Food Research International 167 (2023) 112711

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Arabinoxylan-based substrate preferences and predicted metabolic properties of *Bifidobacterium longum* subspecies as a basis to design differential media

Ines Calvete-Torre [a,b], Carlos Sabater [a,b], Susana Delgado [a,b], Patricia Ruas-Madiedo [a,b], Alicia Rupérez-García [c], Antonia Montilla [c], F. Javier Moreno [c], Abelardo Margolles [a,b,*], Lorena Ruiz [a,b,*]

[a] Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias-Consejo Superior de Investigaciones Científicas IPLA, CSIC, Paseo Río Linares s/n, 33300, Villaviciosa, Asturias, Spain
[b] Functionality and Ecology of Beneficial Microbes (MicroHealth) Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Asturias, Spain
[c] Instituto de Investigación en Ciencias de la Alimentación CIAL, (CSIC-UAM) CEI (CSIC+UAM), Madrid, Spain

ARTICLE INFO

Keywords:
Arabinoxylan
Arabinoxylo-oligosaccharides
Prebiotics
Bifidobacterium

ABSTRACT

Arabinoxylan (AX) and arabinoxylo-oligosaccharides (AXOS) derived therefrom are emergent prebiotics with promising health promoting properties, likely linked to its capacity to foster beneficial species in the human gut. Bifidobacteria appear to be one taxa that is frequently promoted following AX or AXOS consumption, and that is known to establish metabolic cross-feeding networks with other beneficial commensal species. Therefore, probiotic bifidobacteria with the capability to metabolize AX-derived prebiotics represent interesting candidates to develop novel probiotic and synbiotic combinations with AX-based prebiotics. In this work we have deepen into the metabolic capabilities of bifidobacteria related to AX and AXOS metabolization through a combination of *in silico* an *in vitro* tools. Both approaches revealed that *Bifidobacterium longum* and, particularly, *B. longum* subsp. *longum*, appears as the better equipped to metabolize complex AX substrates, although other related subspecies such as *B. longum* subsp. *infantis*, also hold some machinery related to AXOS metabolization. This correlates to the growth profiles exhibited by representative strains of both subspecies in AX or AXOS enriched media. Based on these results, we formulated a differential carbohydrate free medium (CFM) supplemented with a combination of AX and AXOS that enabled to recover a wide diversity of *Bifidobacterium* species from complex fecal samples, while allowing easy discrimination of AX metabolising strains by the appearance of a precipitation halo. This new media represent an appealing alternative to isolate novel probiotic bifidobacteria, rapidly discriminating their capacity to metabolize structurally complex AX-derived prebiotics. This can be convenient to assist formulation of novel functional foods and supplements, including bifidobacterial species with capacity to metabolize AX-derived prebiotic ingredients.

1. Introduction

The increased consumer awareness and demand of foods with health beneficial properties, is fostering the research on novel probiotic strains, microorganisms which when administered in adequate amounts confer a health benefit (Hill et al., 2014), and prebiotics, non-digestible food ingredients that are selectively utilized by host microorganisms conferring a health benefit (Gibson et al., 2017). Prebiotics traditionally refer to non-digestible carbohydrates, and include a wide and rapidly growing diversity of oligo- and polysaccharides that are resistant to intestinal digestion and absorption (Ferreira-Lazarte et al., 2020). Among these, arabinoxylo-oligosaccharides (AXOS) derived from the hydrolysis of arabinoxylan (AX), which constitute the major hemicellulosic component in the cell wall of some cereal plants, have received increasing attention as emergent prebiotics (Mathew et al., 2018; Sabater et al., 2021).

The complex chemical structure of AX and AXOS is not degraded by human digestive enzymes, thus they reach the distal digestive system

* Corresponding authors.
E-mail addresses: amargolles@ipla.csic.es (A. Margolles), lorena.ruiz@ipla.csic.es (L. Ruiz).

https://doi.org/10.1016/j.foodres.2023.112711
Received 30 June 2022; Received in revised form 9 March 2023; Accepted 14 March 2023
Available online 20 March 2023

intact (Broekaert et al., 2011; Rivière et al., 2014; van den Abbeele et al., 2018). AX consist of a linear backbone of 1,500 to 15,000 β(1–4) D-xylopyranoside units, which can occur unsubstituted, monosubstituted with α-L-arabinofuranoside residues positioned on C-(*O*)-2 or C-(*O*)-3, or disubstituted with single α-L-arabinofuranoside units at C-(*O*)-2 and C-(*O*)-3 (Broekaert et al., 2011; Rivière et al., 2014). Xylose residues can be substituted to a lesser extent with glucuronic acid and its 4-O-methyl derivative, or with short oligomers like L-arabinose, D-xylose, D-galactose, D-glucose or uronic acids. Arabinose residues can be esterified with phenolic acids, such as ferulic acid and to a lesser extent *p*-coumaric acid or dehydrodiferulic acid (Broekaert et al., 2011; Rivière et al., 2014). The presence of these modifications mainly depends on the vegetable source and greatly influences the biological properties of AX and AXOS (Aachary & Prapulla, 2011; Sabater et al., 2021). On the other hand, AXOS or *xylo*-oligosaccharides (XOS) may be obtained by enzymatic action with β-endoxylanases produced by various microorganisms, and are characterized by their lower average degree of polymerization and their average degree of arabinose substitution. Therefore, AXOS are more soluble and readily available for fermentation by the gut microbiota than AX (Broekaert et al., 2011; Rivière et al., 2014; Suriano et al., 2017).

