

Identifying glycan consumers in human gut microbiota samples using metabolic labeling coupled with fluorescence-activated cell sorting

Received: 14 October 2022

Accepted: 26 January 2023

Published online: 07 February 2023



Lharbi Dridi¹, Fernando Altamura¹, Emmanuel Gonzalez^{2,3,4}, Olivia Lui¹, Ryszard Kubinski¹, Reilly Pidgeon¹, Adrian Montagut¹, Jasmine Chong⁵, Jianguo Xia⁵, Corinne F. Maurice^{6,7}✉ & Bastien Castagner¹✉

The composition and metabolism of the human gut microbiota are strongly influenced by dietary complex glycans, which cause downstream effects on the physiology and health of hosts. Despite recent advances in our understanding of glycan metabolism by human gut bacteria, we still need methods to link glycans to their consuming bacteria. Here, we use a functional assay to identify and isolate gut bacteria from healthy human volunteers that take up different glycans. The method combines metabolic labeling using fluorescent oligo-saccharides with fluorescence-activated cell sorting (FACS), followed by amplicon sequencing or culturomics. Our results demonstrate metabolic labeling in various taxa, such as *Prevotella copri*, *Collinsella aerofaciens* and *Blautia wexlerae*. In vitro validation confirms the ability of most, but not all, labeled species to consume the glycan of interest for growth. In parallel, we show that glycan consumers spanning three major phyla can be isolated from cultures of sorted labeled cells. By linking bacteria to the glycans they consume, this approach increases our basic understanding of glycan metabolism by gut bacteria. Going forward, it could be used to provide insight into the mechanism of prebiotic approaches, where glycans are used to manipulate the gut microbiota composition.

The gut microbiota is integral to human physiology, as it metabolizes our diet, synthesizes essential vitamins and amino acids, trains the immune system, and protects us from pathogens^{1–5}. Advances in genetic and bioinformatic tools have led to a new understanding of the complexity and diversity of the gut microbiota, as well as its

importance in human diseases⁶. The gut microbiome is enriched in genes involved in glycolysis and carbohydrate metabolism^{2,7}. Through these carbohydrate-active enzymes (CAZymes), gut bacteria metabolize diet-derived complex glycans (dietary fibers) that reach the colon undigested by the host^{8,9}. Consequently, diet is a major determinant of

¹Department of Pharmacology & Therapeutics, McGill University, 3655 Prom. Sir-William-Osler, Montreal, Quebec H3G 1Y6, Canada. ²Canadian Centre for Computational Genomics, McGill Genome Center, 740, Dr. Penfield Avenue, Montreal, Quebec H3A 0G1, Canada. ³Department of Human Genetics, McGill University, 3640 University, Montreal, Quebec H3A 0C7, Canada. ⁴Gerald Bronfman Department of Oncology, McGill University, 5100 Boulevard de Maisonneuve West, Montreal, Quebec H4A 3T2, Canada. ⁵Institute of Parasitology, McGill University, 2111 Lakeshore Rd, Ste-Anne-de-Bellevue, Quebec H9X 3V9, Canada. ⁶McGill Centre for Microbiome Research, McGill University, Montreal, Quebec, Canada. ⁷Department of Microbiology & Immunology, McGill University, 3773 University Street, Montreal, Quebec H3A 2B4, Canada. ✉e-mail: corinne.maurice@mcgill.ca; bastien.castagner@mcgill.ca

the composition and diversity of gut microbiota, more so than genetic factors¹⁰. Indeed, changes in diet typically result in rapid microbial metabolic modifications and an altered microbial community structure^{7,11}. Importantly, even subtle structural differences in glycans can yield distinct microbial metabolic outputs in human supplementation studies¹².

Our understanding of CAZymes is rapidly advancing, as exemplified by the characterization of polysaccharide utilization loci (PULs) in Bacteroidetes, such as the starch utilization system (SUS) extensively studied in *Bacteroides thetaiotaomicron*¹³. The *Sus* locus encodes proteins responsible for the binding (SusDEF) and cell surface degradation (SusG) of starch polysaccharides into oligosaccharides that are transported into the periplasm by the TonB-dependent transporter SusC, where they are further processed into monosaccharides by the glycoside hydrolases (GHs) SusAB¹⁴. Similarly, specific PULs have been described for many other glycans, such as mannan, β -glucan, xyloglucan, and galactomannan^{15–19}. On the other hand, Gram-positive bacteria contain transporters that can internalize oligosaccharide structures such as fructooligosaccharides (FOS) by the four-component MsmEFGK in *Lactobacillus acidophilus*²⁰, or β -mannans by a solute binding protein (MnBP) and two permeases (MPP) in *Roseburia intestinalis*²¹. Despite these advances, many PULs still have unknown substrate specificities, and GH families can have multiple substrates, making it difficult to predict activity based on sequencing^{9,13,22–24}. Thus, functional methods that link glycans with their primary consumers are necessary, especially for Firmicutes and other less studied members of the human gut microbiota²⁵.

Several methods have been developed to address this need, with varying levels of complexity, and have been summarized in excellent reviews focusing either on membrane metabolic labeling²⁶ or metabolic oligosaccharide engineering^{27,28}. As one of the first techniques developed, stable isotope probing (SIP) is a laborious but powerful method to follow metabolism and identify consumers that was applied to starch and galacto-oligosaccharide metabolism in fermentation systems^{29–31}. However, SIP generally requires bacterial growth for labeling, thereby inducing bacterial media biases. Recently, Patnode and colleagues used forward genetic screening, quantitative metabolomics, and artificial food particles to interrogate fiber degradation in mice colonized with synthetic communities of gut bacteria³². This approach revealed the contribution of Bacteroides species to the processing of specific glycans and highlighted interspecies competition. More recently, the same researchers used glycan-covered beads to examine the adhesion of bacteria to polysaccharides and revealed strain-level differences in specificity³³. Notably, they used magnetic beads to recover and identify adherent bacteria from a bacterial coculture and hinted at the possibility of using this method to mine previously uncharacterized consumers³³. However, it is important to note that these approaches remain to be validated in natural more complex communities.

Next-generation physiology approaches that combine non-destructive cellular phenotyping with separation of cells provide a powerful framework to study microbial physiology and metabolism²⁷. Fluorescence-activated cell sorting (FACS) coupled with sequencing (FACSeq) is particularly efficient and can reveal the physiology of individual cells within complex bacterial communities, allowing for the identification of bacteria labeled according to their relative nucleic acid content, cell membrane integrity, and metabolism without prior culturing^{34–37}. The FACSeq method can be applied in various contexts such as identifying gut microbiota taxa associated with immunoglobulin A or cholesterol^{38,39}. Fluorescent glycans were recently used to demonstrate the uptake of α -mannan oligosaccharides in the periplasm of *B. thetaiotaomicron* by epifluorescence microscopy and FACS⁴⁰, while another study revealed the role of uncultured *Verrucomicrobia* in degrading polysaccharides in aquatic systems using fluorescently labeled glycans, FACS, and single-cell genomics⁴¹. More recently, Doud

and colleagues used fluorescently-labeled cellulose particles, FACS, and amplicon sequencing to uncover cellulose-degrading bacteria in geothermal springs⁴². Collectively, these studies highlight the potential of combining culture-independent approaches to link bacterial diversity to specific metabolism.

Here, we utilized metabolic labeling coupled to FACS and 16S rDNA amplicon sequencing to reveal the bacterial uptake of three structurally different oligosaccharides in stools from three healthy unrelated volunteers. Sorted glycan⁺ cells were enriched in glycan foragers from the Bacteroidetes phylum, but also Firmicutes and Actinobacteria, such as *Blautia wexlerae* and *Collinsella aerofaciens*, respectively. A majority of significantly labeled bacteria were confirmed to be glycan consumers for growth and encoded GHs with activity consistent with the glycans used, yet we were able to identify *B. wexlerae* as a previously unreported FOS consumer. However, three *Bacteroidetes* species labeled by a fluorescent glycan did not show a growth phenotype in vitro with this glycan. In parallel, we isolated bacterial glycan consumers that belonged to three different phyla in a human microbiota sample by culturing glycan⁺ cells. While this method does not label bacteria implicated in cross-feeding, it is a useful method to identify consumers of oligosaccharides.

Results

Fluorescent glycan uptake in cultured isolates

To investigate the uptake of fluorescent glycans in gut bacteria, we first synthesized a fluorescein conjugate of β -cyclodextrin, which mimics the helical structure of starch, one of the most common polysaccharides in our diet, and has been used for the structural characterization of *B. thetaiotaomicron* SUS⁴³. The monofunctionalized conjugate (CD-F (1), Fig. 1a) was purified by preparative HPLC and characterized by mass spectrometry (MS). The specific uptake of CD-F was first investigated in *Klebsiella oxytoca*, a gut bacterium from the Proteobacteria phylum that harbors the CymA transporter specific for cyclodextrin⁴⁴. *K. oxytoca* strain MSA1 was grown in minimum medium M9 (MM M9) supplemented with 0.1% β -cyclodextrin. Bacteria in the log phase that were incubated with CD-F showed a significant fluorescence increase compared to that of the negative control (Fig. 1d), which was corroborated by fluorescence microscopy (Fig. 1e) and flow cytometry (Fig. S1a).

