median values), and Supplementary Table S3.

The median butyrate concentration in post-fermentation liquids from HI fecal cultures grown on lactate and acetate was 1.36 g/L, whereas in cultures grown on glucose it was 0.25 g/L. These findings indicate that the fecal microbiota of healthy individuals primarily produce butyrate through the conversion of lactate and acetate (Fig. 2a; Table 1, Supplementary Table S3), consistent with previous reports identifying this pathway as a major route of butyrate production in the gut microbiome [32, 44, 45]. In contrast, butyrate synthesis from both lactate/acetate and glucose was markedly impaired in CD fecal cultures (Fig. 2a, Supplementary Table S3). The median butyrate concentration decreased to 0.11 g/L and 0.07 g/L in CD fecal cultures grown on lactate/acetate and glucose, respectively (Table 1). Reduced butyrate production was not observed in two CD cultures, one of which originated from a patient in clinical remission. Similarly, CD fecal cultures on lactate and acetate exhibited reduced production of butyrate derivatives (isobutyrate and 2-methyl butyrate) and other minor fermentation products, including valerate, isovalerate, caproate, and isocaproate. The concentrations of these minor fermentation products in HI fecal cultures on lactate and acetate, as well as on glucose, were several-fold higher than those originating from the yeast extract and fecal inocula alone (Fig. 2a; Table 1, Supplementary Table S3).

Propionate was the dominant fermentation product in fecal cultures on acetate and lactate; however, its concentration did not discriminate between the health status of the fecal donors (Fig. 2a and b; Table 1). In fecal cultures on glucose, lactate was the dominant product, followed by acetate, propionate, butyrate, and ethanol (Fig. 2a; Table 1, Supplementary Table S3). The concentrations of acetate and propionate were significantly lower in CD cultures than in HI cultures grown on glucose (Fig. 2b). Another noteworthy finding was the elevated concentration of GABA in CD fecal cultures on glucose.

Glucose fermentation reduced the pH from the initial value of 7 to 4–5 (Fig. 2c). In contrast, the pH values resulting from acetate and lactate metabolism ranged between 6.5 and 7, consistent with our previous studies on microbial interactions and metabolic activity in anaerobic microbial communities [51, 52].

With regard to the fermentation media composition, glucose functioned exclusively as a substrate and was utilized by 99.95% in HI cultures and 97% in CD cultures (median values; Table 1, Supplementary Table S3). In contrast, acetate and lactate acted not only as substrates but could also be generated during fermentation, particularly acetate.

Particular attention should also be given to amino acids. Although they originated from both the yeast extract and the fecal inocula, they could also be produced during fermentation and consistently reached higher concentrations in CD cultures. Although amino acids represented only a minor fraction of the metabolite pool and their contribution to the observed fermentation patterns remains unclear, subsequent metabolic potential analyses (see the Metabolic Potential Analysis section) together with literature reports describing altered amino acid metabolism in the CD microbiome suggest that these compounds provide valuable information on the altered metabolic capabilities of the CD fecal microbiota and therefore warrant further investigation (see Discussion).

Principal component analysis (PCA) (Fig. 2d) showed that the metabolite profiles formed distinct patterns depending on both the fecal source and the carbon substrate. Samples were distributed along the first ordination axis, forming a gradient from HI fecal cultures grown on acetate and lactate to CD fecal cultures grown on glucose. HI fecal cultures grown on acetate and lactate were characterized by higher abundances of C2–C6 organic acids, including propionate, butyrate, caproate, valerate, and their derivatives, whereas CD fecal cultures grown on glucose exhibited elevated levels of ethanol, lactate, GABA, and amino acids (Fig. 2a, b, and d).

Differential abundance analysis of metabolite concentrations (Fig. 2b) further illustrated distinct fermentation patterns among the fecal microbiota cultures. Particularly informative were the comparisons *glucose:CD vs. glucose:healthy* and *acetate_lactate:CD vs. acetate_lactate:healthy*, as they revealed disease-associated differences in fermentation products under identical substrate conditions. Differences were observed for all analyzed

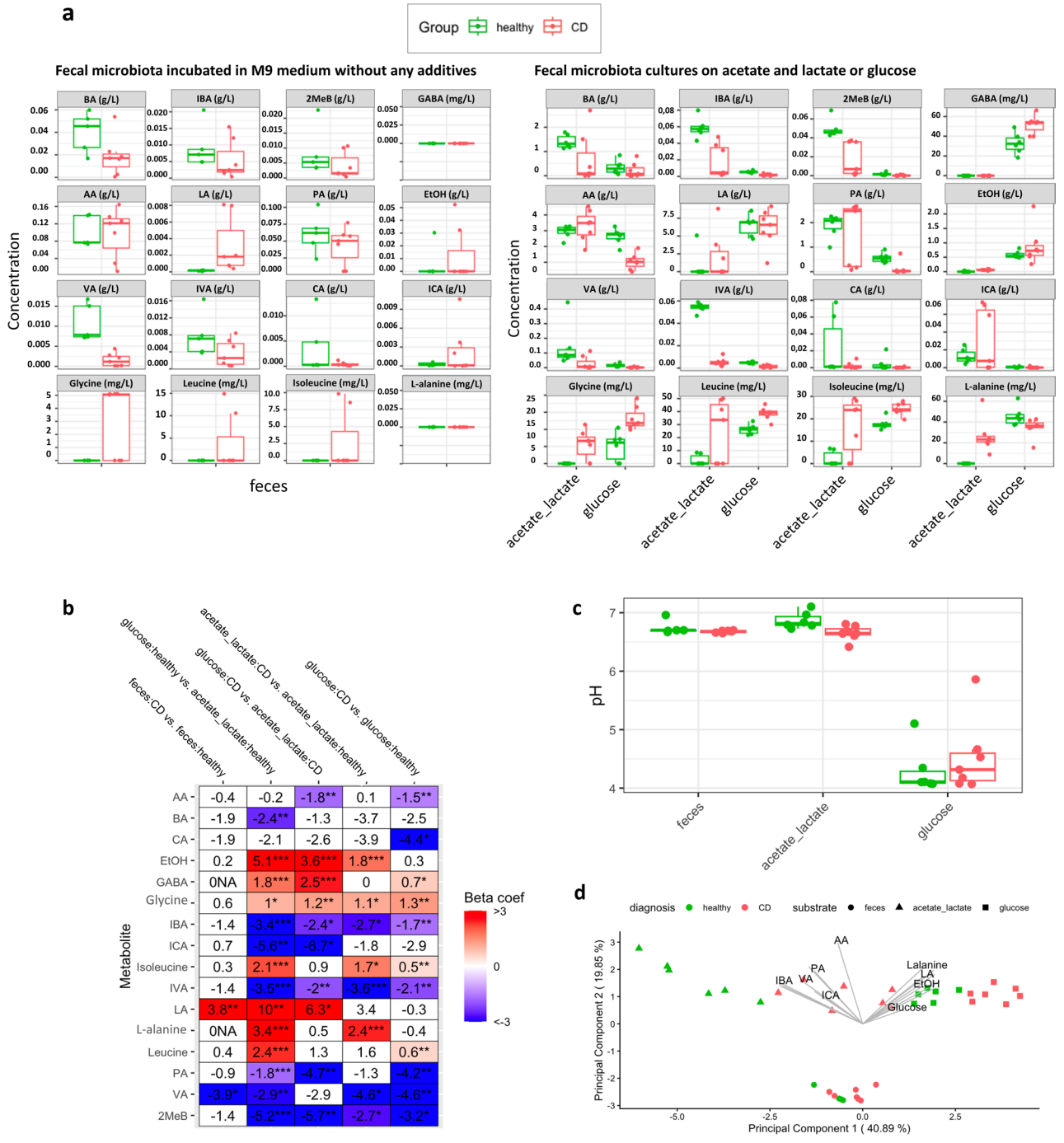


Fig. 2 Outcomes of microbial fermentation activity in fecal microbiota batch cultures. **a** Boxplots showing differences in the concentrations of metabolites across different groups. **b** Differential abundance analysis of metabolite concentrations. Beta-coefficient is equivalent to log2(fold change), P * <0.05, **<0.01, ***<0.001. Red cells indicate a significant increase, blue cells a significant decrease (P * <0.05, **<0.01, ***<0.001). White cells indicate non-significant differences. **c** Boxplot showing the pH of post-fermentation liquids, green data points – HIs, red data points – CD patients. **d** PCA ordination of metabolite measurements. Round points indicate baseline samples (samples without added carbon source). Panels **a**, **c** and **d**, feces – fecal microbiota incubated in M9 medium without any additives (see, Fig. 1); glucose – fecal microbiota cultures on glucose; acetate_lactate – fecal microbiota cultures on acetate and lactate. Panels **a**, **b** and **d**, AA – acetate, BA – butyrate, CA – caproate, EtOH – ethanol, GABA – γ-aminobutyrate, IBA – isobutyrate, ICA – isocaproate, IVA – isovalerate, LA – lactate, PA – propionate, VA – valerate, 2MeB – 2-methyl butyrate. Panel **b**, comparisons: feces:CD vs. feces:healthy – CD fecal microbiota incubated in M9 medium without any additives vs. HI fecal microbiota incubated in M9 medium without any additives; glucose:healthy vs. acetate_lactate:healthy – HI fecal microbiota culture on glucose vs. HI fecal microbiota culture on acetate and lactate; the same applies to the remaining conditions

metabolites, including the major fermentation products (butyrate, lactate, propionate, acetate, and ethanol), butyrate derivatives (isobutyrate and 2-methyl butyrate), and other minor products such as caproate, isocaproate, valerate, isovalerate, GABA, and amino acids. Together, these metabolites were associated with both the health status of the fecal donor and the carbon source supplied in the growth medium.

Fecal microbiota incubated in M9 medium without added substrates also exhibited clear differences between CD and HI samples. CD cultures showed substantially higher lactate concentrations accompanied by lower levels of other C2–C6 organic acids in the post-incubation liquids, although only the decrease in valerate reached statistical significance (Fig. 2b).

Microbial diversity based on 16S rRNA gene sequencing

Given that variations in the fermentation of simple organic carbon substrates are indicative of the donor's health status, we investigated the fecal samples and fecal cultures through 16S rRNA profiling followed by shotgun sequencing of selected samples. The number of different 16S rRNA gene amplicon sequence variants (ASVs) was 1,662. Fecal microbiota cultures grown on glucose exhibited significantly lower ASV richness and Shannon diversity than the corresponding fecal microbiota samples, whereas cultures grown on acetate and lactate did not differ significantly from the corresponding fecal microbiota samples (Fig. 3a). No significant interaction between substrate and disease status was detected in the linear mixed-effects models, indicating that the effects of the carbon substrate on alpha diversity metrics were comparable between HI and CD fecal microbiota cultures. Statistical estimates and model coefficients are provided in Supplementary Tables S4 and S5.

In contrast, analysis of beta diversity revealed that carbon substrate was the primary determinant of community composition, followed by donor health status (Fig. 3b; Table 2). Furthermore, CD fecal microbiota cultures exhibited greater Bray–Curtis dissimilarities between substrate conditions than HI fecal microbiota cultures (Fig. 3c), indicating that although the overall reduction in alpha diversity was comparable between groups, the taxonomic composition of CD communities changed more extensively in response to different carbon substrates (Fig. 3b; Table 2). Notably, CD fecal cultures exhibited higher dissimilarities between substrates compared to the HI fecal cultures (Fig. 3c).

Shotgun metagenomic analysis of the fecal samples and their corresponding batch cultures

For metagenomic analysis, the samples closest to the group centroid calculated with BC dissimilarities from 10 individuals (5 CD patients and 5 HIs) from the set of three biological replicates per substrate were selected for shotgun sequencing (see the Methods section and Supplementary Table S6). Taxonomic profiling of the reads was conducted using MetaPhlAn4, which successfully estimated between 65% and 87% of the total taxonomic composition (see Supplementary Table S6 for the detailed taxonomic assignments).

Fecal cultures transitioned from being dominated by highly diverse mixtures of low-abundance species to an increased contribution of *E. coli* or *Veillonella* species, in communities grown on glucose or acetate and lactate, respectively (Fig. 4a). This shift was especially prominent in CD fecal cultures. The results of differential abundance analysis confirmed these trends and identified other species whose abundance depended on the substrate (Fig. 4b and c).