AX and AXOS are a source of energy for certain saccharolytic bacteria residing in the colon, including *Bacteroides, Bifidobacterium, Clostridium, Lactobacillus* and *Eubacterium* species. Accordingly, the intake of AXOS has been associated to an increase in several commensal and potentially beneficial species including members of the *Lachnospiraceae* and *Oscillospiraceae* families, and of the *Bifidobacterium* genus, that is the most strongly promoted genus following consumption of AXOS (Suriano et al., 2017). *Bifidobacterium* also includes some of the species most commonly used as probiotics in functional foods and nutraceuticals (Ivashkin et al., 2021; Russell et al., 2011; Sarkar and Mandal, 2016). Remarkably, some bifidobacteria can cross-feed with other gut commensals such as *Eubacterium* and *Bacteroides* species, as a mean to cooperatively degrade xylan what results in production of beneficial metabolites by third species (Fernandez-Julia et al., 2022; Liu et al., 2020; Rivière et al., 2014). Among bifidobacteria, *Bifidobacterium longum* is the species with a larger number of fully sequenced genomes available, the majority belonging to the subspecies *B. longum* subsp. *longum* and only a few representing *B. longum* subsp. *infantis*. Genomic data and *in vitro* growth tests have explored the diversity of the metabolic capabilities of these microorganisms with a focus on those traits involved in the colonization of the gastro-intestinal tract and their adaptation to different groups of human hosts based on their dietary patterns (Arboleya et al., 2018; Blanco et al., 2020; Odamaki et al., 2018; Díaz et al., 2021). Overall, *B. longum* subsp. *infantis* exhibits a better adaptation to the intestinal environment of breastfed infants as it is able to metabolise human milk oligosaccharides (HMO) (Sela et al., 2008). On the other hand, metabolization of the most complex carbohydrates that can be present in the adult diet, including AX and AXOS, require a complex arsenal of enzymes such as glycoside hydrolases (GH), which split the glycosidic bonds into poly and oligosaccharides, releasing shorter metabolisable products. Accordingly, the array of GH involved in metabolization of complex vegetable fibres is considerably larger in the genomes of *B. longum* subsp. *longum* (Blanco et al., 2020). Besides, available evidence suggests that, among bifidobacteria, the enzymatic activities involved in the metabolism of arabinans, AX and arabinogalactan may be restricted to some *B. longum* subsp. *longum* strains. This fact may explain the better adaptation of this microorganism to the intestinal tract of adults following a fiber-rich diet, as compared to other bifidobacterial species (Blanco et al., 2020; Komeno et al., 2019). On the other hand, low molecular weight AXOS can be fermented by a broader range of *Bifidobacterium* species, such as *B. adolescentis*, *B. breve* and *B. longum* (Pastell et al., 2009; Rivière et al., 2014).

In this context, the main objective of this work was to deepen into the differential metabolism of AX and AXOS by bifidobacteria and to take advantage of the variability in this metabolic trait to design a differential culture medium for the rapid discrimination of AX-metabolizing *Bifidobacterium* isolates, with a focus on *B. longum* subspecies as representatives of one of the most widely used probiotics. For this purpose, a combination of culture-dependent techniques and *in silico* methods, were used.

2. Materials and methods

2.1. Strains and growth conditions

Four strains of *B. longum* subsp. *longum* (NCIMB8809, and three IPLA collection strains recovered from colonic biopsia or contents from healthy adult volunteers, 1CCM4, 2BCM3 and 7BCM1) and four strains of *B. longum* subsp. *infantis* (DSM20090, DSM20218, CECT4551 and LMG18902) were used. These bifidobacterial strains were identified through Internal Transcribed Spacer (ITS)-bifidobacterial profiling by following a methodology previously described (Milani et al., 2014). Strains were routinely grown in MRS (Biokar Diagnostics, Beauvois, France) supplemented with 0.25% L-cysteine (Sigma- Chemical Co., St. Louis, MO, USA) at 37 °C in an anaerobic chamber MG500 (Don Whitley Scientific, Yorkshire, UK) under an 80% N_2, 10% CO_2 and 10% H_2 atmosphere. Overnight cultures (18 h) were used to prepare the bacterial inoculum for all experiments, which were obtained after collecting cells by centrifugation and suspending them in the same volume of the medium without added carbon source as described below.