We then prepared fluorescently labeled nystose (NYST-F (2), Fig. 1b), a tetrasaccharide derived from inulin, which is a common fructan prebiotic found in wheat, onion, and banana (Fig. 1b)⁴⁵. An unselective functionalization of the nystose hydroxyl groups was performed to obtain a mixture of mono-functionalized nystoses that were conjugated at different positions. This is important since we do not know the putative transporter recognition motif, and a mixture of conjugates is less likely to interfere with binding. Thus, purification of fluorescent nystose (NYST-F) yielded a mixture of products with identical molecular weights that corresponded to the mono-functionalized glycan by mass-spectrometry-coupled liquid chromatography (LC-MS). Cultures of the known consumer *L. acidophilus* (strain ATCC 4356) were grown in custom De Man, Rogosa, Sharpe (cMRS) broth depleted of glucose and supplemented with inulin, and these cultures were also labeled with NYST-F by fluorescence spectroscopy and microscopy (Fig. 1d, e)²⁰.

Metabolic labeling in the human gut microbiota

After demonstrating the uptake of fluorescent glycans in cultured isolates, we then metabolically labeled bacteria from a stool sample from a healthy human volunteer. Fresh stool samples were placed in anaerobic conditions within 30 min of collection, and aliquots were frozen at -80°C to be tested further with multiple different glycans. Under these conditions, most bacteria were shown to remain viable⁴⁶. Bacteria were incubated with CD-F for 1 h under anaerobic conditions. Fluorescence measurement of washed cells showed increased fluorescence after incubation with 4.4 μM CD-F for 1 h (Fig. 1f). Importantly,

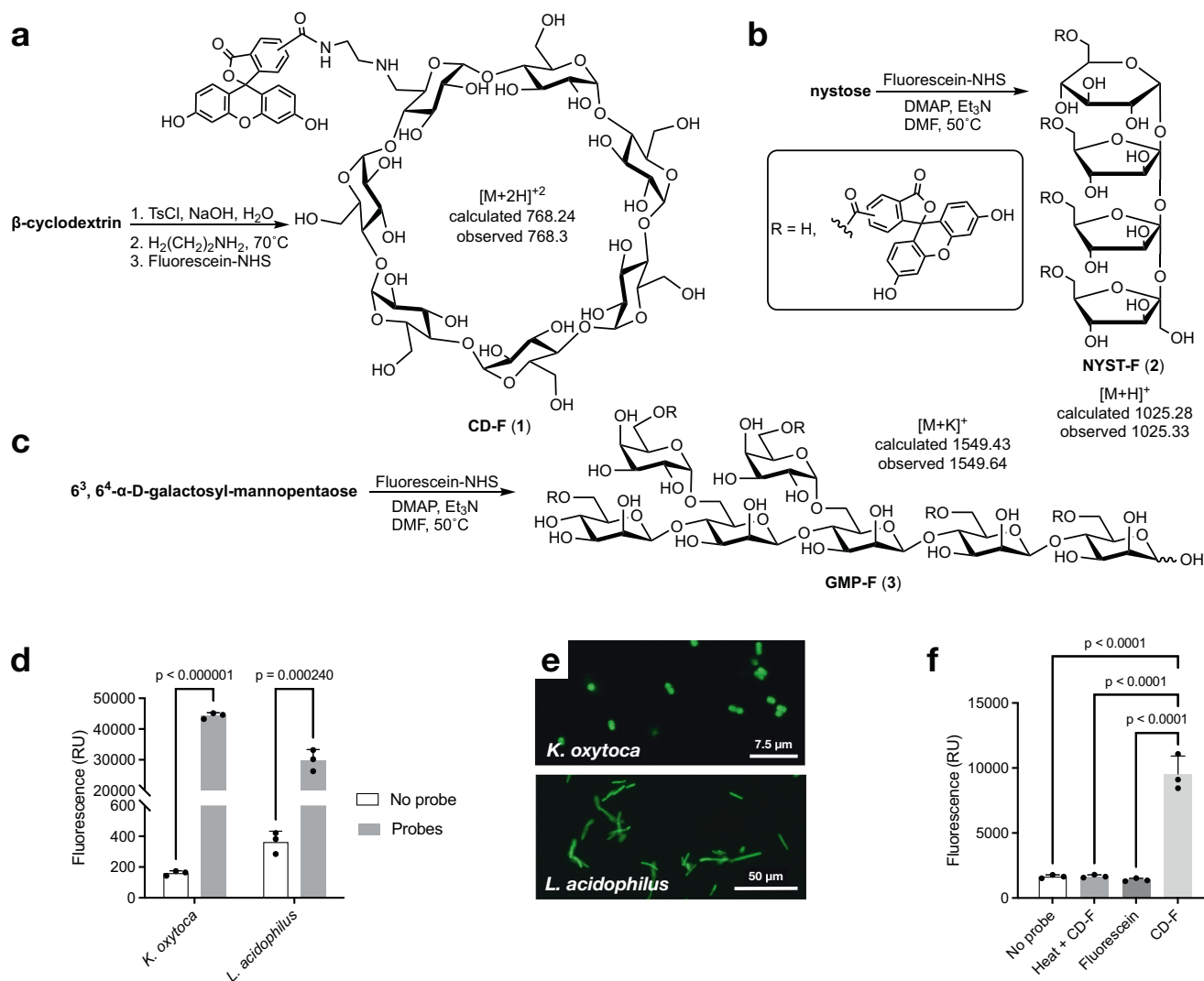


Fig. 1 | Cultured isolates are metabolically labeled by fluorescent oligosaccharides. a–c Synthesis of fluorescent β -cyclodextrin (**a**), nystose (**b**), and galactosyl-mannopentaose (**c**) probes. Mono-functionalized glycans were purified by reverse-phase chromatography, size-exclusion chromatography, or high-performance liquid chromatography (HPLC). The products were characterized by mass spectrometry. **d** Fluorescence measured from washed *K. oxytoca* (Proteobacteria) and *L. acidophilus* (Firmicutes) cultures after incubation in their respective minimum media with CD-F or NYST-F probe for 30 min, as measured on a spectrofluorometer. Data are the mean \pm SD ($n = 3$). Statistical significance compared to the control condition by unpaired t-tests with Bonferroni–Dunn’s multiple

comparison test. **e** Confocal microscopy image of fluorescently labeled *K. oxytoca* and 2-photon microscopy image of *L. acidophilus* after incubation with CD-F and NYST-F. Bacteria in the exponential phase were washed and incubated for 1 h with the probes (500 nM) and then fixed with 4% paraformaldehyde. **f** Fluorescence quantification of bacteria isolated from stool samples after incubation with free fluorescein (4.4 μM) or CD-F (4.4 μM) for 1 h with or without heat shock pretreatment of the gut microbiota for 10 min at 65 $^\circ\text{C}$. Data are the mean \pm SD ($n = 3$). Statistical significance compared to the labeled condition by one-way ANOVA with Bonferroni’s multiple comparison test. Source data and statistical details are provided as a Source data file.

we observed no increase in fluorescence using free fluorescein and when the bacteria were heat-shocked prior to the incubation, suggesting that the fluorescent glycan underwent active transport. Higher concentrations of the fluorescent probe or prolonging the incubation time beyond 1 h did not result in a significant increase in fluorescence (Fig. S1b, c). We then repeated these experiments using flow cytometry to quantify the cell populations that took up the fluorescent glycan. Unlabeled bacteria were used as a negative control to set the threshold of the fluorescent bacterial population. The labeling of a gut microbiota sample with CD-F indicated that ca. 1% of cells were glycan⁺, while no labeling was detected with free fluorescein or after heat inactivation (Fig. 2a).

To demonstrate the specificity of CD-F labeling, we performed a competition experiment with an increasing concentration of unlabeled β -cyclodextrin (CD). Flow cytometry analysis revealed a gradual reduction in labeling that was significant above 436 μM CD,

corresponding to a 100-fold excess over CD-F (Fig. 2b). We then sought to verify whether the labeling was due to the uptake of oligosaccharides or if the glycan was hydrolyzed by cell-surface or secreted glycoside hydrolases prior to uptake. We thus synthesized the monosaccharide (glucose-F, **4**) and disaccharide (maltose-F, **5**) equivalent of the CD-F probe (Fig. S2a). Flow cytometry analysis of metabolic labeling showed very little or no labeling with maltose-F and glucose-F compared to CD-F, thus confirming that only larger oligosaccharides with fluorescent labels were taken up (Fig. S2b).