We conducted a series of comparisons to characterize the CD-related differences in the batch cultures (Fig. 4b; Table 3). From the comparison of CD and HI feces, a noteworthy and surprising observation was the increased levels of *Bifidobacterium bifidum* in the CD fecal samples. However, the prevailing trend was the diminished abundance of numerous species (*e.g. Phocaeicola vulgatus* and *C. catus*) in the CD feces compared to HI feces. Growth on media supplemented with carbon sources accentuated this trend, i.e., a significant reduction in species number in the CD fecal cultures compared to those from HIs (see comparisons of glucose:CD vs. glucose:healthy and acetate_lactate:CD vs. acetate_lactate:healthy in Fig. 4b). Some species showed reduced abundance in both media, e.g. *F. saccharivorans* and the aforementioned *C. catus*, while diminished levels of other species were only observed in one medium, e.g., *F. prausnitzii* on acetate and lactate. *E. coli* and *Enterococcus faecalis* were particularly notable, since their representation was increased significantly in CD fecal culture compared to HI fecal culture on glucose (see comparison of glucose:CD vs. glucose:healthy in Fig. 4b). Both species exhibited higher abundance in CD feces than in HI feces (comparison of CD vs. healthy feces in Fig. 4b), although this difference was not statistically significant. Conversely, *Peptoniphilus harei* was enriched in CD fecal cultures grown on acetate and lactate.

HI fecal culture grown on glucose showed stimulation of specific bacterial species, including *Bifidobacterium adolescentis*, *B. longum*, *Lactiplantibacillus plantarum* and *Collinsella aerofaciens*, an effect not observed in CD fecal cultures.

Comparisons of HI and CD fecal cultures grown on glucose versus acetate and lactate (glucose:healthy vs. acetate_lactate:healthy and glucose:CD vs. acetate_lactate:CD in Fig. 4b) revealed trends that appear to be independent of health status, as they were observed in both groups. For example, acetate and lactate stimulated

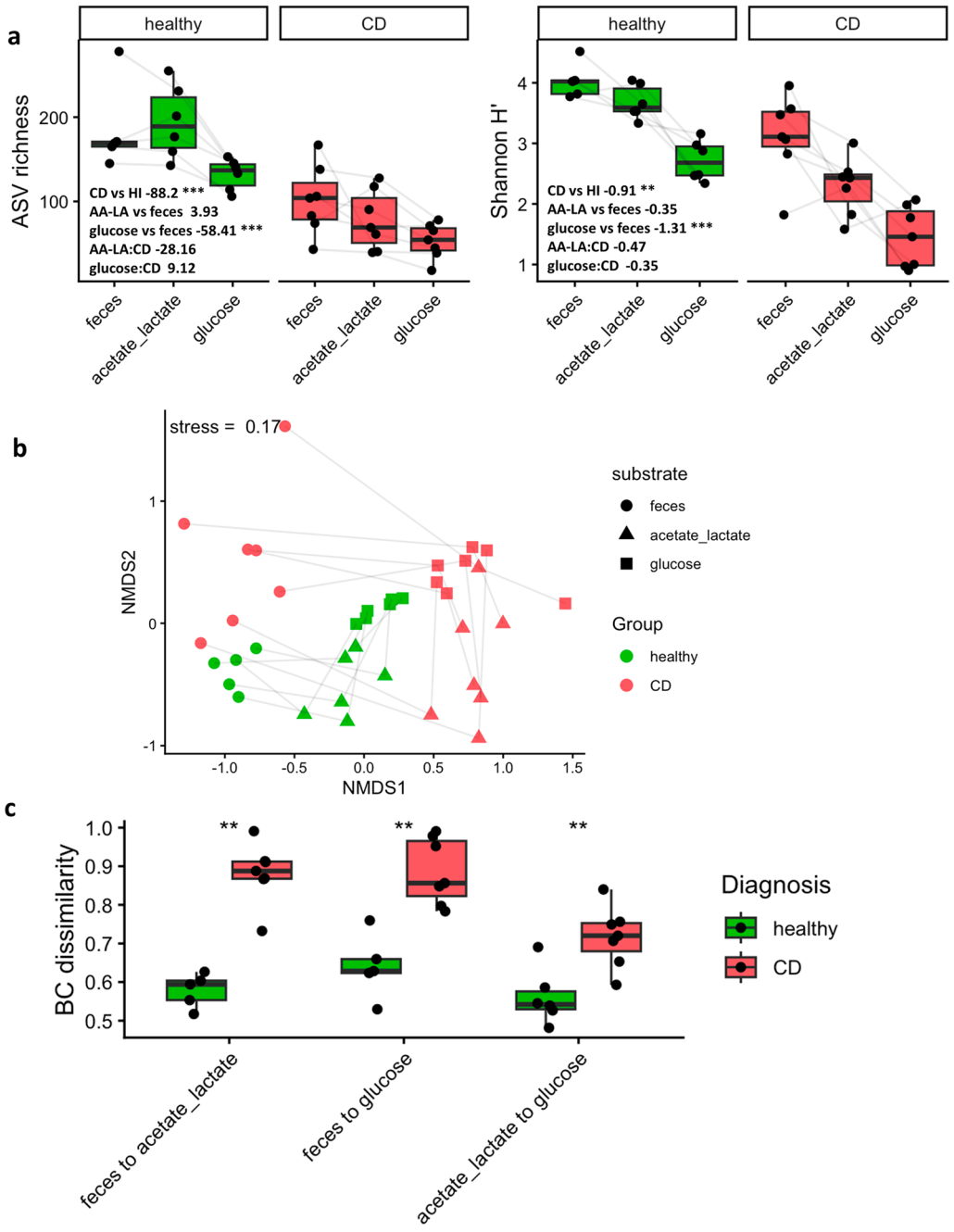


Fig. 3 Results of 16 SrRNA gene sequencing illustrating the biodiversity of the fecal microbiota (label: feces) and fecal microbiota cultures grown on glucose (label: glucose) or acetate and lactate (label: acetate_lactate). **a** Alpha diversity metrics. ASV richness (left) and Shannon diversity (right) are shown for fecal microbiota and fecal cultures, with paired samples from the same individual connected by gray lines. Boxplots summarize the distribution across subjects. See Supplementary Tables S4 and S5 for the results of the linear mixed-effects models. **b** NMDS ordination based on BC dissimilarities between samples. Gray lines connect samples originating from the same donor. **c** Comparison of BC dissimilarities between paired samples from the same donor. The x-axis shows dissimilarities between: (i) fecal microbiota and fecal cultures grown on acetate and lactate, (ii) fecal microbiota and fecal cultures grown on glucose, and (iii) fecal cultures grown on acetate and lactate and those grown on glucose. P values (** $P < 0.01$) were calculated using the Wilcoxon rank-sum test. Statistical significance assessed by PERMANOVA is presented in Table 2.

the growth of *Veillonella* species, such as *V. parvula* and *V. atypica*.

A clarification is warranted here, given that the batch cultures were conducted at 32 °C. Shimokawa et al. [74] investigated the growth of 54 gut microbial species across a wide temperature range (30, 33, 35, 37, 39, 41, 43, 45, and 50 °C). The results suggest that the most dominant commensal gut bacteria are able to grow outside the

Table 2 PERMANOVA results (R^2 and p-values) for Bray–Curtis dissimilarities between sample types (see Fig. 3b and c)

PERMANOVA Results		R^2	*P*
Diagnosis		0.10	**< 0.001**
Substrate		0.29	**< 0.001**
Diagnosis: Substrate		0.05	0.081
PERMANOVA Pairwise, Substrate			
Acetate_lactate vs. Feces		0.20	**< 0.001**
Glucose vs. Feces		0.30	**< 0.001**
Acetate_lactate vs. Glucose		0.18	**< 0.001**
PERMANOVA Pairwise, Combinations			
healthy	Acetate_lactate vs. Feces	0.25	**0.004**
	Glucose vs. Feces	0.44	**0.003**
	Acetate_lactate vs. Glucose	0.30	**0.003**
CD	Acetate_lactate vs. Feces	0.27	**0.002**
	Glucose vs. Feces	0.37	**0.002**
	Acetate_lactate vs. Glucose	0.24	**0.001**
Feces	Healthy vs. CD	0.17	**0.02**
Acetate_Lactate	Healthy vs. CD	0.21	**0.004**
Glucose	Healthy vs. CD	0.30	**0.004**

feces – fecal microbiota, glucose – fecal microbiota cultures on glucose; acetate and lactate – fecal microbiota cultures on acetate and lactate

human body over a temperature range of 30–43 °C, provided that anaerobic conditions are maintained. Notably, temperatures above 43 °C were a strong inhibitory factor for bacterial growth, which is consistent with the mesophilic nature of gut microbiota. At 30 °C, the growth of 49% of species was unaffected, while 20% and 31% of dominant human gut bacterial species showed increased and decreased growth, respectively, compared with growth at 37 °C. At 33 °C, the growth of 65% of species was unaffected, while 14% and 24% of dominant human gut bacterial species showed increased and decreased growth, respectively, compared with growth at 37 °C. In the study by Shimokawa et al. [74], unchanged growth within the temperature range of 30–37 °C was observed for several bacterial species that were reduced in CD fecal cultures, including *Phocaeicola vulgatus* (formerly *Bacteroides vulgatus*), *Ruminococcus torques*, and *Bifidobacterium adolescentis*. For *Dorea longicatena*, even increased growth was observed at 30 and 32 °C. The growth of *Coprococcus catus* and *Anaerostipes hadrus* was similar at 33 °C and 37 °C, but reduced at 30 °C. The growth of *Eubacterium rectale* (currently *Agathobacter rectalis*) and *Dorea formicigenerans* was reduced at both 30 °C and 33 °C; however, this did not represent complete inhibition. Shimokawa et al. [74] did not test *Blautia faecis* and *Roseburia (Agathobacter) faecis*. However, other species from these genera showed partially inhibited growth at 30 °C and 33 °C. Species enriched in CD fecal cultures on glucose, such as *Enterococcus faecalis*, showed increased growth at 30 °C and 32 °C.

Shimokawa et al. [74] did not test all bacterial species for which significant differences were observed in CD fecal cultures in our work. These included *Gemmiger formicilis*, whose strains showed good growth at 30 °C [75]; *Anaerotignum faecicola*, with a recognized growth temperature range of 10–45 °C [76]; *Alistipes putredinis*, capable of growing between 25 and 45 °C [77]; *Faecalibacterium prausnitzii*, reported to grow at 30–42 °C [78]; *Lactococcus lactis*, *Fusicatenibacter saccharivorans*, *Faecalibacillus intestinalis*, *Escherichia coli*, *Peptoniphilus harei*, *Bifidobacterium bifidum*, *Oscillibacter* sp., considered mesophilic according to the BacDive database [79].

The above data show that a temperature of 32 °C is not lethal for fecal microbiota. It can cause either slowed or accelerated growth of certain species, but in the experimental setup used, this effect is assumed to occur to the same extent in both CD and His fecal cultures on both media.

Associations between bacterial species and metabolites

Associations between bacterial species and metabolites, derived from the data presented in Figs. 2 and 4, are shown in Fig. 5. The results indicate that *E. coli* and *L. plantarum* contributed to the increased production of ethanol, lactate, GABA and amino acids. This activity was most pronounced in CD fecal cultures, mainly when grown in glucose-containing medium. Many species associated with higher levels of butyrate, valerate and their derivatives exhibited an inverse correlation with ethanol, lactate, GABA and amino acid synthesis. These included e.g. *C. catus*, *Anaerotignum faecicola*, *Gemmiger formicilis*, *E. rectale*, *Alistipes putredinis*, *Blautia faecis* and *Anaerostipes hadrus*. This pattern was most evident in HI fecal culture on acetate and lactate. Some species, like *Parabacteroides distasonis* and *Bacteroides uniformis*, were associated with increased production of butyrate and valerate without corresponding decreases in other metabolites. Other species, such as *Limosilactobacillus mucosae* and *B. bifidum*, were linked to lower levels of these compounds. Elevated propionate synthesis was associated with *Phascolarctobacterium faecium*, while *Petoniphilus harei* and *Veillonella dispar* were correlated with propionate and acetate production. The production of caproate and iso-caproate often co-occured with butyrate and valerate synthesis or was uniquely associated with certain species, like *Ruminococcus*.