2.2. Growth curves in liquid medium with different carbohydrates

Growth curves were performed in a carbohydrate-free medium (CFM) which contained 10 g/L proteose peptone N°3 (Difco, BD, Biosciences, San Diego, CA), 10 g/L beef extract (Difco), 5 g/L yeast extract (BD, Bacto, NJ, USA), 1 g/L polysorbate 80 (Sigma-Aldrich, Merck, St. Louis, MO, United States), 2 g/L ammonium citrate, 5 g/L sodium acetate (Scharlau, Barcelona, Spain), 0.05 g/L manganese sulphate (Sigma-Aldrich, Merck), 2 g/L dipotassium phosphate (Merck), 0.1 g/L magnesium sulphate (Sigma-Aldrich, Merck) and 2.5 g/L L-cysteine-HCl (Sigma-Aldrich, Merck). A final 1% (w/v) concentration of one of the following different sugars was incorporated to the medium prior to bacterial inoculation: D(+)-xylose (Sigma- Aldrich, Merck, St. Louis, MO, United States), xylan (Megazyme, Wiclow, Ireland), arabinan (Megazyme), L-arabinose (BD, Bacto, NJ, USA), AX (Megazime, Wiclow, Ireland), AXOS enriched powder (kindly provided by Cargill, Minnetonka, Minnesota, U.S. USA) or glucose (Sigma-Aldrich, Merck). Freshly prepared media, supplemented with one of the carbon sources previously listed, were distributed in round-bottom 96-microwell plates. Plates were inoculated with freshly grown overnight cultures of the different bifidobacterial strains, prepared as previously described, at 1% (v/v), corresponding to a final inoculum of 8.11 ± 0.29 and 7.75 ± 0.62 log CFU/mL for *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains, respectively. Growth curves for each strain/carbohydrate combination were conducted on independent biological triplicates. Fermentations were carried out at 37 °C in the anaerobic chamber MG500 under the same atmosphere previously described. Using an EPOCH instrument (Agilent-Biotek, Santa Clara, U.S.A.) installed within the anaerobic chamber, the absorbance was measured at 600 nm every hour during 24 h, in order to monitor the growth of the bifidobacterial strains. Growth rates were estimated by fitting the growth data to the equation $Nt = N0 \times e^{\mu t}$, in which Nt and N0 are the cell densities at time t and time zero in the exponential growth phase, respectively.

Determination of viable cells at early stationary growth phase was performed on independent biological triplicate growth curves performed on 5 ml of CFM supplemented with 1% lactose, AX or AXOS and incubated at 37 °C under the above indicated conditions.

2.3. Analysis of carbohydrates consumption in liquid cultures

In order to determine the pattern of carbohydrates consumption in the presence of complex AX and/or AXOS mixtures, the eight model strains were inoculated at 1% in CFM, CFM with 1% AX (w/v), CFM with 1% AXOS (w/v) and CFM with 1% (w/v) AX and 1% AXOS (w/v). These cultures were grown in the anaerobic chamber at 37 °C, and samples were collected at 0, 6, 10, 24 and 48 h. Then, samples were centrifuged at 4000 rpm and the supernatants were purified with Carrez salts (Moreno et al., 1999) and analyzed by GD-FID following the methodology previously described by Sabater, Corzo, Olano & Montilla (2018), with an oven initial temperature of 120 °C, increased at a rate of 3 °C/min to 380 °C and held for 10 min.

Independent biological duplicates were performed for each culture. The characterization of AX and AXOS was based on the determination of the low molecular weight carbohydrates (LMWC, from mono- to heptasaccharides) content, and the monomeric composition after hydrolysis with trifluoroacetic acid by GC-FID (Sabater, Corzo, Olano, & Montilla, 2018).

2.4. Growth in solid medium

Among the sugars used in the growth curves, AX and AXOS were selected to formulate a solid differential medium to aid the rapid discrimination of AX-metabolizing capabilities in the two *B. longum* strains subspecies. Different concentrations of these substrates (0.5%, 1% and 2% w/v) were incorporated to CFM agar plates, and the capability of the 8 *Bifidobacterium* strains to grow on each media was evaluated. For this purpose, actively growing overnight cultures in MRSc of each strain were washed in CFM without added carbon source, appropriately diluted, and spread on CFM agar plates supplemented with different concentrations of AX or AXOS. Seeded plates were grown in an anaerobic chamber at 37 °C for 48 h prior to evaluation of colony and precipitation halo formation.

2.5. Growth recovery and discrimination of Bifidobacterium longum subsp. longum strains from human fecal microbiota samples using CFM-AX-AXOS

Since the different subspecies under study, *B. longum* subsp. *longum* and *B. longum* subsp. *infantis,* originated colonies with different visual appearance, a novel CFM medium combining 1% (w/v) of both AX and AXOS (CFM-AX-AXOS) was evaluated as a differential media to support growth of both subspecies, while facilitating the discrimination of colonies belonging to the two subspecies by visual inspection. In order to verify, as a proof-of-principle, whether the formulated CFM-AX-AXOS media could aid to identify and discriminate AX-metabolizing *B. longum* subsp. *longum* strains and non AX-metabolizing *B. longum* subsp. *infantis* strains when present in a complex fecal microbiota sample, representative strains of both subspecies were spiked in a human fecal sample. For this purpose, a preserved fecal sample previously analyzed by means of 16S rRNA sequencing and which had been demonstrated not to harbor any bifidobacterial assigned reads (sample HD11 from Hevia et al., 2016), was used. This sample was supplemented with 10^8 cells of either *B. longum* subsp. *infantis* (CECT4551), *B. longum* subsp. *longum* (NCIMB8809), or with a combination of both strains. The artificially *Bifidobacterium* enriched fecal samples were vortex-homogenized in the anaerobic cabinet and serial dilutions were spread on CFM-AX-AXOS plates, which were further incubated anaerobically for at least 48 h.