Furthermore, we confirmed that the labeling observed was largely an active, energy-dependent process by performing labeling at 0 $^\circ\text{C}$, which suppressed labeling for both CD-F and NYST-F (Fig. 2c, d). The utilization of complex polysaccharides is also often repressed by the presence of simple monosaccharides, such as glucose⁴⁷. We therefore performed the labeling experiments in minimum media containing 0.1% glucose. Under these catabolic repression conditions,

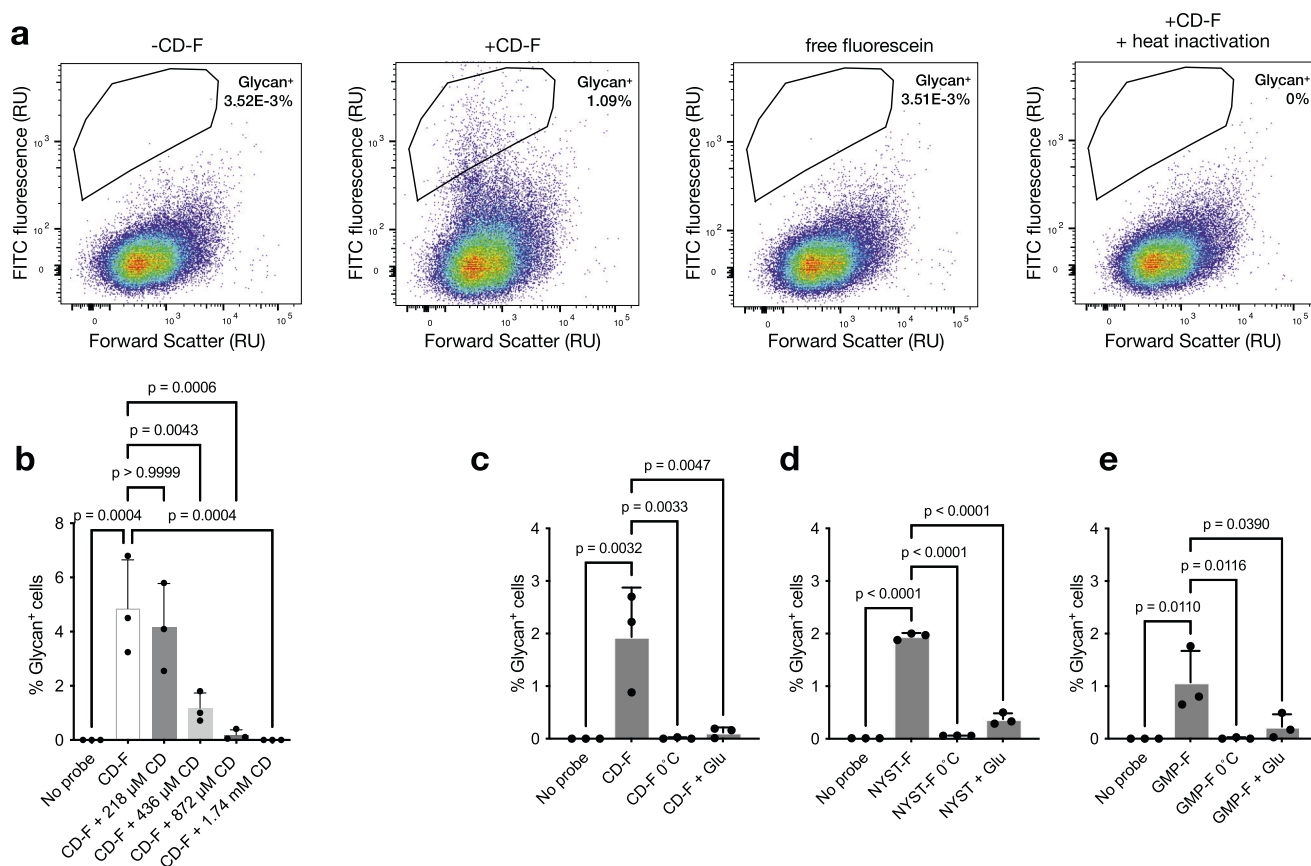


Fig. 2 | Microbiota metabolic labeling is a specific and energy-dependent process. **a** Representative flow cytometric pseudocolor plots of metabolic labeling. Bacteria isolated from stool were incubated with 4.4 μM CD-F or fluorescein with or without heat inactivation for 1 h, washed, and analyzed by flow cytometry. **b** Quantification of glycan⁺ cells in stool samples incubated with 4.4 μM CD-F probe in the presence of increasing concentrations of free β-cyclodextrin. Bar graph representing means ± SDs (*n* = 3). Statistical significance compared to the CD-F condition by one-way ANOVA with Bonferroni's multiple comparison test.

Specificity of CD-F (**c**), NYST-F (**d**), and GMP-F (**e**) labeling. Bacteria isolated from the stool samples were preincubated in MM at 0 °C or in MM supplemented with 0.1% glucose at 37 °C for 1 h and then labeled with CDF, NYST-F, and GMP-F probes at 4.4 μM for 1 h at 37 °C. After washing, the percentage of glycan⁺ bacteria was measured by flow cytometry. The graphs represent the means ± SDs (*n* = 3) of the percentage of labeled bacteria under different conditions. Statistical significance compared to the CD-F condition by one-way ANOVA with Bonferroni's multiple comparison test. Source data and statistical details are provided as a Source data file.

the uptake of our probes was indeed repressed, suggesting a link between probe uptake and metabolism (Fig. 2c, d).

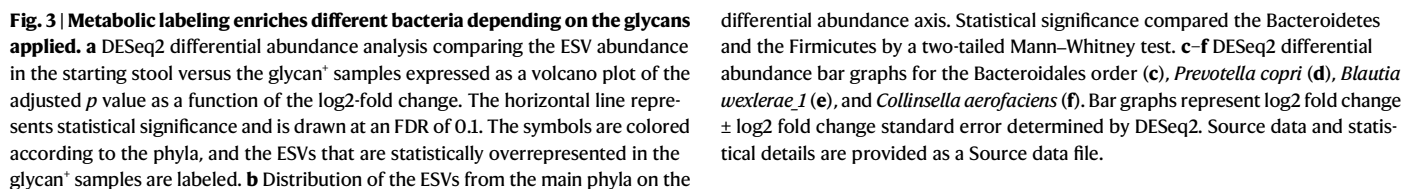
Encouraged by these results, we synthesized a third, structurally different fluorescent oligosaccharide by randomly functionalizing 6³,6⁴-α-D-galactosyl-β(1,4)-D-mannopentaose to obtain GMP-F (**3**, Fig. 1c). Galactomannans are hemicellulose glycans found in our diet (e.g., coffee beans and tomatoes), notably in the form of thickening agents, such as guar gum²¹. Metabolic labeling of bacterial cells by GMP-F was confirmed by flow cytometry and was contrasted by a lack of probe uptake at 0 °C and under catabolic repression conditions (Fig. 2e). Taken together, these results suggest that fluorescent oligosaccharides are taken up by gut microbiota members in a specific and energy-dependent fashion, and largely linked to bacterial metabolism.

Revealing glycan uptake in gut microbiota samples

We then coupled metabolic labeling and FACS with 16S rDNA amplicon sequencing to identify bacteria responsible for glycan uptake. We used our three fluorescent glycans on fecal samples from three unrelated healthy individuals in the same way. Bacteria from stool aliquots were incubated for 1 h with a fluorescent probe in minimum media, washed, and then processed for flow cytometry. Unlabeled control samples were used to set the gating threshold for glycan⁺ cell sorting. Glycan⁺ sorted cells were then lysed before DNA extraction and amplification of the V4 region of the bacterial 16S rRNA gene. Illumina MiSeq sequencing was performed on DNA extracted from 1–3 million sorted

cells and on DNA extracted from the initial three stool samples for comparison. Sequence reads were processed and annotated using the ANCHOR pipeline⁴⁸. Briefly, only paired-end sequences in which the primer set was precisely detected were selected, aligned, and dereplicated before selection of exact sequence variants (ESVs). Annotation queried 4 sequence repositories with strict BLASTn criteria (>99% identity and coverage). Note that all annotations are considered putative and subject to improvement as database errors are resolved and new species are characterized.

We obtained an average of 68,122 read counts per sample, ranging from 29,360 to 85,124. The ANCHOR pipeline identified a total of 334 distinct ESVs. Of these, 93 ESVs could be unambiguously assigned to a species with 100% coverage and >99% identity, and 100% identity was obtained for 86 out of the 93 ESVs. The sorted glycan⁺ samples exhibited lower bacterial α-diversity indices (observed ESVs, Chao1, and Shannon) than those of the starting stool samples, which is consistent with the labeled cells representing a subset of the initial gut microbiota samples (Fig. S3a). A principal component analysis (PCoA) showed a clustering of samples according to the individual (Fig. S3b), indicating that interpersonal differences in microbial communities explained most of the variance (45%). However, constrained ordination (CAP) analysis was performed using the labeled cells versus the initial stool samples as an a priori hypothesis and produced two distinct clusters on an ordination axis; this result explained 9% of the variance (Fig. S3c) and once again supported the enrichment of specific



We then examined *C. aerofaciens*, which was reported to exhibit a weak growth phenotype on levan but not FOS⁵⁵. In the CAZy database, three GH32s are annotated in the genome of *C. aerofaciens* ATCC 25986 (100% identity and coverage to our ESV). The first PUL comprises two GH32 genes flanking a phosphoenolpyruvate:sugar phosphotransferase system (PTS) subunit IIC gene. The second locus contains a GH32 and a PTS subunit IIB gene. These two loci are homologous to known PULs