Metabolic potential analysis

To assess metabolic potential (Fig. 6) using the HUMAnN3 tool, we selected genes encoding enzymes that catalyze reactions in which the measured metabolites are either products or substrates. Additionally, vital fermentation processes, including glycolysis, acetyl-CoA formation, hydrogen formation, and the reductive carbon monoxide dehydrogenase/acetyl-CoA synthase pathway (reductive CODH/ACS), were also analyzed. A complete

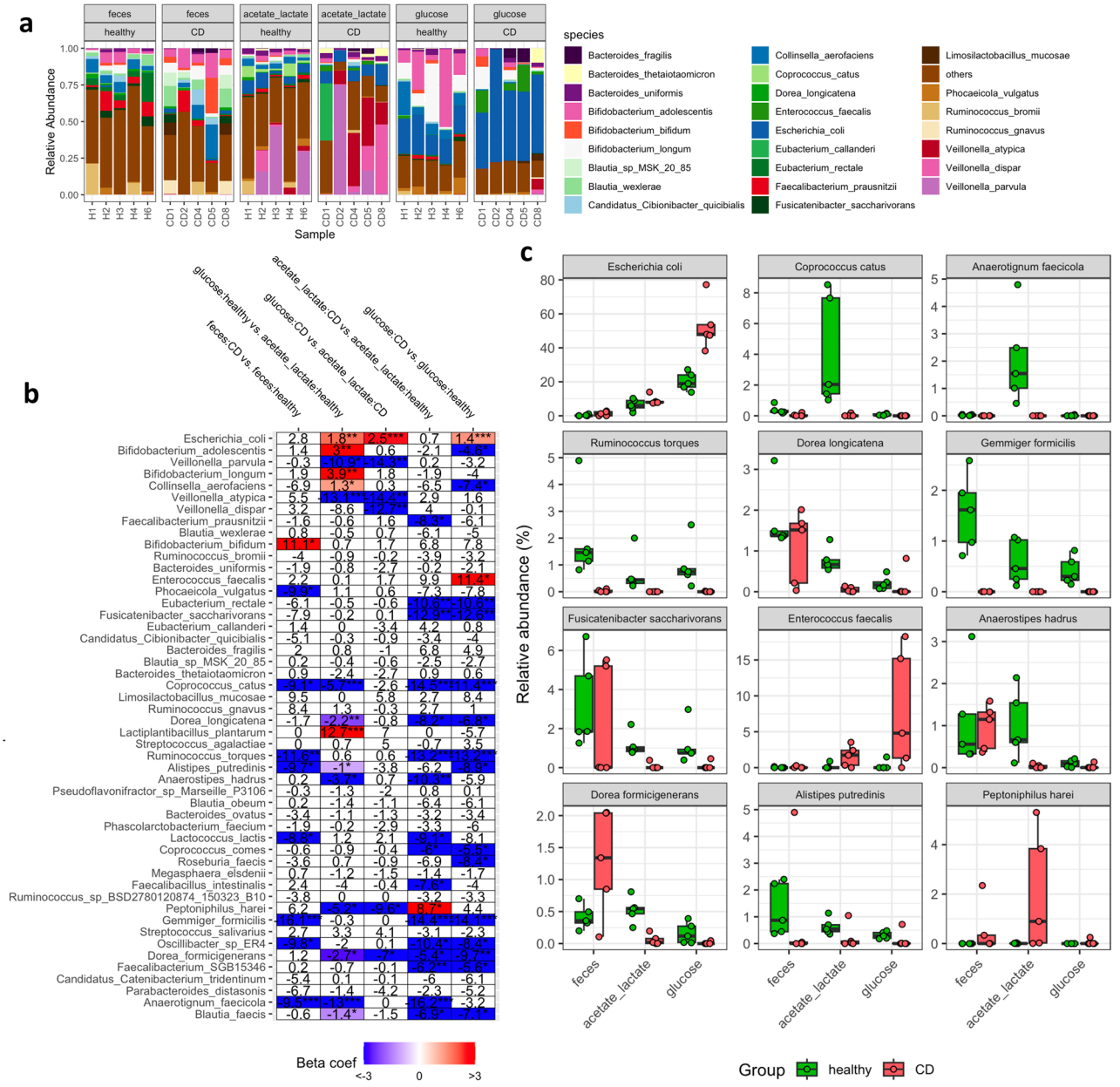


Fig. 4 Taxonomic assignment of shotgun metagenomics reads, the fecal microbiota (label: feces), fecal microbiota cultures on glucose (label: glucose) or acetate and lactate (label: acetate_lactate). a Stacked bar plot of species composition as identified by MetaPhlAn4. Only the top 25 species across the study are presented; all others are grouped into "others." b Differential abundance analysis of bacterial species. Comparisons: feces:CD vs. feces:healthy – CD fecal microbiota vs. HI fecal microbiota; glucose:healthy vs. acetate_lactate:healthy – HI fecal culture on glucose vs. HI fecal culture on acetate and lactate; the same applies to the remaining conditions. Beta-coefficient is equivalent to log2(fold change), P * <0.05, **<0.01, ***<0.001. Red cells indicate a significant increase, blue cells a significant decrease (P * <0.05, **<0.01, ***<0.001). White cells indicate non-significant differences. c Boxplots showing relative abundances across treatments of species selected as examples

list of enzymes and biochemical reactions possible in fecal cultures is provided in Supplementary Table S7.

CD fecal samples, when compared to those from HIs, exhibited an overrepresentation of genes encoding ethanol dehydrogenase (EC 1.1.1.1) and branched-chain amino acid transaminase (EC 2.6.1.42) involved in leucine and isoleucine metabolism. Consequently, ethanol and amino acid overproduction in CD fecal cultures was supported by, respectively, an elevated contribution of ethanol dehydrogenase (in both glucose- and acetate with lactate-supplemented media) and of dipeptidase (EC 3.4.13.18) and branched-chain amino acid transaminase on acetate and lactate.

CD fecal cultures on acetate and lactate exhibited overrepresentation of genes encoding hydrogen-yielding pyruvate: ferredoxin oxidoreductase (EC 1.2.7.1) and

Table 3 Bacterial species showing statistically significant changes in the fecal microbiota of patients with Crohn's disease or in CD fecal cultures. Table drawn based on the data in Fig. 4b

Species	Characteristics
Species reduced in CD feces only	
Phocaeicola vulgatus (formerly *Bacteroides vulgatus*)	Regarded as a relevant member of the core human microbiome that produces acetate, succinate, formate, propionate and other valuable metabolites such as 3-hydroxyphenylacetic acid, which helps maintain lipid homeostasis in the liver and prevents metabolic dysfunction-associated steatotic liver disease [107, 108].
Species reduced both in CD feces and CD fecal cultures grown on glucose (*) and/or acetate and lactate (#)	
Coprococcus catus *#	Mainly a propionate- but also a butyrate-producer in the human microbiome, capable of lactate utilization in the gut [41, 109].
Ruminococcus torques *#	Butyrate producer with decreased abundance in the microbiomes of CD patients, especially those who are C-reactive protein (CRP) positive [110].
Gemmiger formicilis *#	Butyrate producer, reduced in both CD and colorectal cancer patients [86].
Oscillibacter sp. *#	Reduced in IBD patients [111].
Anaerotignum faecicola *	A bacterium isolated from human feces that produces acetate, propionate, butyrate and isobutyrate as fermentation end products [76], with no recognized role in IBD
Alistipes putredinis *	Its role in the gut microbiome and IBD has yet to be confirmed by experimental studies [112]. A human *A. putredinis* strain exhibited anti-inflammatory effects in a mouse model of colitis induced by sodium dextran sulfate and oxazolone [113].
Lactococcus lactis#	A well-characterized bacterium with anti-inflammatory properties, commonly used as a probiotic [114].
Species reduced in CD fecal cultures grown on glucose and acetate with lactate	
Fusicatenibacter saccharivorans	Producer of C2-C6 organic acids, including butyrate, in the human gut, with a decreased contribution to the gut microbiome of patients with colorectal cancer [115] and UC [116].
Eubacterium rectale (currently *Agathobacter rectalis*)	A well-recognized butyrate producer in the gut, reduced in CD and UC patients [87, 117, 118].
Dorea longicatena *D. formicigenerans*	Non-butyrate producers that ferment carbohydrates mainly to formate, acetate and ethanol [119], helping to maintain a healthy gastrointestinal environment by the downregulation of inflammation in the gut mucosa [120]. *D. longicatena* was found to contribute to CD remission [121], whereas *D. formicigenerans* protects against colorectal carcinogenesis [122].
Blautia faecis	Representing the *Blautia* genus, found in fecal samples collected from a healthy human and regarded as a functional genus with potential probiotic properties due to its production of acetate, succinate, lactate, ethanol plus other beneficial effects for the host [123].
Species reduced in CD fecal cultures grown on acetate and lactate	
Faecalibacterium prausnitzii	A key beneficial commensal butyrate producer, with a well-documented diminished contribution in CD patients [88, 124–126].
Faecalibacillus intestinalis	Isolated from feces of healthy humans, with potential probiotic properties [127, 128].
Anaerostipes hadrus	An acetate-utilizing butyrate producer [129].
Species reduced in CD fecal cultures grown on glucose	
Bifidobacterium adolescentis	Primary starch degrader and producer of acetate and lactate, stimulates the growth of butyrogenic bacteria through metabolic cross-feeding; the health-beneficial properties of *B. adolescentis* include enhancement of intestinal barrier function, anti-inflammatory and immune-regulatory effects, as well as the production of neurotransmitters (GABA) and vitamins. A reduced abundance of *B. adolescentis* in the human gut microbiome has been associated with various forms of IBD [130–132].
Roseburia faecis (currently *Agathobacter faecis*)	A commensal bacterium that produces C2-C6 organic acids, particularly butyrate, which affects colonic motility, maintains immunity and has anti-inflammatory properties [133].
Species enriched in CD fecal microbiota only	
Bifidobacterium bifidum	The increases may result from disruption of the microbiome; the reduction in butyrate producers in CD patients appears to disturb the balance between different physiological groups of bacteria, leading to greater representation of members of the genus Bifidobacterium, for example [104].
Species enriched in CD fecal cultures grown on glucose	
Escherichia coli	Well-documented pathobiont contributing to IBD and cancer [89–92].
Enterococcus faecalis	Regarded as a probiotic strain with anti-inflammatory and protective properties. However, there are reports suggesting that it can cause injury to intestinal epithelial cells, induce gut inflammation and promote cancer [102, 103].
Species enriched in CD fecal cultures grown on acetate and lactate	
Peptoniphilus harei	Regarded as a commensal in the human microbiome and also an opportunistic pathogen [99, 100].

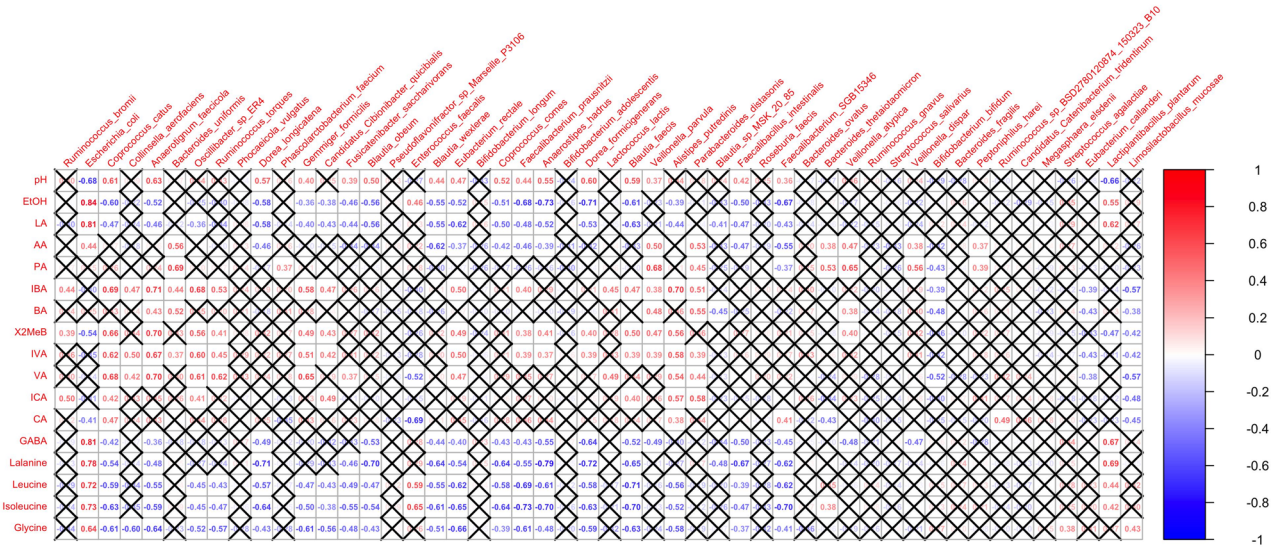


Fig. 5 Spearman correlation matrix based on fecal microbiota cultures' data showing correlations between bacterial species and the measured metabolites. Red: positive correlation, blue: negative correlation. Non-significant correlations ($P < 0.05$ after FDR correction) are marked with an 'X'. AA – acetate, BA – butyrate, CA – caproate, EtOH – ethanol, GABA – γ-aminobutyrate, IBA – isobutyrate, ICA – isocaproate, IVA – isovalerate, LA – lactate, PA – propionate, VA – valerate, 2MeB – 2-methyl butyrate

formate dehydrogenase (EC 1.17.98.4), which suggested a tendency towards excessive production of fermentative gases. This is consistent with clinical observations of bloating and gas production in IBD patients.