The applicability of CFM-AX-AXOS media to recover and discriminate *B. longum* subsp. *infantis* and *B. longum* subsp. *longum* colonies from fecal samples was further validated on freshly collected fecal samples. For this purpose, fecal samples from 8 healthy lactating infants (with ages ranging from 3 months to 2 years), which had not taken any antibiotics in the previous 6 months, were collected. Participants were recruited under the project PID2019-104546RB-100, and the study was approved by the Regional Ethics Committee for Clinical Research of Principality of Asturias (Ref. 343/19). These samples were homogenized in phosphate buffered saline (PBS) and serial dilutions were spread on CFM-AX-AXOS supplemented with lithium mupirocin solution (MUP, Merck, Germany: 50 mg/L) (CFM-AX-AXOSm) to increase its selectivity towards bifidobacteria (Margolles and Ruiz, 2021). Plates were incubated in an anaerobic chamber under the aforementioned conditions.

In order to confirm the species and subspecies assignation of the colonies recovered from CFM-AX-AXOS media, a random selection of colonies (at least 10 from each fecal sample) were recovered and identified through partial 16S rRNA sequencing. For this purpose, colonies were subjected to small-scale total DNA extraction with chloroform (Ruiz-Barba et al., 2005) and 16S rRNA region was amplified and sequenced by Sanger technology as previously described by using primers 27F and 1492R (Frank et al., 2008). Further confirmation of the assignation to *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* subspecies, was performed through PCR detection of the gene encoding the urease (subunit C), as a genetic marker commonly associated to *B. longum* subsp. *infantis* (Schimmel et al., 2021). For this purpose the oligonucleotides ureC-Fwd (5′- GCCGGATTGAAGATCCACG-3′) and ureC-Rev (5′-CCGCGATGGTGTCATTG-3′), designed to amplify an internal fragment of the urease subunit C gene in *B. longum* subsp. *infantis*, were used.

2.6. Genome sequencing of Bifidobacterium longum strains

Commercial kit DNeasy Blood & Tissue from QIAGEN was used for DNA extraction (Qiagen GmbH, Germany) of *B. longum* subsp. *longum* strains (1CCM4, 2BCM3 and 7BCM1) and *B. longum* subsp. *infantis* strains (DSM20090, DSM20218 and LMG18902). DNA extraction protocol was followed according to the manufactureŕs instructions with the following modifications. A prior enzymatic lysis step was conducted by suspending the cells in a lysis buffer composed of 20 mM Tris-HCl (GE Healthcare, Illinois, USA) (pH 8), 2 mM sodium EDTA (AlfaAesar, Massachusetts, USA), 1.2% triton X-100 (Sigma-Aldrich, Merck), 20 mg/mL lysozyme (Sigma-Aldrich, Merck) and 25 U/mL mutanolysin (Sigma-Aldrich, Merck), and incubating the suspensions for 30 min at 37 °C. Afterwards, lysed suspension was used for DNA purification by following the manufacturer's instructions.

Genome sequencing of *B. longum* strains was performed at GenProbio S.R.L. (Parma, Italy), with an Illumina MiSeq Sequencing System. Genome assemblies of bifidobacterial genomes were performed with SPAdes v3.14.0 by means of MEGAnnotator pipeline (Lugli et al. 2016). Reordering of the final contigs compared with National Center for Biotechnology Information (NCBI) template genomes of *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* were performed with Mauve v2.3.1. Average coverage ranged from 71 to 133 while the number of contigs ranged from 19 to 46. Raw sequences are deposited in Sequence Read Archive (SRA) of the NCBI (https://www.ncbi.nlm.nih.gov/sra, accessed on 9 March 2023) under BioProject code PRJNA851776, BioSample codes SAMN29251405 - SAMN29251419. Reference genomes from *B. longum* subsp. *longum* NCIMB8809 (GenBank identifier CP011964.1) and *B. longum* subsp. *infantis* CECT4551 (GenBank identifier AP010889.1) were collected from public repositories.

2.7. 16S rRNA and bifidobacterial ITS profiling of infant fecal samples

DNA from the infant fecal samples collected for *Bifidobacterium* strains isolation, was extracted by using the DNeasy PowerSoil Pro Kit (Qiagen GmbH, Germany) according to manufacturer's instructions. Then, 16S rRNA V3 regions were amplified using the primer's pair Probio_Uni and /Probio_Rev, as previously described (Milani et al., 2013) and samples were submitted to 2 × 250 bp paired-end sequencing in an Illumina MiSeq System instrument (Illumina) at GenProbio S.R.L. (Parma, Italy). Sequence reads were quality filtered by the Illumina

software and then trimmed, and filtered sequences were processed with a custom script based on the QIIME software suite (Caporaso et al., 2010). On the other hand, the bifidobacterial community composition of the fecal samples was analyzed through targeted amplicon sequencing of ITS regions, which were sequenced and analyzed according to previously described procedures (Milani et al., 2014). Raw sequences generated are deposited in the SRA of the NCBI (https://www.ncbi.nlm.nih.gov/sra, accessed on 9 March 2023) under BioProject code PRJNA851776.