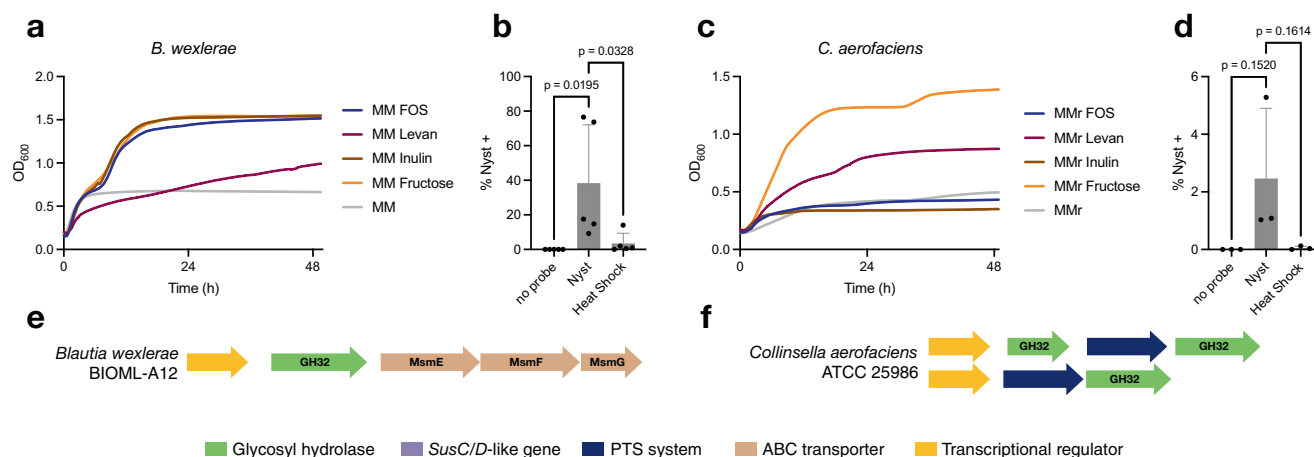


Fig. 4 | *Blautia wexlerae* and *Collinsella aerofaciens* are FOS and levan consumers, respectively. Growth of *B. wexlerae* (**a**) and *C. aerofaciens* (**c**) in MM supplemented with different fructooligosaccharides for 48 h. The viability of bacteria was evaluated by growth in MM supplemented with fructose. All growth measurements are the means of triplicates. Exponential cultures of *B. wexlerae* ($n = 5$) (**b**) and *C. aerofaciens* ($n = 3$) (**d**) in MM supplemented with FOS were incubated for 1 h with NYST-F. The percentage of NYST⁺ bacteria was measured by flow cytometry. Data from three to five independent experiments are shown as the

means \pm SD. Statistical significance compared to the GMP-F condition by one-way ANOVA with Bonferroni's multiple comparison test. Map of potential FOS metabolism-associated genes. Identification of putative genes involved in the metabolism of FOS in *B. wexlerae* (**e**) and *C. aerofaciens* (**f**). BLAST alignments were performed on the complete genome available in the NCBI database with the known glycoside hydrolases that are involved in GMP and FOS metabolism. Source data and statistical details are provided as a Source data file.

involved in FOS metabolism in the closely related *Anaerostipes hadrus*, suggesting that they are involved in fructose polymer metabolism (Figs. 4f, S4b)⁵⁶. We obtained *C. aerofaciens* ATCC 25986 and confirmed that it could grow slowly in minimum media containing yeast and beef extract supplemented with levan, but not FOS or inulin (Fig. 4c). Nevertheless, *C. aerofaciens* isolated cultures were labeled by NYST-F (Fig. 4d). This result shows that while *C. aerofaciens* consumes oligo-fructose, it is specific for the β -2,6 linkages found in levan, as opposed to the β -2,1 linkage in FOS and inulin.

B. vulgatus and *B. caccae* were also significantly labeled by GMP-F (Fig. 3a) despite a lack of GH26 in the genomes of sequenced isolates in the CAZy database²⁴. In addition, *B. caccae* is known to not grow on this substrate⁵⁵. We thus cultured *B. vulgatus* (ATCC 8482) and *B. caccae* CLD22004 (which was isolated from one of the fecal samples, *vide infra*) and confirmed the lack of growth in minimum media (MM) with 0.1% GMP as a sole carbon source, whereas growth was observed in MM supplemented with glucose (Fig. S5). We further confirmed that both strains were labeled by GMP-F after growth in BHI, as seen by flow cytometry (2.18% and 1.25% of glycan⁺ cells for *B. vulgatus* and *B. caccae*, respectively), which was abrogated by heat shock (Fig. S5). These data suggest that some fluorescent glycan uptake took place, but probably by a PUL with a related substrate, or that the metabolism was not sufficient to sustain the growth of these isolates.

Similarly, *Alistipes communis* (a.k.a. *Alistipes obesi*)⁵⁷ was labeled by galactomannopentaose (Fig. 3a). Little is known about galactomannan metabolism in *A. communis*, but this bacterium is known to exhibit α -galactosidase activity⁵⁸. The *A. communis* 5CBH24 strain (100% identity and coverage to our ESV) encodes a classic SusC/D-like-containing PUL with 2 GH26s (with β 1-4-mannanase activity), a GH130 (a mannosylphosphorylase), and a GH97 (α -galactosidase activity), which is consistent with galactomannan metabolism (Fig. S5j) and resembles the known galactomannan PUL in *B. ovatus*¹⁸. However, while *A. communis* 5CBH24, could grow slowly in Columbia media depleted with carbohydrate, its growth was increased by supplementing glucose but not galactomannan (Fig. S5g), suggesting that this strain is incapable of utilizing this substrate for growth.

Taken together, these results demonstrate that 66% (6/9) of the ESVs significantly overexpressed in the glycan⁺ samples were known or

confirmed consumers of the glycan, but that 33% (3/9) could not use the glycans for growth in vitro.

Isolation of glycan consumers from stool samples

Considering that glycan consumers were enriched in the sorted labeled cells, we further used this subset of cells to isolate specific glycan consumers from human fecal samples. This gave us access to the bacterial strains present in the sample and allowed us to study their growth phenotype directly. First, fecal bacteria were labeled with GMP-F for 1 h, and glycan⁺ cells were sorted for 5 min (reaching approximately 27k sorted cells) before immediately being resuspended in reduced PBS in anaerobic conditions. Diluted bacteria were then cultured on BHI for 72 h. A GMP consumer (strain CLD22001) was isolated from this culture, as demonstrated by its ability to grow in MM with 0.1% GMP as a sole carbon source but not MM alone (Fig. 5a). We then performed DNA extraction and amplification of the whole 16-S rDNA region followed by Sanger sequencing. By performing BLAST alignment of the V1V9 sequence to the NCBI nucleotide database collection, strain CLD22001 was identified as a *Bacteroides xylanisolvens* with 99.79% identity and 100% coverage with *B. xylanisolvens* (strain funn3). Multiple members of the *B. xylanisolvens* harbor the galactomannan PUL that was characterized in *B. ovatus*, allowing them to grow on galactomannan^{18,19}. By flow cytometry, we confirmed that *B. xylanisolvens* CLD22001 cells were labeled by GMP-F when grown on MM containing GMP (Fig. 5a). In the same way, we isolated *B. caccae* strain CLD22004, that has 100% coverage and 98.95% identity with strain CL03T12C61. We demonstrated that this strain was labeled with GMP-F but could not grow on GMP (Fig. S5a–c).

We then cultured NYST-F⁺ sorted cells to isolate FOS consumers. Here, the sorted labeled bacteria were immediately resuspended in reduced ABB medium supplemented with 0.1% FOS under anaerobic conditions. After 48 h of incubation, the cultured bacteria were transferred in a dilution of 1/100 into MM supplemented with 0.1% FOS as the sole carbon source and incubated for 48 h under anaerobic conditions. The FOS consumers were then isolated on ABB or TSA supplemented with 5% sheep blood for 72 h and then characterized as described above. We isolated a *Bacteroides uniformis* (strain CLD22005, with 100% coverage and identity with strain CL11T00C07) that could grow in MM containing FOS but not MM alone (Fig. 5b). The