Surprisingly, certain genes encoding enzymes related to butyrate, propionate or caproate synthesis were detected in only a few of the analyzed bacterial species. This finding underscores the need for further research and the development of more advanced analytical tools. However, in the CD fecal samples, there was a reduced contribution of the gene encoding acetate ligase (EC 6.2.1.13), which plays a role in acetate and propionate metabolism. Furthermore, distinct metabolic responses emerged in glucose-supplemented cultures. Decreased contribution of genes encoding propionate CoA-transferase (EC 2.8.3.1), butyrate-acetoacetate CoA-transferase (EC 2.8.3.9), and 2-oxoisocaproate dehydrogenase (EC 1.2.4.4) were observed in HI fecal cultures on glucose. In comparison, the CD fecal cultures on glucose showed overrepresentation of butyrate-acetoacetate CoA-transferase (EC 2.8.3.9) and acetyl-CoA C-acetyltransferase (EC 2.3.1.9), suggesting a metabolic shift in these microbial communities favoring butyrate synthesis from glucose rather than acetate and lactate. Notably, a hallmark of a HI feces is the efficient conversion of lactate and acetate into butyrate, a process that appears to be disrupted in CD fecal microbial culture (Supplementary Table S3).

Among all analyzed taxa, *E. coli* emerged as the most metabolically versatile species. It dominated in glucose-containing media, particularly in CD fecal culture, highlighting its ability to exploit diverse metabolic pathways. This metabolic flexibility may contribute to its persistence and overrepresentation in CD fecal culture, reinforcing its role as a key player in CD-associated dysbiosis.

Together, these findings illustrate the metabolic divergence between CD- and HI feces, revealing substrate-driven microbial and functional shifts in the batch cultures. The impressive metabolic versatility of *E. coli* is corroborated by the findings of this research.

Discussion

Rationale for the experimental approach

In this study, we utilized an experimental approach in which the fermentative capacities of fecal microbiota derived from HIs and CD patients were examined using in vitro batch cultures. The growth media contained simple carbon sources, such as glucose, acetate and lactate. In this way, we established conditions for C2-C6 organic acids production: (i) glycolytic fermentations, which include butyrate and propionate fermentation; (ii) acetate and lactate conversion to butyrate [44, 45, 80, 81], and (iii) propionate synthesis from lactate [46].

Despite the substantial contribution of LAB to the gut microbiome, lactate concentrations in fecal samples from healthy individuals remain very low (< 5 mM) [45], whereas butyrate, propionate, and acetate are the dominant C2-C6 organic acids, typically present at a molar ratio of 3:1:1, respectively [31–34].

The conversion of lactate and acetate is a process resulting from lactate cross-feeding, symbiotic interactions between lactate-, acetate-, and butyrate-producing bacteria. This represents an important metabolic pathway for butyrate production in the gut microbiome [32, 45], although it is not always taken into account [47, 48].

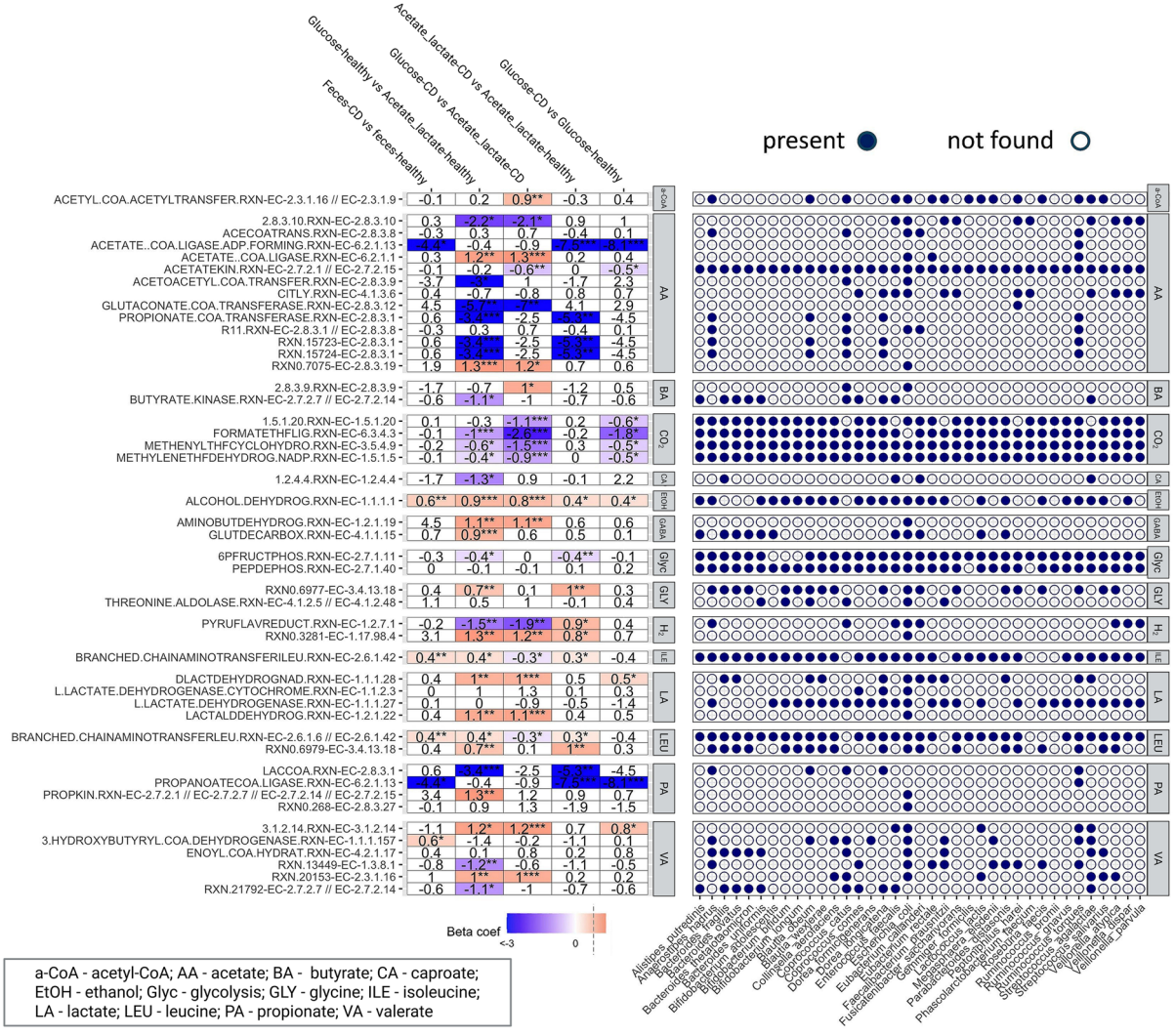


Fig. 6 Differential abundance analysis of selected metabolites and fermentation processes, and enzymes catalyzing reactions associated with them. Comparisons: feces:CD vs. feces:healthy – CD feces microbiota vs. HI fecal microbiota; glucose:healthy vs. acetate_lactate:healthy – HI fecal culture on glucose vs. HI fecal culture on acetate and lactate; the same applies to the remaining conditions. Beta-coefficient is equivalent to log2(fold change), P * <0.05, **<0.01, ***<0.001. Red cells indicate a significant increase, blue cells a significant decrease (P * <0.05, **<0.01, ***<0.001). White cells indicate non-significant differences. The right-hand panel shows which of the genes encoding the different enzymes are present in selected species. The abbreviations used are listed on the right side of the figure. The abbreviations of the metabolites used are provided on the right side of the figure

In in vitro experiments, butyrate production from acetate and lactate have been investigated in batch fermentation cultures: (i) in co-cultures of gut-derived bacteria, lactate-, acetate-, and butyrate-producers grown on media containing starch [45, 82], inulin [83], and fructooligosaccharides [82], highlighting their prebiotic properties, as well as glucose [80]; and (ii) in HI fecal cultures on fermentation media supplemented with lactate [84] or with both lactate and acetate [85]. In the latter case, the substrates were labeled (^{13}C-labeled lactate and ^{2}H-labeled acetate), and the results demonstrated that butyrate was the major net product of these processes. Propionate was the second key product [82, 85]. The cited studies confirm the appropriateness of the selected carbon sources used in the media in this work.

Despite the relatively small cohort of participants, we observed clear, logical, and statistically significant trends. A key finding was that the fermentation pattern of CD fecal cultures is altered compared to HIs due to disease-associated differences in microbial composition and function (Fig. 7). Previous studies with similar sample sizes (3–10 feces donors) have successfully identified robust metabolic signatures and reproducible trends [40–43, 82, 85].

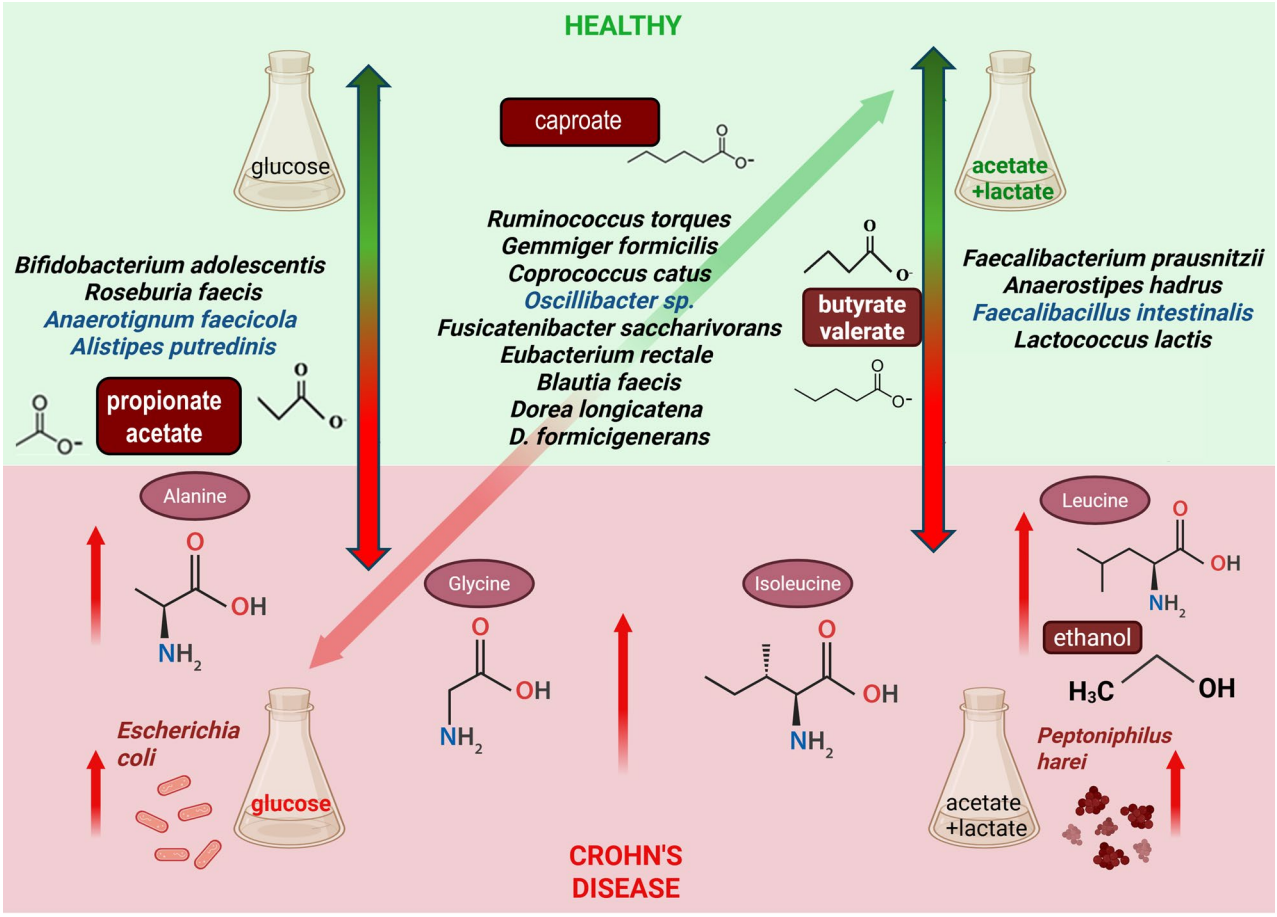


Fig. 7 Summary of study findings: CD fecal microbiota cultures features reduced synthesis of C2-C6 organic acids like butyrate, valerate, caproate and propionate, coupled with elevated production of ethanol and amino acids. These metabolic shifts correspond to the altered microbial composition, including depletion of beneficial commensals and overrepresentation of the recognized CD-associated bacteria, particularly *E. coli*. In vitro fermentations reveal that acetate and lactate promotes the growth of health-associated taxa, while glucose favors CD-linked microbes, highlighting distinct metabolic imbalances. Identifying key metabolites and microbial shifts may offer the potential for noninvasive diagnostics in CD. Green indicates a healthy state, while red represents dysbiosis associated with Crohn's disease. Downward arrows indicate a decrease and upward arrows signify an increase. Bacteria marked in blue are those whose role in the microbiota/microbiome requires further investigation. The diagonal gradient arrow indicates that in the HI microbiota cultures, genomes associated with acetate and lactate were more abundant than those associated with glucose

Features of CD-related dysbiosis observed in fecal microbiota batch cultures

A comparison of CD and HI fecal samples confirmed the well-recognized decrease in microbial richness and diversity characteristic of CD-associated dysbiosis [1, 8, 10, 11, 19–29]. Using the z-score measure, we showed that in CD fecal cultures, regardless of the carbon source in the media, the feces-associated genomes were reduced compared to those in HI fecal cultures. Furthermore, in the communities in HI fecal cultures, genes associated with acetate and lactate metabolism were more abundant than those associated with glucose metabolism. Table 3 lists bacterial species exhibiting a significant CD-related decrease in abundance in both fecal microbiotas and fecal microbiota cultures grown on specific carbon sources. These species include key butyrate- and propionate-producing bacteria, including *R. torques*, *G. formicilis*, *E. rectale* (*A. rectalis*), *F. saccharivorans*, *R. faecis* (*A. faecis*), *F. prausnitzii*, *(A) hadrus*, *C. catus* and non-butyrate producers such as *P. vulgatus* (*B. vulgatus*), *L. lactis*, *D. longicatena* and *D. formicigenerans*, *(B) faecis*, *B. adolescentis* all of which are associated with maintaining gut homeostasis [86–88].