2.8. Bioinformatic analysis

To gain a better understanding of metabolic capabilities of *Bifidobacterium* species associated to AX and AXOS consumption, several bioinformatic analyses were carried out. In this sense, a comprehensive dataset (n = 707) comprising reference genomes and genome assemblies from *Bifidobacterium* species that have the Qualified Presumption of Safety (QPS) status according to the European Safety Authority (EFSA) was retrieved from the European Nucleotide Archive (ENA) (Supplementary material Table S1): *B. adolescentis* (n = 293), *B. animalis* subsp. *animalis* (n = 9), *B. animalis* subsp. *lactis* (n = 36), *B. bifidum* (n = 102), *B. breve* (n = 68), *B. longum* subsp. *infantis* (n = 30), *B. longum* subsp. *longum* (n = 169). In addition, reference genomes and genome assemblies from other *Bifidobacterium* species from human origin (n = 320) were included in the study for comparative purposes: *B. angulatum* (n = 5), *B. catenulatum* (n = 12), *B. dentium* (n = 22), *B. faecale* (n = 1), *B. gallicum* (n = 4), *B. pseudocatenulatum* (n = 271), *B. scardovii* (n = 5).

These reference sequences from a total 12 representative *Bifidobacterium* species were mapped against the Carbohydrate-Active enZYmes Database (CAZy, https://www.cazy.org/last accessed: February 11, 2022) using "run_dbcan" software developed by Zhang et al. (2018). This computational pipeline integrates HMMER software for biosequence analysis using profile hidden Markov models, allowing functional domain annotation of bacterial glycosidases. To ensure the quality of the data generated, only glycosidase domains showing coverage values higher than 0.95 were chosen. *Bifidobacterium* species were grouped according to the glycosidase domains found in their genomes through complete linkage hierarchical clustering. In this method, all pairwise dissimilarities between the elements in each cluster (i.e., glycosidase profiles of *Bifidobacterium* species) are calculated using the basic function "hclust" from R v.3.6.2 programming environment. Then, the largest dissimilarity value is chosen as the distance between clusters. Therefore, this method produces more compact clusters. Then, heatmaps illustrating the presence and absence of functional domains were generated.

Once glycosidase profiles of bifidobacteria were elucidated at species level, a second bioinformatic analysis focusing on the genome sequences of the model *B. longum* subsp. *longum* strains (1CCM4, 2BCM3, 7BCM1 and NCIMB8809) and *B. longum* subsp. *infantis* strains (CECT4551, DSM20090, DSM20218 and LMG18902) utilized in the *in vitro* growth experiments previously described was performed. These sequences were analysed following the computational pipeline above described.

3. Results

3.1. In silico study of glycosidase profiles of bifidobacteria related to carbon sources containing arabinose and xylose

As a first approximation, an *in silico* analysis was conducted to delineate the variation in the genetic attributes that could sustain the metabolism of AX, AXOS, XOS and glucan-oligosaccharides, such as cello-oligosaccharides (COS), commonly found in AX rich biomasses, across genomes representative of various species and subspecies of the genus *Bifidobacterium*. For this purpose, functional domain prediction and annotation of glycosidase activities capable of degrading the aforementioned carbohydrate structures was conducted in genomes from *Bifidobacterium* species that have the QPS status (n = 707), as well as other bifidobacterial species commonly present in the human gut (n = 320) (Fig. 1).

According to the results obtained, most bifidobacterial species that have the QPS status exhibit similarities in their carbohydrate metabolism capabilities; yet they show notable differences in the presence of specific glycosidase domains. Specific domains involved in the degradation of AX, AXOS, XOS, β-*gluco*-oligosaccharides, such as COS, and some of its constituent residues such as xylose or arabinose, were identified across the whole dataset, although their prevalence significantly differed across species. For instance, glycosidase domains acting on arabinose residues were less prevalent in *B. animalis* subsp. *animalis* and *B. gallicum* genomes; while those acting on xylose residues were less prevalent in the genomes from *B. longum* subsp. *infantis, B. bifidum, B. adolescentis* and *B. pseudocatenulatum* (Supplementary material Table S2). On the other hand, among the species recognized with the QPS status, *B. longum* subsp. *infantis, B. breve* and *B. bifidum* genomes displayed the lowest prevalence of functional domains involved in AXOS and XOS hydrolysis, suggesting a poor capacity to metabolise those substrates. Interestingly, a higher number of genomes from *Bifidobacterium longum* subsp. *longum* showed glycosidases of interest as compared to *B. longum* subsp. *infantis* (Supplementary material Table S2). These activities involve functional domains acting on: i) AXOS, COS and XOS (89.2 and 66.1% genomes from *Bifidobacterium longum* subsp. *longum* and *B. longum* subsp. *infantis*, respectively), ii) AXOS and XOS (69.4 and 13.6%, respectively), iii) XOS (69.8 and 20.0%, respectively). Therefore, notable differences in the metabolism of arabinose- and xylose-containing polysaccharides of *Bifidobacterium longum* subsp. *longum* and *B. longum* subsp. *infantis* have been determined. These metabolic activities could be of great interest to discriminate these two subspecies and to design target-specific prebiotics for *B. longum* subsp. *longum*.