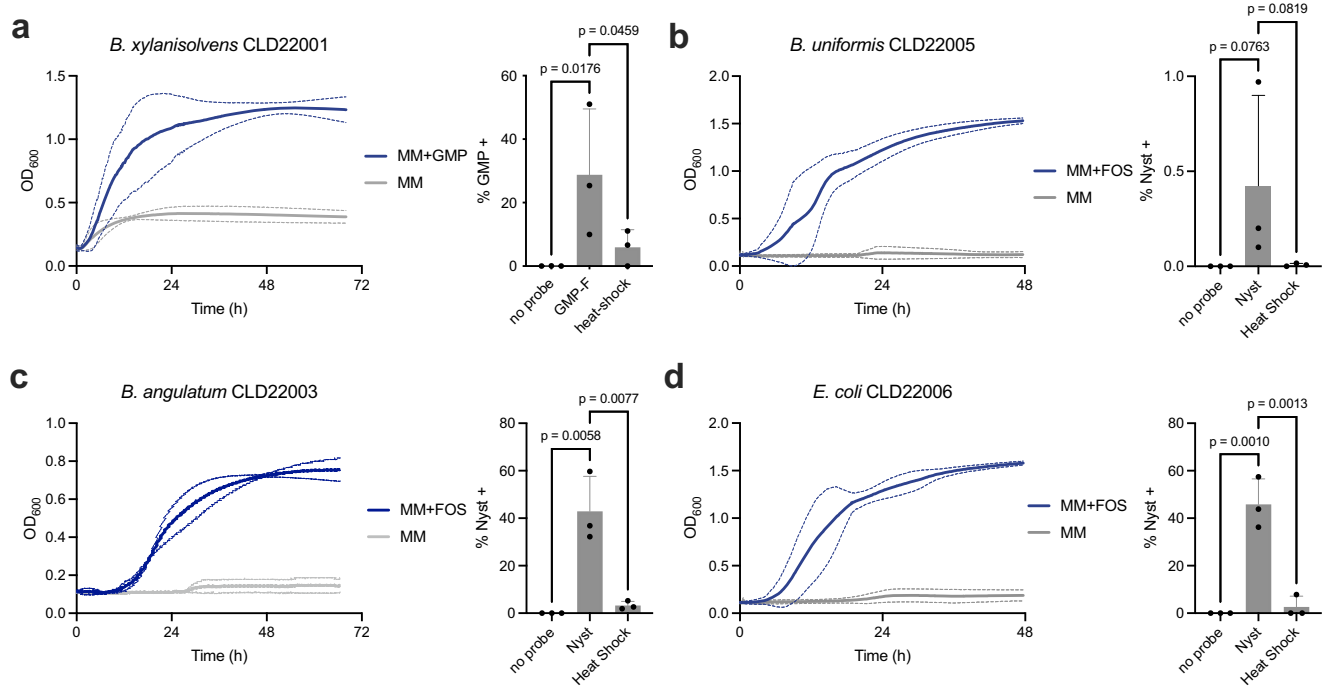


Fig. 5 | Consumer bacteria from three major phyla can be isolated by culturing glycan⁺ cells. a Growth of *B. xylanisolvens* CLD22001 in minimum medium supplemented or not with 0.1% galactomannopentaose. The growth curves are the mean and standard error of three independent replicates. GMP-F uptake by *B. xylanisolvens* CLD22001. The exponential cultures of *B. xylanisolvens* CLD22001 in MM supplemented with GMP were incubated for 1 h with GMP-F. The percentage of GMP⁺ bacteria was measured by flow cytometry. Data from three independent experiments are shown as the means \pm SD. Statistical significance compared to the GMP-F condition by one-way ANOVA with Bonferroni's multiple comparison test.

Growth of *B. uniformis* CLD22005 (**b**), *B. angulatum* CLD22003 (**c**), and *E. coli* CLD22006 (**d**) on MM supplemented or not with 0.1% FOS. Quantification of NYST-F uptake by *B. uniformis* CLD22005 (**b**), *B. angulatum* CLD22003 (**c**), and *E. coli* CLD22006 (**d**) by flow cytometry, with or without heat shock before labeling. All measurements were performed in triplicate, and the mean \pm SD are displayed. Statistical significance compared to the probe condition by one-way ANOVA with Bonferroni's multiple comparison test. Source data and statistical details are provided as a Source data file.

labeling of cultured isolates was low but abolished by heat shock (Fig. 5b). *B. uniformis* (CL11T00C07) contains a predicted PUL 32 containing 4 GH32s; a GH172; and a carbohydrate binding module family 38 (CBM38), which is known for inulin binding, along with the SusCD transporter, and these results are consistent with FOS consumption. Consequently, *B. uniformis* is known to grow on fructose and inulin⁵⁹. In addition, strain CLD22003 that grew on MM supplemented with FOS was identified as *Bifidobacterium angulatum* (Actinobacteria) with 100% coverage and 99.79% identity with multiple strains including DMS 20098. The FOS metabolism of *B. angulatum* was confirmed by growth on MM supplemented with FOS as a sole carbohydrate source and a strong uptake of the NYST-F probe, as measured by cytometry (Fig. 5c). The genus *Bifidobacterium* is well-known for FOS metabolism in studies involving diets enriched by FOS supplementation, and *B. angulatum* is a known consumer in vitro^{60,61}. Finally, we obtained a few Proteobacteria, including strain CLD22006 with a 16-S gene matching several *Escherichia coli* strains with 100% coverage and identity, including strains TUM13867. The *E. coli* strain could grow in MM containing FOS, and NYST-F labeling was robust (Fig. 5d). Enterobacteria, such as *E. coli*, have been reported to metabolize FOS⁶².

These results demonstrate that functional sorting and culturing can lead to the isolation and identification of glycan consumers from stool samples. While we investigated three different glycan structures in detail here, the method is applicable to other structures. Indeed, we expanded our glycan repertoire by preparing 5 additional fluorescein-conjugated oligosaccharides based on xylan, mannan, arabinoxylan, arabinan, and FOS (Fig. S6a) and confirmed by flow cytometry that these additional glycans were taken up by bacteria in a fecal sample (Fig. S6b).

Discussion

As we progress beyond a descriptive understanding of the human gut microbiota, functional assays are necessary to examine the role of specific bacteria within the community and in relation to the host²⁵. Diet-derived complex glycans are a major driver of the diversity and metabolism of gut microbes, but we still have an incomplete understanding of specific glycan metabolism in the gut. However, this knowledge is crucial for understanding the mechanisms of prebiotic applications, in which microbiota-accessible glycans are used to manipulate gut microbial composition and metabolism^{63–66}. Identifying gut bacteria that consume prebiotic glycans is an important step to ultimately reveal bacterial factors or metabolites that mediate host health benefits⁶⁷.

Here, we used a functional assay that combined active metabolic labeling with FACSeq or culturomics to shed light on glycan uptake in human gut microbiota samples, without prior knowledge of the metabolic genes involved. Metabolic labeling of bacteria by fluorescent glycans is possible because oligosaccharides are taken up into bacteria, allowing a small fluorescent tag to go unnoticed^{45,40}. Indeed, the prototypical SUS hydrolyzes polysaccharides at the cell surface into oligosaccharides that are taken up within the cell for further metabolism. This uptake prevents other bacteria from benefiting from any metabolic byproducts. Our experiments showed that the glucose monosaccharide probe was not taken up in contrast to larger oligosaccharides, which were transported into bacterial cells. This suggests that the fluorescein tag interferes with the transport of small monosaccharides but not larger oligosaccharides. However, it is also possible that ring opening or partial hydrolysis of cyclodextrin occurs prior to intracellular uptake. We minimized the potential detrimental effects of dye conjugation by random functionalization at different positions, thus increasing the

chances that the glycan recognition motif of the carbohydrate-binding domain or transporter was not masked by the fluorophore. In contrast with selfish polysaccharide utilization, some gut bacteria hydrolyze polysaccharides at the cell surface, releasing monosaccharides that are readily utilized by other species⁶⁸. Our approach only detects oligosaccharide uptake and does not identify bacterial cross feeding or downstream secondary metabolism, which remain to be determined. In addition, it is possible for an oligosaccharide to be partially hydrolyzed by one bacterium and taken up by another^{68,69}.

This proof-of-principle study was performed using three different glycans on samples from three unrelated healthy volunteers. The differential abundance analysis highlighted the uptake ability of several bacteria, and some of these bacteria were already known to consume starch, galactomannan, and FOS. Our work clearly highlighted the generalist ability of the Bacteroidales order, in line with their known large CAZyme repertoire, while glycan uptake in the Firmicutes was more sparsely distributed and limited to only one of the glycans tested, again consistent with the fact that they have a smaller repertoire of CAZymes than Bacteroidetes⁸.

Importantly, this pipeline allowed us to identify Firmicutes *B. wexlerae* as a consumer of FOS and inulin. This metabolism is likely mediated by a PUL containing a GH32 and an ABC transporter with homology to the FOS PUL of *L. acidophilus* (Fig. S4b). Expanding the screening to different glycans and different samples will likely yield other previously unknown consumers. We have already demonstrated the synthesis of 5 additional probes and demonstrated their labeling of gut bacteria in one fecal sample (Fig. S6), highlighting the feasibility of our approach.

The labeling observed here was largely abrogated at 0 °C or in the presence of glucose, suggesting an active process linked to metabolism. However, three bacteria (*A. communis*, *B. vulgatus*, and *B. caccae*) labeled by GMP-F lacked the ability to grow on galactomannan in vitro. While GMP-F may bind to surface proteins without uptake in this case, the loss in fluorescent signal upon temperature inactivation supports the active uptake of glycans. It is possible that the glycan activates and is bound to or taken up by an active PUL on a structurally related glycan and that GH specificity precludes efficient metabolism. Indeed, *C. aerofaciens* was labeled by nystose containing β -2,1 fructose linkages, but could not grow on this substrate and instead metabolized levan containing β -2,6-fructose linkages for growth. Furthermore, *A. communis* harbors a PUL with a strong resemblance to the known galactomannan PUL of *B. ovatus*, suggesting activity on an α -galactose- and β -mannose-containing substrate, even if growth on galactomannan was not observed⁴⁸. The absence of growth in bacterial strains encoding the appropriate PUL in their genome has been observed for arabinogalactan in *P. copri* and may be due to slight differences in substrate or inaccurate specificity based on gene annotation⁵⁰. Alternatively, glycans can be recognized by proteins implicated in quorum-sensing pathways or host sensing pathways, as has been suggested for fucose in pathogens^{70,71}. Therefore, metabolic labeling alone, even with genes consistent with an activity, is not sufficient to infer metabolism for growth, and further studies are needed to fully characterize the possible metabolic pathways involved and their function for the bacterial cell.