Furthermore, the CD fecal culture on glucose was characterized by a marked increase in the growth of *E. coli*. Adherent-invasive *E. coli* is a well-documented pathobiont contributing to IBD and cancer [89, 90]. In addition, the ability to utilize propionate and the induction of virulent properties by propionate have been documented in CD-associated invasive *E. coli* [91, 92]. Our analysis of metabolic potential also revealed a propionate degradation pathway (PROPKIN-RXN) in *E. coli*.

Since seven CD patients consistently reproduced recognized signatures of CD-associated dysbiosis, this

supports the reliable identification of metabolites in fecal batch cultures and confirms that the observed fermentation patterns are robust and reproducible.

CD fecal cultures grown on both carbon sources produced lower amounts of butyrate, propionate, valerate, caproate and their derivatives. Importantly, our study highlights the limited ability of these cultures to convert acetate and lactate into butyrate. Butyrate was produced in all six HI fecal cultures grown on acetate and lactate. In contrast, this was observed in only two out of seven CD fecal cultures (patients CD2 and CD7) (Supplementary Table S3). In addition, patient CD7 was in endoscopic remission, had normal blood CRP levels, and no fecal calprotectin was detected in their feces. The inability of CD fecal cultures to transform lactate and acetate into butyrate is a clear trend. It is well established that many cross-feeding interactions are disrupted or diminished in disease-related dysbiosis, including IBD [93]. Moreover, we indirectly observed an increase in lactate levels in CD feces (when the samples were incubated in M9 medium without any organic compounds, see Fig. 2a and b), which is consistent with literature reports indicating elevated lactate concentrations in the feces of IBD patients [1, 29, 30, 34].

In the same experimental material, we also observed reduced levels of valerate and caproate in CD samples. Although the concentrations of these metabolites in the gut are typically low, they are known to decrease in IBD and increase following probiotic therapy, suggesting a role in maintaining intestinal homeostasis [94, 95].

Bacterial taxa exhibiting a decreased abundance in CD feces and CD fecal cultures were positively correlated with the level of butyrate, caproate, valerate and their derivatives in post-fermentation liquids. They were also negatively correlated with the level of certain amino acids and ethanol. CD-associated dysbiosis is characterized by alterations in the production of amino acids, which have been used for diagnostic purposes as biomarkers [27, 29, 37, 96]. It is noteworthy that fermentation of amino acids is a pathway of butyrate, 2-methyl butyrate, isobutyrate and isovalerate production in the gut microbiome [32]. In this study, an increased concentration of amino acids was observed in CD fecal cultures grown on glucose as well as on acetate and lactate. Amino acids can be synthesized from pyruvate and acetyl-CoA produced during glucose fermentation, as was shown for alanine and leucine in *Corynebacterium glutamicum* [97, 98]. In our batch cultures, acetyl-CoA and pyruvate could be generated from glucose as well as from acetate and lactate. Acetyl-CoA can be converted to ethanol by acetaldehyde dehydrogenase (EC 1.2.1.10) and ethanol dehydrogenase (EC 1.1.1.1), which may explain its increased production in CD fecal culture on acetate and lactate. The higher concentrations of amino acids and ethanol produced by CD fecal cultures were corroborated by analysis of their metabolic potential and the correlations between the aforementioned metabolites and the bacterium considered a key hallmark of CD-related dysbiosis, *E.coli*. Due to its substantial abundance and metabolic versatility, we propose that *E. coli* should become a focus of research on CD.

Other noteworthy trends observed in fecal microbiota batch cultures

The hallmark CD-related bacterium detected in our CD fecal cultures was *E. coli*, whose growth was stimulated by glucose. At the same time, acetate and lactate promoted the growth of *P. harei*, making it a putative bacterial indicator of CD condition. *P. harei* is regarded as a commensal in the human microbiome and also an opportunistic pathogen found in bacterial vaginosis and abdominal aortic aneurysm [99, 100]. Members of one of the most abundant genera in both fecal cultures grown on acetate and lactate, *Veillonella*, are recognized as propionate-producing human gut commensals, although they may also be associated with IBD [101].

The overabundance of *E. faecalis* in CD fecal culture grown on glucose is difficult to interpret. On the one hand, this species has been described as a probiotic strain with anti-inflammatory and protective properties, while on the other, it can cause injury to intestinal epithelial cells, induce gut inflammation and promote cancer [102, 103]. Its elevated level may reflect CD-related dysbiosis resulting from a decline in butyrate-producing bacteria and imbalances among key physiological bacterial groups. This dysregulation could also account for the observed higher prevalence of *B. bifidum* in the feces of CD patients compared to HIs [104]. Conflicting results regarding the *Bifidobacterium* species observed in this and other studies [1] highlight the need for further research on species-specific effects within this genus. It cannot be ruled out that this increase was related to the conditions under which the batch cultures were conducted. In the study by Shimokawa et al. [74], *E. faecalis* and species of the genus *Bifidobacterium* showed better growth at 30 °C and 33 °C than at 37 °C.

Another interesting observation concerned GABA produced by CD fecal cultures on glucose. GABA is a metabolic product of the gut microbiome, typically reduced in IBD, which is also a neurotransmitter crucial for the brain-gut axis [105, 106]. The increased concentration of GABA among metabolites characteristic of CD fecal cultures on glucose was unexpected and may result from the excessive growth of *B. bifidum* in cultures, as discussed above.

Potential applications

Our study is exploratory basic research that includes concept verification, in vitro fermentation cultures of the selected fecal samples, and the observation of statistically significant trends. Microbiota from healthy individuals and patients with Crohn's disease behave differently under the same standardized conditions. The fact that several well-established features characterizing CD-associated gut dysbiosis can be detected in simple assays without the need to recreate intestinal environment makes this approach novel and compelling. What may serve as a confirmation of this assumption is patient CD7, who was in endoscopic remission with normal serum CRP and fecal calprotectin and whose fecal microbiota showed the capacity to convert lactate and acetate into butyrate (Supplementary Table S3). However, further research is required, including (i) studies on larger cohorts that take into account age, sex, disease status, and treatments; (ii) the use of additional molecular biology techniques such as transcriptomics, gene abundance analysis, metabolic flux analysis, as well as the establishment of reference ranges for fermentation products; (iii) changing the cultivation conditions to more closely reflect intestinal conditions, including an incubation temperature of 37 °C, and using a richer culture medium for fecal microbiota as well as performing parallel experiments using systems such as the Simulator of the Human Intestinal Microbial Ecosystem. The advantages of such in vitro batch cultures include their short duration and the ability to run multiple cultures from the same inoculum simultaneously, with adequate replication under stable, controlled conditions. The results presented here may (i) shed new light on novel ways of using fecal microbiota samples to assess the state of the gut microbiome and (ii) serve as a future perspective for the non-invasive monitoring of gut microbiome status, with potential applications in the diagnosis and evaluation of treatment effects in CD. Studies investigating the conversion of lactate to acetate in batch cultures [45, 80, 82–85] point to the importance of the butyrogenic properties of dietary components and the therapeutic potential of nutritional interventions aimed at increasing butyrate production by the gut microbiome.

Conclusions

The dynamic and metabolic activity of fecal microbiota grown in batch cultures on glucose or on acetate with lactate enabled reliable differentiation between CD and healthy states. Cultivation on glucose-containing medium emphasizes traits associated with CD-dysbiosis, while growth on acetate and lactate medium promotes traits linked to a healthy microbiome. CD-related changes include reduced production of butyrate, valerate, caproate and their derivatives, and increased synthesis of ethanol and amino acids (leucine, isoleucine, alanine, glycine). The ability to convert lactate and acetate into butyrate characterized a HI fecal microbiota and was impaired in the microbiotas from CD patients. Variable metabolic activity of CD fecal cultures was associated with reduced richness and diversity, and the loss of key producers of C2-C6 acids such as butyrate. The species with reduced abundance showed a positive correlation with fermentation products of decreased concentration and a negative correlation with metabolites of increased concentration. Bacterial taxa typical for CD-associated dysbiosis predominated in batch cultures grown on glucose, especially *E. coli*, a species characterized by its metabolic versatility. The huge metabolic potential of *E. coli* makes it of special concern due to its significance in CD-associated dysbiosis. CD-related bacteria correlated positively with the production of ethanol and the examined amino acids. This positive correlation was confirmed through functional profiling based on the analysis of selected biochemical reactions, which indicated an increased potential for ethanol and amino acid synthesis in CD fecal cultures. The proposed approach, after further validation, may be useful for monitoring microbiome status.

Abbreviations

A	Acetate
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ASVs	Amplicon sequence variants
BA	Butyrate
BC dissimilarities	Bray Curtis dissimilarities
BMI	Body mass index
CA	Caproate
CD	Leśniowski-Crohn's disease
CRP	C Reactive Protein
EtOH	Ethanol
FMT	Fecal microbiota transplantation
GABA	γ-aminobutyrate
GFR	Glomerular filtration rate
GI	Gastrointestinal tract
HI	Healthy individual
HB	Haemoglobin
IBA	Isobutyrate
IBD	Intestinal bowel disease
IC	Isocaproate
IVA	Isovalerate
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LA	Lactatenot
MAGs	Metagenome assembled genomes
NMDS	Nonmetric multidimensional scaling
PA	Propionate
PCA	Principal component analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
SD	Standard deviation
UC	Ulcerative colitis
VA	Valerate
2MeB	2-methyl butyrate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12934-026-03052-4.

Supplementary Material 1. Table S1. Clinical characteristics of the Crohn's disease patients.

Supplementary Material 2. Table S2. Results of the preliminary experiment. Concentrations of fermentation products generated from different substrate variants by the fecal microbiota of a healthy donor in 3-day batch cultures.

Supplementary Material 3. Table S3. Concentrations of metabolites (fermentation products) in post-fermentation liquids from fecal microbiota cultures. Data used for the preparation of Fig. 2, Fig. 5 and Table 1.

Supplementary Material 4. Table S4. Detailed results of linear mixed effects model (ASV richness). Data used for the preparation of Fig. 3.

Supplementary Material 5. Table S5. Detailed results of linear mixed effects model (Shannon's diversity). Data used for the preparation of Fig. 3.

Supplementary Material 6. Table S6. Detailed taxonomic composition of the selected fecal microbiota and fecal cultures grown on glucose- and acetate with lactate-containing media, based on shotgun metagenomic sequencing data.

Supplementary Material 7. Table S7. Complete list of selected enzymes and biochemical reactions possible under the conditions applied in fecal batch cultures, prepared using HUMAnN3. Data used for the preparation of Fig. 6.

Acknowledgements
We would like to thank Dr. John Gittins for editorial assistance.

Author contributions
A.S., A.D.-I. planned the work and conceived and designed the experiments. R.F. and S.J.-C. selected the study groups, obtained stool samples, and revised the manuscript. A.D.-I., A.S., and K.J. performed the batch experiments. A.D.-I. isolated microbial DNA. A.W., N.R.H., and D.L. performed DNA sequencing. G.S. analyzed metagenomic data, including data processing and proposing the conceptual framework for data interpretation and presentation. E.S. and K.J. performed analyses of microbial metabolites. A.S., G.S., A.D.-I. wrote the manuscriptAll authors read and approved the final manuscript.