3.2. A focus on metabolism of carbon sources containing arabinose and xylose by a collection of strains of B. longum subsp. longum and B. longum subsp. infantis

3.2.1. AX and AXOS metabolism in a selection of B. longum strains: In silico and in vitro analyses

A complementary *in silico* analysis on the genomes of a selection of eight *B. longum* model strains available at the IPLA collection was performed so as to deepen in the genetic signatures that may determine a differential AX/AXOS metabolization by both subspecies. The glycosidase-encoding genes from these strains, enable to cluster them according to their subspecies classification (*B. longum* subsp. *longum* and *B. longum* subsp. *infantis*) suggesting characteristic patterns in the carbohydrate metabolism of both subspecies (Fig. 2). Whereas glycosidase domains capable of degrading arabinose monomers from AXOS as well as unspecific domains acting on AXOS, COS and XOS were present in the genome sequences of all strains, functional domains associated to AXOS and XOS metabolism, such as arabinofuranosidases and xylanases, were mainly found in *B. longum* subsp. *longum* strains (72.7–100.0% of total domains acting on AXOS and XOS were present in the genomes from *B. longum* subsp. *longum* strains; Fig. 2 and Supplementary material Table S4). Besides, a wider variety of these domains was found in genomes from strains 1CCM4 and NCIMB8809 (100.0 and 90.9% of total domains in 1CCM4 and NCIMB8809 genomes, respectively; Supplementary material Table S4) than in the strains 7BCM1 and 2BCM3 (72.7 and 81.8% of total domains in 7BCM1 and 2BCM3 genomes, respectively; Supplementary material Table S4) (Fig. 2). The characteristic enzyme patterns elucidated for *B. longum* subsp. *longum* at strain level (Fig. 2) are consistent with those obtained at species level (Fig. 1), and they suggest that *B. longum* subsp. *longum* might be better equipped to metabolise more structurally complex AX chains than *B. longum* subsp. *infantis,* in agreement with previous bioinformatic studies (Blanco et al., 2020).