We therefore expanded our approach to include bacterial cultures after the sorting of glycan⁺ cells to isolate consumers in the studied fecal sample. This allowed us to directly validate growth in vitro using the bacterial strains present in the samples and avoid the issue of inferring a strain based on a portion of the 16S rRNA gene. In a proof-of-principle study, we isolated 4 clones from 3 different phyla that could consume GMP and FOS as a sole carbon source in culture. The clones were identified by Sanger sequencing of the 16S rRNA gene as a *B. xylanisolvens* (Bacteroidetes) growing on GMP and *B. angulatum* (Actinobacteria), *B. uniformis* (Bacteroidetes), and *E. coli* (Proteobacteria) growing on FOS. While these consumers were known, more

extensive culturomics with different and more specific growth media could yield unknown consumers⁷². In addition, the aerobic environment of the sorting step is likely detrimental to many strict anaerobes, and sorting under anoxic conditions will most likely improve the recovery of live bacteria labeled by fluorescent glycans.

Existing approaches to study bacterial glycan metabolism have typically focused on simplified complex communities, which can be hard to reproduce despite providing unparalleled insight^{32,33}. Our approach complements such studies by using whole human fecal samples, which could also be patient-derived to capture different bacterial taxa. As our pipeline is functional and includes a cell sorting step, dead or damaged cells in the original samples are not labeled even if they encode the appropriate metabolic machinery. Thus, our method is complementary to genomic methods that do not discriminate between active, inactive, live, or damaged cells³⁴. Expanding this approach to other glycans and samples can help further annotate PUL specificity and discover metabolic genes and pathways.

Due to its noninvasive nature, human stool sampling is a powerful surrogate for the gut microbiota, as bacteria can remain metabolically active when rapidly placed in anoxic conditions^{35,73,74}. However, it should be recognized that stool samples do not provide information on the location of specific gut bacteria along the digestive tract or across the depth of the mucus layer and that its composition represents shed bacteria⁷⁵. Furthermore, stool samples vary in composition according to stool consistency, which reflects differences in transit time and potentially different niche sampling⁷⁶. Nevertheless, this pipeline should be applicable to other types of gut microbiota, such as colonoscopy biopsies.

Here, we described the use of a functional pipeline that identifies bacteria that take up specific glycans in complex gut microbiota samples, thus expanding our functional understanding of glycan metabolism in the human gut microbiota. Applying this pipeline to other glycan structures and to other human populations with distinct diets, as well as individuals with gastrointestinal diseases, could help identify putative consumers of dietary glycans and prebiotic structures. Furthermore, combining this method with culturomics is useful to isolate bacterial consumers without knowledge of the specific genes involved, potentially leading to the discovery of novel CAZymes. The method could also be coupled to other genomic analyses, such as metagenomics and single-cell genomics, to characterize glycan metabolism in non-Bacteroidetes bacteria and discriminate between metabolism for growth vs metabolism without growth.

Methods

Bacterial strains

Lactobacillus acidophilus (Moro) Hansen and Møcquot (ATCC® 4356™) (*L.a*) and *Klebsiella oxytoca* MSA1 (*K.o*), which were purchased from the American Type Culture Collection (Rockville, MD), were cultured at 37 °C in De Man, Rogosa, Sharpe (MRS) medium and Luria-Bertani (LB) medium, respectively.

Synthesis

The detailed synthesis of the fluorescein-conjugated glycans is provided in the supporting information, along with the HPLC chromatograms.

Human stool sample collection

Protocol A04-M27-15B was approved by the McGill Faculty of Medicine Institutional Review Board. We have received informed written consent from the participants for the use of human samples. Healthy participants with a body mass index between 18.5–30, no diagnosed gastrointestinal disease, no ongoing therapeutic treatment, and no antibiotic usage 3 months prior to the start of the study were eligible. Subject information was recorded at the time of sampling. The age of donors ranged from 21–40 years. The initial experiments were performed on one sample from a male donor. The metabolic labeling

FACSeq pipeline was performed on samples from 1 female and 2 males aged 30–40 years, and the BMI ranged from 19–27 kg/m². Fresh fecal samples were collected and placed immediately in an anaerobic chamber, aliquoted, and stored at –80 °C until use.

Metabolic labeling of cultured *Klebsiella oxytoca*

Cultures in minimum medium M9 (MM M9) of *K.o* supplemented with 0.1% β -cyclodextrin were collected during exponential phase growth via centrifugation, washed in MM M9, and suspended in 195 μ L of MM M9. *K.o.* were treated with 500 nM CD-F probes or an equal volume of MM M9 cells as a control. The cells were incubated with shaking for 30 min at 37 °C in the dark. Cells were collected via centrifugation at 15,000 $\times g$ for 5 min and were washed 2 times with 1 mL of MM M9. Pellets were resuspended in 200 μ L of MM M9, and fluorescence was measured by using a Spark 10 M Tecan plate reader at an excitation wavelength of 485 nm following emission at 535 nm. Fluorescence measurements were performed in triplicate, and the mean values and standard deviations are displayed in the figures.

Metabolic labeling of cultured *Lactobacillus acidophilus*

Cultures in custom MRS (cMRS) (peptone (10 g/L), yeast extract (5 g/L), sodium acetate (5 g/L), ammonium citrate (92 g/L), potassium phosphate (2 g/L), magnesium sulfate (0.2 g/L), manganese sulfate (0.05 g/L), Tween 80 (1 mL/L)) of *L.a* supplemented with 0.1% inulin were collected during exponential phase growth via centrifugation, washed in cMRS and suspended in 195 μ L of cMRS. *L.a* were treated with 500 nM of NYST-F probes or an equal volume of cMRS as a control. The cells were incubated with shaking for 30 min at 37 °C in the dark. Cells were collected via centrifugation at 15,000 $\times g$ for 5 min and washed 2 times with 1 mL of phosphate-buffered saline (PBS). Pellets were resuspended in 200 μ L of PBS, and fluorescence was measured by using a Spark 10 M Tecan plate reader at an excitation wavelength of 485 nm following emission at 535 nm. Fluorescence measurements were performed in triplicate, and the mean values and standard deviations are displayed in the figures.

Fluorescence microscopy

After carbohydrate labeling, *K.o.* or *L.a.* were fixed for 30 min with a solution of 4% paraformaldehyde, washed with a PBS solution and mixed at equal volumes with ProLong Gold antifade reagent (Invitrogen). Fixed bacteria were mounted on a slide and coverslip, and images were acquired with a LEICA SP8 confocal microscope (for *K.o*) and a Leica DM100 microscope (for *L.a*) and processed by Leica LAS X software.

Flow cytometry analysis

Flow cytometry analysis was performed on a 5-laser LSR Fortessa 20-parameter analyzer. Cell sorting was performed on 3 lasers, 13 detector FACS Aria-III or 4 lasers, and 18-detector FACS Aria Fusion. Data were analyzed using BD FACSDiva or FlowJo software. To set the thresholds to specifically detect the cells labeled by the fluorescent probes, we used unlabeled cells as negative controls. The detection threshold for signals in the FITC channel was set at 10^3 compared to the maximum signal generated by the negative control at 3×10^2 . The gating threshold may be adjusted at different levels to adjust the sensitivity of the assay depending on the probes. Fifty thousand or 100,000 events per sample were analyzed, and FITC fluorescence was measured using the FITC channel with excitation at 488 nm and emission at 535 nm. FSC and SSC (forward and side scatter, respectively) gating was performed to exclude doublets. A total of 1 to 3×10^6 cells for each sample were sorted.

Carbohydrate labeling

All labeling experiments were performed under anaerobic conditions (87% N₂, 10% CO₂, and 3% H₂). A 0.1 g fecal sample was weighed

and diluted to 1:10 g/mL in minimum medium (MM) (6.6 mM KH₂PO₄ (pH 7.2), 15 mM NaCl, 100 μ M MgCl₂, 175 μ M CaCl₂, 50 μ M MnSO₄, 5 mM (NH₄)₂SO₄, 15 μ M FeSO₄, 24 μ M NaHCO₃, 1 g/L^{–1} L-cysteine, 1.9 μ M hematin, 6 μ M Hemin, and 200 ng/mL^{–1} vitamin B₁₂), vortexed thoroughly and centrifuged for 3 min at 700 $\times g$. The supernatant was saved, and the pellet was discarded. The supernatant was centrifuged for 5 min at 6500 $\times g$, the supernatant was discarded, and the pellet was washed with MM. The pellet was resuspended in 200 μ L of MM. Carbohydrate probes were added at a final concentration of 4.4 μ M. Incubation was performed at 37 °C for one hour protected from light. The cells were then centrifuged for 5 min at 6500 $\times g$, the supernatant was discarded, and the pellet was washed twice with reduced PBS (rPBS). The final pellet was resuspended in 500 μ L of rPBS and kept at 4 °C protected from light. The bacterial suspension was then diluted in rPBS for flow cytometry analysis.