Funding
This work was supported by (i) internal IBB PAS grant no. MG-2/21 – 18; (ii) PANDA Core for Genomics and Microbiome Research, University of Arizona, and (iii) Institute of Medicine, Medical College of Rzeszow University.

Data availability
All data generated or analysed during this study are included in this published article (and its supplementary information files). The raw DNA sequences generated in this study have been deposited in NCBI databases with the following accession numbers: PRJNA1160194 for 16SrRNA and PRJNA1160072 for the metagenomic data.

Declarations

Ethics approval and consent to participate
The study was approved by the Bioethics Committee of the University of Rzeszow, Poland (No. 2022/067).

Consent for publication
Not applicable

Competing interests
Dr. Anna Sikora is an Associate Editor of the journal Microbial Cell Factories. The peer-review of this paper was therefore overseen by an independent member of the editorial board while the submission was subject to the exact same review process as applied to any other manuscript.

Author details
[1]Institute of Biochemistry and Biophysics-Polish Academy of Sciences, Pawińskiego 5a, Warsaw 02-106, Poland
[2]PANDA Core for Genomics and Microbiome Research, University of Arizona, 1501 N Campbell Ave, AHSC building, room 4354. Tucson, Arizona 85724, USA
[3]Institute of Medicine, Medical College of Rzeszow University, Kopisto 2a, Rzeszow 35-959, Poland
[4]Department of Gastroenterology with the Centre for Comprehensive Treatment of Inflammatory Bowel Diseases, Clinical Hospital, No. 2, Lwowska 60, Rzeszow 35-301, Poland
[5]Institute of Health Sciences, Medical College of Rzeszow University, Kopisto 2a, Rzeszow 35-959, Poland

Received: 28 January 2026 / Accepted: 23 June 2026
Published online: 03 August 2026

References

1. Pandey H, Jain D, Tang DWT, Wong SH, Lal D. Gut microbiota in pathophysiology, diagnosis, and therapeutics of inflammatory bowel disease. Intest Res. 2024;22:15–43. https://doi.org/10.5217/ir.2023.00080.
2. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486:207–14. https://doi.org/10.1038/nature11234.
3. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome Med. 2016;8:51. https://doi.org/10.1186/s13073-016-0307-y.
4. Kundu P, Blacher E, Elinav E, Pettersson S. Our Gut Microbiome: The Evolving Inner Self. Cell. 2017;171:1481–93. https://doi.org/10.1016/j.cell.2017.11.024.
5. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474:1823–36. https://doi.org/10.1042/BCJ20160510.
6. Beura S, Kundu P, Das AK, Ghosh A. Metagenome-scale community metabolic modelling for understanding the role of gut microbiota in human health. Comput Biol Med. 2022;149:105997. https://doi.org/10.1016/j.compbiomed.2022.105997.
7. Kuziel GA, Rakoff-Nahoum S. The gut microbiome. Curr Biol. 2022;32:R257–64. https://doi.org/10.1016/j.cub.2022.02.023.
8. Lopetuso LR, Petito V, Graziani C, Schiavoni E, Paroni Sterbini F, Poscia A, et al. Gut Microbiota in Health, Diverticular Disease, Irritable Bowel Syndrome, and Inflammatory Bowel Diseases: Time for Microbial Marker of Gastrointestinal Disorders. Dig Dis. 2018;36:56–65. https://doi.org/10.1159/000477205.
9. Lynch SV, Pedersen O. The Human Intestinal Microbiome in Health and Disease. Phimister EG, editor. N Engl J Med. 2016;375:2369–79. https://doi.org/10.1056/NEJMra1600266
10. Madhogaria B, Bhowmik P, Kundu A. Correlation between human gut microbiome and diseases. Infect Med. 2022;1:180–91. https://doi.org/10.1016/j.imj.2022.08.004.
11. Ozbey D, Saribas S, Kocazeybek B. Gut microbiota in Crohn's disease pathogenesis. World J Gastroenterol [Internet]. 2025. https://doi.org/10.3748/wjg.v31.i6.101266. [cited 2025 Mar 18];31.
12. Shaffer M, Armstrong AJS, Phelan VV, Reisdorph N, Lozupone CA. Microbiome and metabolome data integration provides insight into health and disease. Translational Res. 2017;189:51–64. https://doi.org/10.1016/j.trsl.2017.07.001.
13. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutr Rev. 2012;70:S38–44. https://doi.org/10.1111/j.1753-4887.2012.00493.x.
14. Zhang T, Li X, Li J, Sun F, Duan L. Gut microbiome-targeted therapies as adjuvant treatments in inflammatory bowel diseases: a systematic review and network meta-analysis. J Gastro Hepatol. 2025;40:78–88. https://doi.org/10.1111/jgh.16795.
15. Buie MJ, Quan J, Windsor JW, Coward S, Hansen TM, King JA, et al. Global Hospitalization Trends for Crohn's Disease and Ulcerative Colitis in the 21st Century: A Systematic Review With Temporal Analyses. Clin Gastroenterol Hepatol. 2023;21:2211–21. https://doi.org/10.1016/j.cgh.2022.06.030.
16. Bruner LP, White AM, Proksell S. Inflammatory Bowel Disease. Primary Care: Clinics in Office Practice. 2023;50:411–27. https://doi.org/10.1016/j.pop.2023.03.009
17. Le Berre C, Honap S, Peyrin-Biroulet L. Ulcerative colitis. Lancet. 2023;402:571–84. https://doi.org/10.1016/S0140-6736(23)00966-2.
18. Vich Vila A, Imhann F, Collij V, Jankipersadsing SA, Gurry T, Mujagic Z, et al. Gut microbiota composition and functional changes in inflammatory bowel

disease and irritable bowel syndrome. Sci Transl Med. 2018;10:eaap8914. https://doi.org/10.1126/scitranslmed.aap8914.
19. Borren NZ, Plichta D, Joshi AD, Bonilla G, Peng V, Colizzo FP, et al. Alterations in Fecal Microbiomes and Serum Metabolomes of Fatigued Patients With Quiescent Inflammatory Bowel Diseases. Clin Gastroenterol Hepatol. 2021;19:519–e5275. https://doi.org/10.1016/j.cgh.2020.03.013.
20. Frost F, Kacprowski T, Rühlemann M, Pietzner M, Bang C, Franke A, et al. Long-term instability of the intestinal microbiome is associated with metabolic liver disease, low microbiota diversity, diabetes mellitus and impaired exocrine pancreatic function. Gut. 2021;70:522–30. https://doi.org/10.1136/gutjnl-2020-322753.
21. Ghavami SB, Rostami E, Sephay AA, Shahrokh S, Balaii H, Aghdaei HA, et al. Alterations of the human gut Methanobrevibacter smithii as a biomarker for inflammatory bowel diseases. Microb Pathog. 2018;117:285–9. https://doi.org/10.1016/j.micpath.2018.01.029.
22. Glassner KL, Abraham BP, Quigley EMM. The microbiome and inflammatory bowel disease. J Allergy Clin Immunol. 2020;145:16–27. https://doi.org/10.1016/j.jaci.2019.11.003.
23. Hall AB, Yassour M, Sauk J, Garner A, Jiang X, Arthur T, et al. clade enriched in inflammatory bowel disease patients. Genome Med. 2017;9:103. https://doi.org/10.1186/s13073-017-0490-5.
24. Khan I, Ullah N, Zha L, Bai Y, Khan A, Zhao T, et al. Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD Treatment Targeting the Gut Microbiome. Pathogens. 2019;8:126. https://doi.org/10.3390/pathogens8030126.
25. Kriss M, Hazleton KZ, Nusbacher NM, Martin CG, Lozupone CA. Low diversity gut microbiota dysbiosis: drivers, functional implications and recovery. Curr Opin Microbiol. 2018;44:34–40. https://doi.org/10.1016/j.mib.2018.07.003.
26. Lee M, Chang EB. Inflammatory Bowel Diseases (IBD) and the Microbiome—Searching the Crime Scene for Clues. Gastroenterology. 2021;160:524–37. https://doi.org/10.1053/j.gastro.2020.09.056.
27. Mu C, Zhao Q, Zhao Q, Yang L, Pang X, Liu T, et al. Multi-omics in Crohn's disease: New insights from inside. Comput Struct Biotechnol J. 2023;21:3054–72. https://doi.org/10.1016/j.csbj.2023.05.010.
28. Stange EF, Schroeder BO. Microbiota and mucosal defense in IBD: an update. Expert Rev Gastroenterol Hepatol. 2019;13:963–76. https://doi.org/10.1080/17474124.2019.1671822.
29. Upadhyay KG, Desai DC, Ashavaid TF, Dherai AJ. Microbiome and metabolome in inflammatory bowel disease. J Gastro Hepatol. 2023;38:34–43. https://doi.org/10.1111/jgh.16043.
30. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell. 2016;165:1332–45. https://doi.org/10.1016/j.cell.2016.05.041.
31. Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, et al. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr. 2018;57:1–24. https://doi.org/10.1007/s00394-017-1445-8.
32. Saban Güler M, Arslan S, Ağagündüz D, Cerqua I, Pagano E, Berni Canani R, et al. Butyrate: A potential mediator of obesity and microbiome via different mechanisms of actions. Food Res Int. 2025;199:115420. https://doi.org/10.1016/j.foodres.2024.115420.
33. Van Der Hee B, Wells JM. Microbial Regulation of Host Physiology by Short-chain Fatty Acids. Trends Microbiol. 2021;29:700–12. https://doi.org/10.1016/j.tim.2021.02.001.
34. Deleu S, Machiels K, Raes J, Verbeke K, Vermeire S. Short chain fatty acids and its producing organisms: An overlooked therapy for IBD? eBioMedicine. 2021;66:103293. https://doi.org/10.1016/j.ebiom.2021.103293.
35. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, et al. Author Correction: Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol. 2019;4:898–898. https://doi.org/10.1038/s41564-019-0442-5.
36. Gallagher K, Catesson A, Griffin JL, Holmes E, Williams HRT. Metabolomic Analysis in Inflammatory Bowel Disease: A Systematic Review. J Crohn's Colitis. 2021;15:813–26. https://doi.org/10.1093/ecco-jcc/jjaa227.
37. Vich Vila A, Hu S, Andreu-Sánchez S, Collij V, Jansen BH, Augustijn HE, et al. Faecal metabolome and its determinants in inflammatory bowel disease. Gut. 2023;72:1472–85. https://doi.org/10.1136/gutjnl-2022-328048.
38. Saedi S, Derakhshan S, Hasani A, Khoshbaten M, Poortahmasebi V, Milani PG, et al. Recent Advances in Gut Microbiome Modulation: Effect of Probiotics, Prebiotics, Synbiotics, and Postbiotics in Inflammatory Bowel Disease Prevention and Treatment. Curr Microbiol. 2025;82:12. https://doi.org/10.1007/s00284-024-03997-y.
39. Zhang X-F, Guan X-X, Tang Y-J, Sun J-F, Wang X-K, Wang W-D, et al. Clinical effects and gut microbiota changes of using probiotics, prebiotics or synbiotics in inflammatory bowel disease: a systematic review and meta-analysis. Eur J Nutr. 2021;60:2855–75. https://doi.org/10.1007/s00394-021-02503-5.
40. Chen W, Tan D, Yang Z, Tang J, Bai W, Tian L. Fermentation patterns of prebiotics fructooligosaccharides-SCFA esters inoculated with fecal microbiota from ulcerative colitis patients. Food Chem Toxicol. 2023;180:114009. https://doi.org/10.1016/j.fct.2023.114009.
41. Xu L, Yu Q, Ma L, Su T, Zhang D, Yao D, et al. In vitro simulated fecal fermentation of mixed grains on short-chain fatty acid generation and its metabolized mechanism. Food Res Int. 2023;170:112949. https://doi.org/10.1016/j.foodres.2023.112949.
42. Qiu J, Ye B, Feng L. Improvement of intestinal microbial structure in patients with cerebral infarction through in vitro fermentation of anthocyanins from *Lycium ruthenicum* Murray. Food Sci Nutr. 2024;12:7481–91. https://doi.org/10.1002/fsn3.4263.
43. Shehata E, Day-Walsh P, Kellingray L, Narbad A, Kroon PA. Spontaneous and Microbiota-Driven Degradation of Anthocyanins in an In Vitro Human Colon Model. Mol Nutr Food Res. 2023;67:2300036. https://doi.org/10.1002/mnfr.202300036.
44. Duncan SH, Barcenilla A, Stewart CS, Pryde SE, Flint HJ. Acetate Utilization and Butyryl Coenzyme A (CoA):Acetate-CoA Transferase in Butyrate-Producing Bacteria from the Human Large Intestine. Appl Environ Microbiol. 2002;68:5186–90. https://doi.org/10.1128/AEM.68.10.5186-5190.2002.
45. Duncan SH, Louis P, Flint HJ. Lactate-Utilizing Bacteria, Isolated from Human Feces, That Produce Butyrate as a Major Fermentation Product. Appl Environ Microbiol. 2004;70:5810–7. https://doi.org/10.1128/AEM.70.10.5810-5817.2004.
46. Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, et al. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. ISME J. 2014;8:1323–35. https://doi.org/10.1038/ismej.2014.14.
47. Kircher B, Woltemate S, Gutzki F, Schlüter D, Geffers R, Bähre H, et al. Predicting butyrate- and propionate-forming bacteria of gut microbiota from sequencing data. Gut Microbes. 2022;14:2149019. https://doi.org/10.1080/19490976.2022.2149019.
48. Thulasinathan B, Suvilesh KN, Maram S, Grossmann E, Ghouri Y, Teixeiro EP, et al. The impact of gut microbial short-chain fatty acids on colorectal cancer development and prevention. Gut Microbes. 2025;17:2483780. https://doi.org/10.1080/19490976.2025.2483780.
49. Miller JH. Experiments in Molecular Genetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1972.
50. Tao Z, Yuan H, Liu M, Liu Q, Zhang S, Liu H, et al. Yeast Extract: Characteristics, Production, Applications and Future Perspectives. J Microbiol Biotechnol. 2023;33:151–66. https://doi.org/10.4014/jmb.2207.07057.
51. Detman A, Laubitz D, Chojnacka A, Kiela PR, Salamon A, Barberán A, et al. Dynamics of dark fermentation microbial communities in the light of lactate and butyrate production. Microbiome. 2021;9:158. https://doi.org/10.1186/s40168-021-01105-x.
52. Detman A, Mielecki D, Chojnacka A, Salamon A, Błaszczyk MK, Sikora A. Cell factories converting lactate and acetate to butyrate: Clostridium butyricum and microbial communities from dark fermentation bioreactors. Microb Cell Fact. 2019;18:36. https://doi.org/10.1186/s12934-019-1085-1.
53. Sheikh IA, Bianchi-Smak J, Laubitz D, Schiro G, Midura-Kiela MT, Besselsen DG, et al. Transplant of microbiota from Crohn's disease patients to germ-free mice results in colitis. Gut Microbes. 2024;16:2333483. https://doi.org/10.1080/19490976.2024.2333483.
54. Deschamps C, Fournier E, Uriot O, Lajoie F, Verdier C, Comtet-Marre S, et al. Comparative methods for fecal sample storage to preserve gut microbial structure and function in an in vitro model of the human colon. Appl Microbiol Biotechnol. 2020;104:10233–47. https://doi.org/10.1007/s00253-020-10959-4.
55. Tremaroli V, Karlsson F, Werling M, Ståhlman M, Kovatcheva-Datchary P, Olbers T, et al. Roux-en-Y Gastric Bypass and Vertical Banded Gastroplasty Induce Long-Term Changes on the Human Gut Microbiome Contributing to Fat Mass Regulation. Cell Metabol. 2015;22:228–38. https://doi.org/10.1016/j.cmet.2015.07.009.
56. Tran THT, Boudry C, Everaert N, Théwis A, Portetelle D, Daube G et al. Adding mucins to an *in vitro* batch fermentation model of the large intestine induces changes in microbial population isolated from porcine feces depending on the substrate. Nakatsu C, editor. FEMS Microbiology Ecology. 2016;92:fiv165. https://doi.org/10.1093/femsec/fiv165