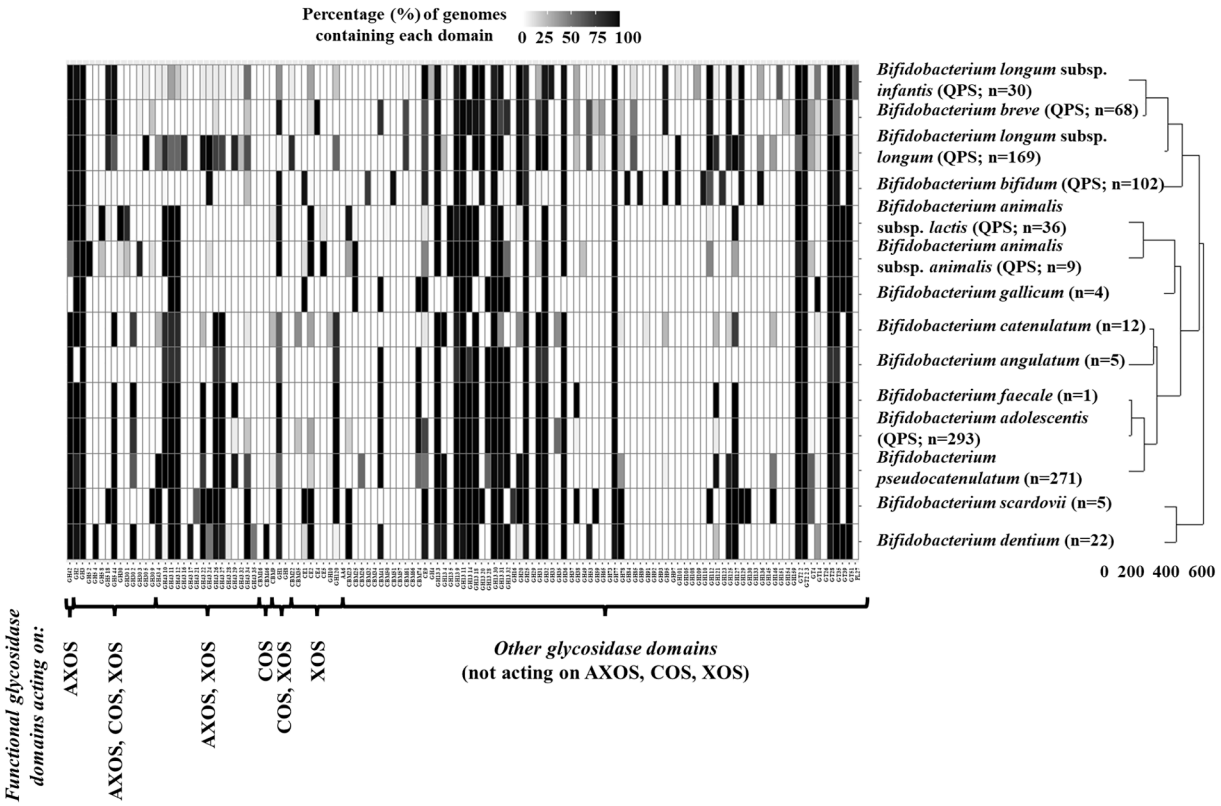

Fig. 1. Heatmap showing the presence of different glycosidases (indicated as grey and black cells) in the reference genomes and genome assemblies (n = 1027) of 14 *Bifidobacterium* species that may be present in human gut microbiota. The percentage (%) of reference genomes and genome assemblies from each species containing each functional domain is shown. Specifically, functional domains involving glycosidases capable of degrading arabinoxylan and arabinoxylo-oligosaccharides (**AXOS**), cello-oligosaccharides (**COS**) and *xylo*-oligosaccharides (**XOS**) are illustrated. Glycosidase functional domains showing coverage values higher than 0.95 were annotated. Codes corresponding to the CAZy family of each enzyme have been assigned. More information about these families can be found in the Carbohydrate-Active enZYmes Database (CAZy). Similarities between *Bifidobacterium* species are also illustrated in a dendrogram and expressed as distances between their characteristic glycosidase profiles calculated by the complete linkage method (vertical axis). Some of these species have the Qualified Presumption of Safety (**QPS**) status.

In order to establish whether the differential metabolic traits *in silico* predicted correlate with actual AX/AXOS metabolization patterns, the ability of the eight *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains to grow in the presence of a range of arabinose and xylose containing oligo- and polysaccharides was elucidated *in vitro*. Results demonstrated that most *B. longum* subsp. *longum* tested strains were able to grow on the majority of the substrates under study (xylose, arabinose, arabinan, AX and AXOS), initiating their exponential growth phase at around 6 h and reaching OD_{600} values above 0.5 within 24 h (Fig. 3). Remarkably, all the strains tested grew in media supplemented with AXOS (exhibiting growth rates of 0.35 ± 0.03 and 0.31 ± 0.02 in *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains, respectively); although only *B. longum* subsp. *longum* strains grew in AX-supplemented media, reflected by growth rates of 0.42 ± 0.06 and 0.02 ± 0.01 in *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains, respectively. The differential ability of representative *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains to grow on AX was confirmed through colony counting from independent cultures grown for 14 h in the presence of AX (Supplementary Fig. S1). Of note, no significant differences in the growth rates were observed in the carbon sources used as positive control (lactose: 0.51 ± 0.12 and 0.43 ± 0.09 in *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains, respectively; maltose: 0.46 ± 0.07 and 0.39 ± 0.14 in *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains, respectively), neither in the final viable counts obtained following growth in lactose (9.78 ± 0.07 and 9.49 ± 0.03 log CFU/mL in *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains, respectively) (Supplementary Fig. S1). These results suggest that, in agreement with previous studies and with the *in silico* analysis previously described, the enzymatic machinery in *B. longum* subsp. *longum* strains enable the cells to metabolize more complex polysaccharides, such as AX, unlike other bifidobacterial species and subspecies (Komeno et al., 2019).

3.2.2. Carbohydrate analysis and metabolization by a collection of *B. longum* strains

Characterisation of AX and AXOS.

In an attempt to identify the specific AX and AXOS fractions that were metabolized differentially by the *B. longum* subsp. *longum* and *B. longum* susbp. *infantis* strains investigated GC-FID analysis was performed on both AX and AXOS powders, to determine LMWC and monomeric composition prior acid hydrolysis (Supplementary material Table S3), in order to have a more detailed information on their composition. The analysis of the AXOS powder allowed the detection and quantification of AXOS chains with a degree of polymerisation (DP) ranging from 2 to 7, which comprised 76% of the total carbohydrates present in the mixture, based on monomeric composition (Supplementary material Table S3). In addition, β-*gluco*-oligosaccharide fractions (mainly, COS) exhibiting polymeric series (DP from 2 to 5) were also found at lower concentrations than AXOS, comprising 22% of total carbohydrates, based on monomer composition. Concerning the AX powder, no oligosaccharide fraction could be determined in the GC-FID analysis. Monomeric composition analysis evidenced that AX was an arabinose-enriched substrate (arabinose:xylose ratio 1:2) while glucose was present in negligible amounts (arabinose + xylose:glucose ratio 59:1). On the contrary, AXOS powder contained a much more reduced content in arabinose (arabinose:xylose ratio 1:14), as compared to AX,