Competition experiment with free cyclodextrin

Metabolic labeling was performed as above but with the addition of unlabeled β -cyclodextrin (218 μ M, 436 μ M, 872 μ M, and 1.74 mM) with the CD-F probe. The fluorescence level was measured by flow cytometry.

Heat inactivation

Heat pretreatment was performed after the preparation of the bacterial pellet from the fecal sample as described above. The incubation was for 10 min at 65 °C followed by cooling at 37 °C before metabolic labeling was performed as described above.

Catabolic repression

An incubation in MM supplemented with 0.1% glucose for one hour was performed before metabolic labeling as described above.

DNA extraction, 16S ribosomal RNA gene amplification, and MiSeq sequencing

DNA was extracted from all samples using the AllPrep PowerFecal DNA/RNA kit (Qiagen, Canada) following the manufacturer's instructions. DNAs were quantified by the Qubit Fluorometric Quantification method (Invitrogen). The V4 region (based on *Escherichia coli*) of the 16S ribosomal RNA (rRNA) was targeted for amplification by PCR using the forward primer 515F GTGCCAGCM GCCGCGGTAA and the reverse primer 806R GGACTACHVGGGT WTCTAAT. The CS1 (ACACTGACGACATGGTCTACA) and CS2 (TACGGTAGCAGAGACTTGGTCT) tags were used to add a barcode and Illumina adapters. Amplification was performed using Q5 High Fidelity DNA polymerase (New England BioLabs) with PCR cycles as follows: initial denaturation step of 98 °C for 30 s, before 23 cycles of 98 °C for 10 s, 58 °C for 15 s and 72 °C for 30 s, with a final extension at 72 °C for 2 min. The MiSeq platform was used for 2 \times 250 bp paired-end sequencing of the resulting PCR products.

Sequencing analysis

The sequencing data analysis was performed using the ANCHOR pipeline⁴⁸. Briefly, the potential amplicons were assembled from high-quality reads. Assembled contigs of all lengths were conserved to control for unexpected amplicon length. Dereplicated sequences were evaluated, and sequences with a count higher than 9 were used as a confident basis for analysis (anchor threshold). These were processed and annotated against four major sequence repositories (curated NCBI bacterial and Archaea RefSeq database, NCBI nr/nt, SILVA, Ribosomal Database Project) with >99% identity and coverage thresholds. α - and β -diversity and ordination were analyzed using R scripts with the vegan library, and differential abundance analysis was performed using DESeq2⁴⁹.

4. Fujimura, K. E. & Lynch, S. V. Microbiota in allergy and asthma and the emerging relationship with the gut microbiome. *Cell Host Microbe* **17**, 592–602 (2015).
5. McKay, K. A. et al. From bugs to brains: the microbiome in neurological health. *Mult. Scler. Relat. Disord.* **12**, 1–3 (2017).
6. Knight, R. et al. The microbiome and human biology. *Annu. Rev. Genom. Hum. Genet.* **18**, 65–86 (2017).
7. David, L. A. et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).
8. El Kaoutari, A., Armougom, F., Gordon, J. I., Raoult, D. & Henrissat, B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat. Rev. Microbiol.* **11**, 497–504 (2013).
9. Koropatkin, N. M., Cameron, E. A. & Martens, E. C. How glycan metabolism shapes the human gut microbiota. *Nat. Rev. Microbiol.* **10**, 323–335 (2012).
10. Rothschild, D. et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature* **555**, 210–215 (2018).
11. Wu, M. et al. Genetic determinants of in vivo fitness and diet responsiveness in multiple human gut *Bacteroides*. *Science* **350**, aac5992 (2015).
12. Deehan, E. C. et al. Precision microbiome modulation with discrete dietary fiber structures directs short-chain fatty acid production. *Cell Host Microbe* **27**, 389–404.e386 (2020).
13. Martens, E. C., Koropatkin, N. M., Smith, T. J. & Gordon, J. I. Complex glycan catabolism by the human gut microbiota: the *Bacteroidetes* Sui-like paradigm. *J. Biol. Chem.* **284**, 24673–24677 (2009).
14. Glenwright, A. J. et al. Structural basis for nutrient acquisition by dominant members of the human gut microbiota. *Nature* **541**, 407–411 (2017).
15. Cuskin, F. et al. Human gut *Bacteroidetes* can utilize yeast mannan through a selfish mechanism. *Nature* **517**, 165–169 (2015).
16. Tamura, K. et al. Molecular mechanism by which prominent human gut *bacteroidetes* utilize mixed-linkage beta-glucans, major health-promoting cereal polysaccharides. *Cell Rep.* **21**, 417–430 (2017).
17. Larsbrink, J. et al. A discrete genetic locus confers xyloglucan metabolism in select human gut *Bacteroidetes*. *Nature* **506**, 498–502 (2014).
18. Bagenholm, V. et al. Galactomannan catabolism conferred by a polysaccharide utilization locus of *Bacteroides ovatus*: ENZYME SYNERGY AND CRYSTAL STRUCTURE OF A beta-MANNANASE. *J. Biol. Chem.* **292**, 229–243 (2017).
19. Reddy, S. K. et al. A beta-mannan utilization locus in *Bacteroides ovatus* involves a GH36 alpha-galactosidase active on galactomannans. *FEBS Lett.* **590**, 2106–2118 (2016).
20. Barrangou, R., Altermann, E., Hutkins, R., Cano, R. & Klaenhammer, T. R. Functional and comparative genomic analyses of an operon involved in fructooligosaccharide utilization by *Lactobacillus acidophilus*. *Proc. Natl Acad. Sci. USA* **100**, 8957–8962 (2003).
21. La Rosa, S. L. et al. The human gut firmicute *Roseburia intestinalis* is a primary degrader of dietary beta-mannans. *Nat. Commun.* **10**, 905 (2019).
22. Terrapon, N. et al. PULDB: the expanded database of polysaccharide utilization loci. *Nucleic Acids Res.* **46**, D677–D683 (2018).
23. Cockburn, D. W. & Koropatkin, N. M. Polysaccharide degradation by the intestinal microbiota and its influence on human health and disease. *J. Mol. Biol.* **428**, 3230–3252 (2016).
24. Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M. & Henrissat, B. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* **42**, D490–D495 (2014).
25. Koppel, N. & Balskus, E. P. Exploring and understanding the biochemical diversity of the human microbiota. *Cell Chem. Biol.* **23**, 18–30 (2016).
26. Siegrist, M. S., Swarts, B. M., Fox, D. M., Lim, S. A. & Bertozzi, C. R. Illumination of growth, division and secretion by metabolic labeling of the bacterial cell surface. *FEMS Microbiol. Rev.* **39**, 184–202 (2015).
27. Hatzenpichler, R., Krukenberg, V., Spietz, R. L. & Jay, Z. J. Next-generation physiology approaches to study microbiome function at single cell level. *Nat. Rev. Microbiol.* **18**, 241–256 (2020).
28. Banahene, N., Kavunja, H. W. & Swarts, B. M. Chemical reporters for bacterial glycans: development and applications. *Chem. Rev.* **122**, 3336–3413 (2022).
29. Maathuis, A. J. H., van den Heuvel, E. C., Schoterman, M. H. C. & Venema, K. Galacto-oligosaccharides have prebiotic activity in a dynamic in vitro colon model using a C-13-labeling technique. *J. Nutr.* **142**, 1205–1212 (2012).
30. Herrmann, E. et al. Determination of resistant starch assimilating bacteria in fecal samples of mice by in vitro RNA-based stable isotope probing. *Front. Microbiol.* **8**, 1331 (2017).
31. Berry, D. & Loy, A. Stable-isotope probing of human and animal microbiome function. *Trends Microbiol.* **26**, 999–1007 (2018).
32. Patnode, M. L. et al. Interspecies competition impacts targeted manipulation of human gut bacteria by fiber-derived glycans. *Cell* **179**, 59–73.e13 (2019).
33. Patnode, M. L. et al. Strain-level functional variation in the human gut microbiota based on bacterial binding to artificial food particles. *Cell Host Microbe* **29**, 664–673.e665 (2021).
34. Maurice, C. F., Haiser, H. J. & Turnbaugh, P. J. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell* **152**, 39–50 (2013).
35. Maurice, C. F. & Turnbaugh, P. J. Quantifying and identifying the active and damaged subsets of indigenous microbial communities. *Methods Enzymol.* **531**, 91–107 (2013).
36. Taguer, M., Shapiro, B. J. & Maurice, C. F. Translational activity is uncoupled from nucleic acid content in bacterial cells of the human gut microbiota. *Gut Microbes* **13**, 1–15 (2021).
37. Klassen, L. et al. Quantifying fluorescent glycan uptake to elucidate strain-level variability in foraging behaviors of rumen bacteria. *Microbiome* **9**, 23 (2021).
38. Palm, N. W. et al. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* **158**, 1000–1010 (2014).
39. Le, H. H., Lee, M. T., Besler, K. R., Comrie, J. M. C. & Johnson, E. L. Characterization of interactions of dietary cholesterol with the murine and human gut microbiome. *Nat. Microbiol.* **7**, 1390–1403 (2022).
40. Hehemann, J. H. et al. Single cell fluorescence imaging of glycan uptake by intestinal bacteria. *ISME J.* **13**, 1883–1889 (2019).
41. Martinez-Garcia, M. et al. Capturing single cell genomes of active polysaccharide degraders: an unexpected contribution of Verrucomicrobia. *PLoS ONE* **7**, e35314 (2012).
42. Doud, D. F. R. et al. Function-driven single-cell genomics uncovers cellulose-degrading bacteria from the rare biosphere. *ISME J.* **14**, 659–675 (2020).
43. Koropatkin, N. M., Martens, E. C., Gordon, J. I. & Smith, T. J. Starch catabolism by a prominent human gut symbiont is directed by the recognition of amylose helices. *Structure* **16**, 1105–1115 (2008).
44. van den Berg, B., Prathyusha Bhamidimarri, S., Dahyabhai Prajapati, J., Kleinekathofer, U. & Winterhalter, M. Outer-membrane translocation of bulky small molecules by passive diffusion. *Proc. Natl Acad. Sci. USA* **112**, E2991–E2999 (2015).
45. Roberfroid, M. B. Inulin-type fructans: functional food ingredients. *J. Nutr.* **137**, 2493S–2502S (2007).
46. Vandeputte, D., Tito, R. Y., Vanleeuwen, R., Falony, G. & Raes, J. Practical considerations for large-scale gut microbiome studies. *FEMS Microbiol. Rev.* **41**, S154–S167 (2017).