57. Venema K, Van Den Abbeele P. Experimental models of the gut microbiome. Best Pract Res Clin Gastroenterol. 2013;27:115–26. https://doi.org/10.1016/j.bpg.2013.03.002.
58. Østlie HM, Helland MH, Narvhus JA. Growth and metabolism of selected strains of probiotic bacteria in milk. Int J Food Microbiol. 2003;87:17–27. https://doi.org/10.1016/S0168-1605(03)00044-8.
59. Ostrowska J, Samborowska E, Jaworski M, Toczyłowska K, Szostak-Węgierek D. The Potential Role of SCFAs in Modulating Cardiometabolic Risk by Interacting with Adiposity Parameters and Diet. Nutrients. 2024;16:266. https://doi.org/10.3390/nu16020266.
60. Pinu F, Villas-boas SG. Rapid Quantification of Major Volatile Metabolites in Fermented Food and Beverages Using Gas Chromatography-Mass Spectrometry. Metabolites. 2017;7:37. https://doi.org/10.3390/metabo7030037.
61. Sun S, Wang H, Xie J, Su Y. Simultaneous determination of rhamnose, xylitol, arabitol, fructose, glucose, inositol, sucrose, maltose in jujube (Zizyphus jujube Mill.) extract: comparison of HPLC–ELSD, LC–ESI–MS/MS and GC–MS. Chem Cent J. 2016;10:25. https://doi.org/10.1186/s13065-016-0171-2.
62. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012;6:1621–4. https://doi.org/10.1038/ismej.2012.8.
63. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl Environ Microbiol. 2007;73:5261–7. https://doi.org/10.1128/AEM.00062-07.
64. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2012;41:D590–6. https://doi.org/10.1093/nar/gks1219.
65. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20. https://doi.org/10.1093/bioinformatics/btu170.
66. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9. https://doi.org/10.1038/nmeth.1923.
67. Blanco-Míguez A, Beghini F, Cumbo F, McIver LJ, Thompson KN, Zolfo M, et al. Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. Nat Biotechnol. 2023;41:1633–44. https://doi.org/10.1038/s41587-023-01688-w.
68. Beghini F, McIver LJ, Blanco-Míguez A, Dubois L, Asnicar F, Maharjan S, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. eLife. 2021;10:e65088. https://doi.org/10.7554/eLife.65088.
69. Team R, Development Core. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. [Internet]. R Foundation for Statistical Computing; 2020. http://www.r-project.org.
70. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. vegan: Community Ecology Package. [Internet]. 2018. https://cran.r-project.org/package=vegan
71. Paulson JN, Stine OC, Bravo HC, Pop M. Differential abundance analysis for microbial marker-gene surveys. Nat Methods. 2013;10:1200–2. https://doi.org/10.1038/nmeth.2658.
72. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001;26:32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.
73. Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH et al. LP <>Coelho editor 2021 Multivariable association discovery in population-scale meta-omics studies. PLoS Comput Biol 17 e1009442 https://doi.org/10.1371/journal.pcbi.1009442.
74. Shimokawa H, Sakakibara H, Ami Y, Hirano R, Kurihara S. The effect of culturing temperature on the growth of the most dominant bacterial species of human gut microbiota and harmful bacterial species. Bioscience Microbiota Food Health. 2025;44:182–95. https://doi.org/10.12938/bmfh.2024-087.
75. Gossling J, Moore WEC. Gemmiger formicilis, n.gen., n.sp., an Anaerobic Budding Bacterium from Intestines. Int J Syst Bacteriol. 1975;25:202–7. https://doi.org/10.1099/00207713-25-2-202.
76. Choi S-H, Kim J-S, Park J-E, Lee KC, Eom MK, Oh BS, et al. Anaerotignum faecicola sp. nov., isolated from human faeces. J Microbiol. 2019;57:1073–8. https://doi.org/10.1007/s12275-019-9268-3.
77. Rautio M, Eerola E, Väisänen-Tunkelrott M-L, Molitoris D, Lawson P, Collins MD, et al. Reclassification of Bacteroides putredinis (Weinberg et al., 1937) in a New Genus Alistipes gen. nov., as Alistipes putredinis comb. nov., and Description of Alistipes finegoldii sp. nov., from Human Sources. Syst Appl Microbiol. 2003;26:182–8. https://doi.org/10.1078/072320203322346029.
78. Sakamoto M, Sakurai N, Tanno H, Iino T, Ohkuma M, Endo A. Genome-based, phenotypic and chemotaxonomic classification of Faecalibacterium strains: proposal of three novel species Faecalibacterium duncaniae sp. nov., Faecalibacterium hattorii sp. nov. and Faecalibacterium gallinarum sp. nov. Int J Syst Evolutionary Microbiol [Internet]. 2022. https://doi.org/10.1099/ijsem.0.005379. [cited 2026 Apr 7];72.
79. Söhngen C, Bunk B, Podstawka A, Gleim D, Overmann J. BacDive—the Bacterial Diversity Metadatabase. Nucl Acids Res. 2014;42:D592–9. https://doi.org/10.1093/nar/gkt1058.
80. Muñoz-Tamayo R, Laroche B, Walter É, Doré J, Duncan SH, Flint HJ, et al. Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species: Modelling butyrate production by colonic bacterial species. FEMS Microbiol Ecol. 2011;76:615–24. https://doi.org/10.1111/j.1574-6941.2011.01085.x.
81. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 2009;294:1–8. https://doi.org/10.1111/j.1574-6968.2009.01514.x.
82. Belenguer A, Duncan SH, Calder AG, Holtrop G, Louis P, Lobley GE, et al. Two Routes of Metabolic Cross-Feeding between *Bifidobacterium adolescentis* and Butyrate-Producing Anaerobes from the Human Gut. Appl Environ Microbiol. 2006;72:3593–9. https://doi.org/10.1128/AEM.72.5.3593-3599.2006.
83. Moens F, Verce M, De Vuyst L. Lactate- and acetate-based cross-feeding interactions between selected strains of lactobacilli, bifidobacteria and colon bacteria in the presence of inulin-type fructans. Int J Food Microbiol. 2017;241:225–36. https://doi.org/10.1016/j.ijfoodmicro.2016.10.019.
84. Belenguer A, Holtrop G, Duncan SH, Anderson SE, Calder AG, Flint HJ, et al. Rates of production and utilization of lactate by microbial communities from the human colon: Lactate metabolism by colonic microbiota. FEMS Microbiol Ecol. 2011;77:107–19. https://doi.org/10.1111/j.1574-6941.2011.01086.x.
85. Morrison DJ, Mackay WG, Edwards CA, Preston T, Dodson B, Weaver LT. Butyrate production from oligofructose fermentation by the human faecal flora: what is the contribution of extracellular acetate and lactate? Br J Nutr. 2006;96:570–7. https://doi.org/10.1079/BJN20061853.
86. Ning L, Zhou Y-L, Sun H, Zhang Y, Shen C, Wang Z, et al. Microbiome and metabolome features in inflammatory bowel disease via multi-omics integration analyses across cohorts. Nat Commun. 2023;14:7135. https://doi.org/10.1038/s41467-023-42788-0.
87. Pittayanon R, Lau JT, Leontiadis GI, Tse F, Yuan Y, Surette M, et al. Differences in Gut Microbiota in Patients With vs Without Inflammatory Bowel Diseases: A Systematic Review. Gastroenterology. 2020;158:930–e9461. https://doi.org/10.1053/j.gastro.2019.11.294.
88. Gonzalez CG, Mills RH, Zhu Q, Sauceda C, Knight R, Dulai PS, et al. Location-specific signatures of Crohn's disease at a multi-omics scale. Microbiome. 2022;10:133. https://doi.org/10.1186/s40168-022-01331-x.
89. Sugihara K, Kamada N. Metabolic network of the gut microbiota in inflammatory bowel disease. Inflamm Regener. 2024;44:11. https://doi.org/10.1186/s41232-024-00321-w.
90. Mirsepasi-Lauridsen HC, Vallance BA, Krogfelt KA, Petersen AM. *Escherichia coli* Pathobionts Associated with Inflammatory Bowel Disease. Clin Microbiol Rev. 2019;32:e00060–18. https://doi.org/10.1128/CMR.00060-18.
91. Pobeguts OV, Ladygina VG, Evsyutina DV, Eremeev AV, Zubov AI, Matyushkina DS, et al. Propionate Induces Virulent Properties of Crohn's Disease-Associated Escherichia coli. Front Microbiol. 2020;11:1460. https://doi.org/10.3389/fmicb.2020.01460.
92. Ormsby MJ, Logan M, Johnson SA, McIntosh A, Fallata G, Papadopoulou R, et al. Inflammation associated ethanolamine facilitates infection by Crohn's disease-linked adherent-invasive Escherichia coli. EBioMedicine. 2019;43:325–32. https://doi.org/10.1016/j.ebiom.2019.03.071.
93. Marcelino VR, Welsh C, Diener C, Gulliver EL, Rutten EL, Young RB, et al. Disease-specific loss of microbial cross-feeding interactions in the human gut. Nat Commun. 2023;14:6546. https://doi.org/10.1038/s41467-023-42112-w.
94. Di Martino L, Osme A, Ghannoum M, Cominelli F. A Novel Probiotic Combination Ameliorates Crohn's Disease–Like Ileitis by Increasing Short-Chain Fatty Acid Production and Modulating Essential Adaptive Immune Pathways. Inflamm Bowel Dis. 2023;29:1105–17. https://doi.org/10.1093/ibd/izac284.
95. Markowiak-Kopeć P, Śliżewska K. The Effect of Probiotics on the Production of Short-Chain Fatty Acids by Human Intestinal Microbiome. Nutrients. 2020;12:1107. https://doi.org/10.3390/nu12041107.
96. Jagt JZ, Verburgt CM, De Vries R, De Boer NKH, Benninga MA, De Jonge WJ, et al. Faecal Metabolomics in Paediatric Inflammatory Bowel Disease: A