47. Schwalm, N. D. 3rd, Townsend, G. E. 2nd & Groisman, E. A. Multiple signals govern utilization of a polysaccharide in the gut bacterium *Bacteroides thetaiotaomicron*. *Mbio* **7**, <https://doi.org/10.1128/mbio.01342-16> (2016).
48. Gonzalez, E., Pitre, F. E. & Brereton, N. J. B. ANCHOR: a 16S rRNA gene amplicon pipeline for microbial analysis of multiple environmental samples. *Environ. Microbiol.* **21**, 2440–2468 (2019).
49. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
50. Fehlner-Peach, H. et al. Distinct polysaccharide utilization profiles of human intestinal *Prevotella copri* isolates. *Cell Host Microbe* **26**, 680–690 e685 (2019).
51. Li, J. et al. A versatile genetic toolbox for *Prevotella copri* enables studying polysaccharide utilization systems. *EMBO J.* e108287, <https://doi.org/10.15252/embj.2021108287> (2021).
52. Yan, H. et al. Dietary fat content and fiber type modulate hind gut microbial community and metabolic markers in the pig. *PLoS ONE* **8**, e59581 (2013).
53. Pham, V. T. et al. Antioxidant vitamins and prebiotic FOS and XOS differentially shift microbiota composition and function and improve intestinal epithelial barrier in vitro. *Nutrients* **13**, 1125 (2021).
54. Barrangou, R. et al. Global analysis of carbohydrate utilization by *Lactobacillus acidophilus* using cDNA microarrays. *Proc. Natl Acad. Sci. USA* **103**, 3816–3821 (2006).
55. Desai, M. S. et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* **167**, 1339–1353.e1321 (2016).
56. Tanno, H. et al. Characterization of fructooligosaccharide metabolism and fructooligosaccharide-degrading enzymes in human commensal butyrate producers. *Gut Microbes* **13**, 1869503 (2021).
57. Sakamoto, M. et al. *Alistipes communis* sp. nov., *Alistipes dispar* sp. nov. and *Alistipes onderdonkii* subsp. *vulgaris* subsp. nov., isolated from human faeces, and creation of *Alistipes onderdonkii* subsp. *onderdonkii* subsp. nov. *Int. J. Syst. Evol. Microbiol.* **70**, 473–480 (2020).
58. Hugon, P. et al. Non contiguous-finished genome sequence and description of *Alistipes obesi* sp. nov. *Stand. Genom. Sci.* **7**, 427–439 (2013).
59. Sonnenburg, E. D. et al. Specificity of polysaccharide use in intestinal bacteroides species determines diet-induced microbiota alterations. *Cell* **141**, 1241–1252 (2010).
60. Liu, F. et al. Fructooligosaccharide (FOS) and galactooligosaccharide (GOS) increase *Bifidobacterium* but reduce butyrate producing bacteria with adverse glycemic metabolism in healthy young population. *Sci. Rep.* **7**, 11789 (2017).
61. Perrin, S., Fougny, C., Grill, J. P., Jacobs, H. & Schneider, F. Fermentation of chicory fructo-oligosaccharides in mixtures of different degrees of polymerization by three strains of bifidobacteria. *Can. J. Microbiol.* **48**, 759–763 (2002).
62. Hartemink, R., Van Laere, K. M. & Rombouts, F. M. Growth of enterobacteria on fructo-oligosaccharides. *J. Appl. Microbiol.* **83**, 367–374 (1997).
63. Gibson, G. R. et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 491–502 (2017).
64. Bindels, L. B., Delzenne, N. M., Cani, P. D. & Walter, J. Towards a more comprehensive concept for prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **12**, 303–310 (2015).
65. Zeevi, D. et al. Personalized nutrition by prediction of glycemic responses. *Cell* **163**, 1079–1094 (2015).
66. Zmora, N., Zeevi, D., Korem, T., Segal, E. & Elinav, E. Taking it personally: personalized utilization of the human microbiome in health and disease. *Cell Host Microbe* **19**, 12–20 (2016).
67. Chaudhari, S. N., McCurry, M. D. & Devlin, A. S. Chains of evidence from correlations to causal molecules in microbiome-linked diseases. *Nat. Chem. Biol.* **17**, 1046–1056 (2021).
68. Rakoff-Nahoum, S., Coyne, M. J. & Comstock, L. E. An ecological network of polysaccharide utilization among human intestinal symbionts. *Curr. Biol.* **24**, 40–49 (2014).
69. Hehemann, J. H. et al. Adaptive radiation by waves of gene transfer leads to fine-scale resource partitioning in marine microbes. *Nat. Commun.* **7**, 12860 (2016).
70. Pacheco, A. R. et al. Fucose sensing regulates bacterial intestinal colonization. *Nature* **492**, 113–117 (2012).
71. Vogt, S. L., Pena-Diaz, J. & Finlay, B. B. Chemical communication in the gut: effects of microbiota-generated metabolites on gastrointestinal bacterial pathogens. *Anaerobe* **34**, 106–115 (2015).
72. Lau, J. T. et al. Capturing the diversity of the human gut microbiota through culture-enriched molecular profiling. *Genome Med.* **8**, 72 (2016).
73. Browne, H. P. et al. Culturing of ‘unculturable’ human microbiota reveals novel taxa and extensive sporulation. *Nature* **533**, 543–546 (2016).
74. Taguer, M., Quillier, O. & Maurice, C. F. Effects of oxygen exposure on relative nucleic acid content and membrane integrity in the human gut microbiota. *PeerJ* **9**, e10602 (2021).
75. Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* **14**, 20–32 (2016).
76. Falony, G. et al. Population-level analysis of gut microbiome variation. *Science* **352**, 560–564 (2016).
77. Dhariwal, A. et al. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Res.* <https://doi.org/10.1093/nar/gkx295> (2017).
78. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890 (2018).
79. Menzel, P., Ng, K. L. & Krogh, A. Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nat. Commun.* **7**, 11257 (2016).

Acknowledgements

This research was funded by a Canadian Glycomics Network (GlycoNet) Catalyst Grant (DO-16) to B.C. and C.F.M. and by the Canadian Institutes of Health Research (CIHR) grant PJT-437944 to B.C. and C.F.M. B.C. holds a tier II Canada Research Chair (CRC) in Therapeutic Chemistry. C.F.M. holds a tier II CRC in Gut Microbial Physiology and is a Azrieli Global Scholar in the Humans & Microbiome program. J.X. holds a tier II CRC in Bioinformatics and Big Data Analytics. The sequencing was performed at the McGill University and Genome Quebec Innovation Centre. Flow cytometry was performed at the Flow Cytometry Core Facility of the Life Sciences Complex of McGill University, which is supported by funding from the Canadian Foundation for Innovation. Fluorescence image acquisition and molecular biology experiment (PCR and nanodrop quantification) were performed with the McGill University Imaging and Molecular Biology Platform (IMBP) equipment.

Author contributions

L.D. designed and carried out the microbiology experiments. F.A., O.L., R.K., R.P., and A.M. performed the synthesis and characterization of the probes as well as preliminary uptake experiments. E. G. performed the bioinformatic analyses. J.C. and J.X. performed the metagenomic analysis. B.C. and C.F.M. conceived and supervised the project. B.C. wrote the manuscript with C.F.M., L.D., F.A., O.L., and R.K. All authors discussed the results and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41467-023-36365-8>.

Correspondence and requests for materials should be addressed to Corinne F. Maurice or Bastien Castagner.

Peer review information *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work.

Reprints and permissions information is available at <http://www.nature.com/reprints>

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

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, 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 license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license 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 license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2023