Systematic Review. J Crohn's Colitis. 2022;16:1777–90. https://doi.org/10.1093/ecco-jcc/jjac079.
97. Wang Y, Xu J, Jin Z, Xia X, Zhang W. Improvement of acetyl-CoA supply and glucose utilization increases l -leucine production in *Corynebacterium glutamicum*. Biotechnol J. 2022;17:2100349. https://doi.org/10.1002/biot.202100349.
98. Yamamoto S, Gunji W, Suzuki H, Toda H, Suda M, Jojima T, et al. Overexpression of Genes Encoding Glycolytic Enzymes in Corynebacterium glutamicum Enhances Glucose Metabolism and Alanine Production under Oxygen Deprivation Conditions. Appl Environ Microbiol. 2012;78:4447–57. https://doi.org/10.1128/AEM.07998-11.
99. Wan X, Wang S, Wang M, Liu J, Zhang Y. Identification of Peptoniphilus harei From Blood Cultures in an Infected Aortic Aneurysm Patient: Case Report and Review Published Literature. Front Cell Infect Microbiol. 2021;11:755225. https://doi.org/10.3389/fcimb.2021.755225.
100. Diop K, Diop A, Michelle C, Richez M, Rathored J, Bretelle F, et al. Description of three new *Peptoniphilus* species cultured in the vaginal fluid of a woman diagnosed with bacterial vaginosis: *Peptoniphilus pacaensis* sp. nov., *Peptoniphilus raoultii* sp. nov., and *Peptoniphilus vaginalis* sp. nov. MicrobiologyOpen. 2019;8:e00661. https://doi.org/10.1002/mbo3.661.
101. Akhtar M, Chen Y, Ma Z, Zhang X, Shi D, Khan JA, et al. Gut microbiota-derived short chain fatty acids are potential mediators in gut inflammation. Anim Nutr. 2022;8:350–60. https://doi.org/10.1016/j.aninu.2021.11.005.
102. Quaglio AEV, Grillo TG, Oliveira ECSD, Stasi LCD, Sassaki LY. Gut microbiota, inflammatory bowel disease and colorectal cancer. WJG. 2022;28:4053–60. https://doi.org/10.3748/wjg.v28.i30.4053.
103. De Almeida CV, Taddei A, Amedei A. The controversial role of *Enterococcus faecalis* in colorectal cancer. Th Adv Gastroenterol. 2018;11:1756284818783606. https://doi.org/10.1177/1756284818783606.
104. Wang W, Chen L, Zhou R, Wang X, Song L, Huang S et al. Increased Proportions of Bifidobacterium and the Lactobacillus Group and Loss of Butyrate-Producing Bacteria in Inflammatory Bowel Disease. Forbes BA, editor. J Clin Microbiol. 2014;52:398–406. https://doi.org/10.1128/JCM.01500-13
105. Strandwitz P, Kim KH, Terekhova D, Liu JK, Sharma A, Levering J, et al. GABA-modulating bacteria of the human gut microbiota. Nat Microbiol. 2018;4:396–403. https://doi.org/10.1038/s41564-018-0307-3.
106. Wang J, Liu G, Xu K, Ai K, Huang W, Zhang J. The role of neurotransmitters in mediating the relationship between brain alterations and depressive symptoms in patients with inflammatory bowel disease. Hum Brain Mapp. 2023;44:5357–71. https://doi.org/10.1002/hbm.26439.
107. Jin S, Chen P, Yang J, Li D, Liu X, Zhang Y, et al. *Phocaeicola vulgatus* alleviates diet-induced metabolic dysfunction-associated steatotic liver disease progression by downregulating histone acetylation level via 3-HPAA. Gut Microbes. 2024;16:2309683. https://doi.org/10.1080/19490976.2024.2309683.
108. Clausen U, Vital S-T, Lambertus P, Gehler M, Scheve S, Wöhlbrand L, et al. Catabolic Network of the Fermentative Gut Bacterium *Phocaeicola vulgatus* (Phylum Bacteroidota) from a Physiologic-Proteomic Perspective. Microb Physiol. 2024;34:88–107. https://doi.org/10.1159/000536327.
109. Sheridan PO, Louis P, Tsompanidou E, Shaw S, Harmsen HJ, Duncan SH, et al. Distribution, organization and expression of genes concerned with anaerobic lactate utilization in human intestinal bacteria. Microb Genomics [Internet]. 2022. https://doi.org/10.1099/mgen.0.000739. [cited 2024 Oct 30];8.
110. Takahashi K, Nishida A, Fujimoto T, Fujii M, Shioya M, Imaeda H, et al. Reduced Abundance of Butyrate-Producing Bacteria Species in the Fecal Microbial Community in Crohn's Disease. Digestion. 2016;93:59–65. https://doi.org/10.1159/000441768.
111. Pisani A, Rausch P, Bang C, Ellul S, Tabone T, Marantidis Cordina C et al. JM <>Auchtung editor 2022 Dysbiosis in the Gut Microbiota in Patients with Inflammatory Bowel Disease during Remission. Microbiol Spectr 10 e00616–22 https://doi.org/10.1128/spectrum.00616-22.
112. Parker BJ, Wearsch PA, Veloo ACM, Rodriguez-Palacios A. The Genus Alistipes: Gut Bacteria With Emerging Implications to Inflammation, Cancer, and Mental Health. Front Immunol. 2020;11:906. https://doi.org/10.3389/fimmu.2020.00906.
113. Ishikawa D, Zhang X, Nomura K, Shibuya T, Hojo M, Yamashita M, et al. Anti-inflammatory Effects of Bacteroidota Strains Derived From Outstanding Donors of Fecal Microbiota Transplantation for the Treatment of Ulcerative Colitis. Inflamm Bowel Dis. 2024;izae080. https://doi.org/10.1093/ibd/izae080.
114. Javid H, Oryani MA, Akbari S, Amiriani T, Ravanbakhsh S, Rezagholinejad N, et al. *L. Plantarum* and *L. Lactis* as a Promising Agent in Treatment of Inflammatory Bowel Disease and Colorectal Cancer. Future Microbiol. 2023;18:1197–209. https://doi.org/10.2217/fmb-2023-0076.
115. He T, Cheng X, Xing C. The gut microbial diversity of colon cancer patients and the clinical significance. Bioengineered. 2021;12:7046–60. https://doi.org/10.1080/21655979.2021.1972077.
116. Takeshita K, Mizuno S, Mikami Y, Sujino T, Saigusa K, Matsuoka K, et al. A Single Species of Clostridium Subcluster XIVa Decreased in Ulcerative Colitis Patients. Inflamm Bowel Dis. 2016;22:2802–10. https://doi.org/10.1097/MIB.0000000000000972.
117. Mukherjee A, Lordan C, Ross RP, Cotter PD. Gut microbes from the phylogenetically diverse genus *Eubacterium* and their various contributions to gut health. Gut Microbes. 2020;12:1802866. https://doi.org/10.1080/19490976.2020.1802866.
118. Rosero JA, Killer J, Sechovcová H, Mrázek J, Benada O, Fliegerová K et al. Reclassification of Eubacterium rectaleHauduroy (1937) Prévot 1938 in a new genus Agathobacter gen. nov. as Agathobacter rectalis comb. nov., and description of Agathobacter ruminis sp. nov., isolated from the rumen contents of sheep and cows. International Journal of Systematic and Evolutionary Microbiology. 2016;66:768–73. https://doi.org/10.1099/ijsem.0.000788
119. Taras D, Simmering R, Collins MD, Lawson PA, Blaut M. Reclassification of Eubacterium formicigenerans Holdeman and Moore 1974 as Dorea formicigenerans gen. nov., comb. nov., and description of Dorea longicatena sp. nov., isolated from human faeces. Int J Syst Evol MicroBiol. 2002;52:423–8. https://doi.org/10.1099/00207713-52-2-423.
120. Nishida K, Sawada D, Kawai T, Kuwano Y, Fujiwara S, Rokutan K. Para-psychobiotic *Lactobacillus gasseri* CP 2305 ameliorates stress-related symptoms and sleep quality. J Appl Microbiol. 2017;123:1561–70. https://doi.org/10.1111/jam.13594.
121. Mondot S, Lepage P, Seksik P, Allez M, Tréton X, Bouhnik Y, et al. Structural robustness of the gut mucosal microbiota is associated with Crohn's disease remission after surgery. Gut. 2016;65:954–62. https://doi.org/10.1136/gutjnl-2015-309184.
122. Zhang X, Yu D, Wu D, Gao X, Shao F, Zhao M, et al. Tissue-resident Lachnospiraceae family bacteria protect against colorectal carcinogenesis by promoting tumor immune surveillance. Cell Host Microbe. 2023;31:418–e4328. https://doi.org/10.1016/j.chom.2023.01.013.
123. Liu X, Mao B, Gu J, Wu J, Cui S, Wang G, et al. *Blautia* —a new functional genus with potential probiotic properties? Gut Microbes. 2021;13:1875796. https://doi.org/10.1080/19490976.2021.1875796.
124. Quévrain E, Maubert MA, Michon C, Chain F, Marquant R, Tailhades J, et al. Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. Gut. 2016;65:415–25. https://doi.org/10.1136/gutjnl-2014-307649.
125. Leylabadlo HE, Ghotaslou R, Feizabadi MM, Farajnia S, Moaddab SY, Ganbarov K, et al. The critical role of Faecalibacterium prausnitzii in human health: An overview. Microb Pathog. 2020;149:104344. https://doi.org/10.1016/j.micpath.2020.104344.
126. Lenoir M, Martín R, Torres-Maravilla E, Chadi S, González-Dávila P, Sokol H, et al. Butyrate mediates anti-inflammatory effects of *Faecalibacterium prausnitzii* in intestinal epithelial cells through *Dact3*. Gut Microbes. 2020;12:1826748. https://doi.org/10.1080/19490976.2020.1826748.
127. Zhang Z, Mocanu V, Deehan EC, Hotte N, Zhu Y, Wei S, et al. Recipient microbiome-related features predicting metabolic improvement following fecal microbiota transplantation in adults with severe obesity and metabolic syndrome: a secondary analysis of a phase 2 clinical trial. Gut Microbes. 2024;16:2345134. https://doi.org/10.1080/19490976.2024.2345134.
128. Seo B, Jeon K, Baek I, Lee YM, Baek K, Ko G. Faecalibacillus intestinalis gen. nov., sp. nov. and Faecalibacillus faecis sp. nov., isolated from human faeces. Int J Syst Evol MicroBiol. 2019;69:2120–8. https://doi.org/10.1099/ijsem.0.003443.
129. Endo A, Tanno H, Kadowaki R, Fujii T, Tochio T. Extracellular fructooligosaccharide degradation in Anaerostipes hadrus for co-metabolism with non-fructooligosaccharide utilizers. Biochem Biophys Res Commun. 2022;613:81–6. https://doi.org/10.1016/j.bbrc.2022.04.134.
130. Leser T, Baker A. Bifidobacterium adolescentis – a beneficial microbe. Benef Microbes. 2023;14:525–51. https://doi.org/10.1163/18762891-20230030.
131. Kowalska-Duplaga K, Gosiewski T, Kapusta P, Sroka-Oleksiak A, Wędrychowicz A, Pieczarkowski S, et al. Differences in the intestinal microbiome of healthy children and patients with newly diagnosed Crohn's disease. Sci Rep. 2019;9:18880. https://doi.org/10.1038/s41598-019-55290-9.
132. Duranti S, Ruiz L, Lugli GA, Tames H, Milani C, Mancabelli L, et al. Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of GABA. Sci Rep. 2020;10:14112. https://doi.org/10.1038/s41598-020-70986-z.

133. Tamanai-Shacoori Z, Smida I, Bousarghin L, Loreal O, Meuric V, Fong SB, et al. Roseburia Spp : Marker Health? Future Microbiol. 2017;12:157–70. https://doi.org/10.2217/fmb-2016-0130.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.