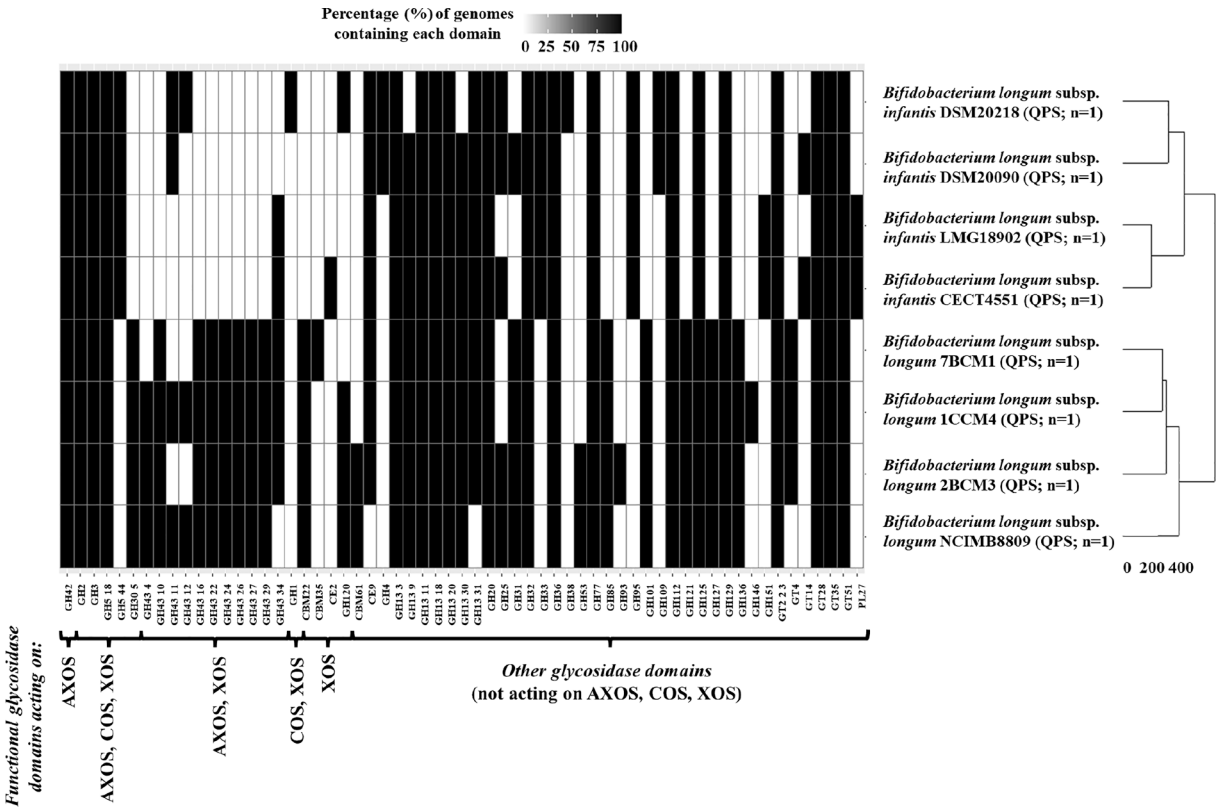

Fig. 2. Heatmap showing the presence of different glycosidases (indicated as black cells) in the genome sequences of 4 *Bifidobacterium longum* subsp. *longum* strains (1CCM4, 2BCM3, 7BCM1 and NCIMB8809) and 4 *B. longum* subsp. *infantis* strains (CECT4551, DSM20090, DSM20218 and LMG18902). The percentage (%) of genomes from each species containing each functional domain is shown. Specifically, functional domains involving glycosidases capable of degrading arabinoxylan and arabinoxylo-oligosaccharides (AXOS), cello-oligosaccharides (COS) and *xylo*-oligosaccharides (XOS) are illustrated. Glycosidase functional domains showing coverage values higher than 0.95 were annotated. Codes corresponding to the CAZy family of each enzyme have been assigned. More information about these families can be found in the Carbohydrate-Active enZYmes Database (CAZy). Similarities between *Bifidobacterium longum* strains are also illustrated in a dendogram and expressed as distances between their characteristic glycosidase profiles calculated by the complete linkage method (vertical axis). These strains have the Qualified Presumption of Safety (QPS) status.

also having considerable levels of glucose residues derived from COS (arabinose + xylose:glucose ratio 3.5:1).

3.3. AXOS and AX metabolism by Bifidobacterium longum

GC-FID analysis was also conducted to determine AXOS consumption on culture supernatants of the eight model strains following growth in the presence of either AXOS or a combination of AX and AXOS (Fig. 4). On the one hand, all *B. longum* subsp. *longum* strains showed a higher consumption of AXOS along the first 24 h of growth, as compared to the *B. longum* subsp. *infantis* strains, both in AXOS and AXOS + AX supplemented media. With regard to *B. longum* subsp. *infantis*, their AXOS carbohydrate consumption rates were generally lower than those exhibited by *B. longum* subsp. *longum* cultures. On the other hand, all *B. longum* subsp. *longum* strains showed a similar behavior after 48 h in AXOS-supplemented medium. However, most *B. longum* subsp. *longum* strains showed lower AXOS consumption when grown in the presence of AXOS + AX, than those observed in media supplemented only with AXOS. This fact may be attributed to its plausible utilization of AX, supported by a larger number of glycosidase domains involved in AX metabolization, and that could lead to the preferential consumption of enriched-arabinose polysaccharide fractions from AX instead of smaller AXOS molecules. This also suggests that monomeric composition could play a key role in AX and AXOS fermentability. It is also worth noting that AX metabolization by *B. longum* subsp. *longum* strains may release AXOS, leading to AXOS augmentation, as observed for strains 1CCM4 and 7BCM1 at 48 h (Fig. 4**D**), thus masking the actual AXOS consumption in cultures. On the contrary, AX structures might not be easily metabolized by most *B. longum* subsp. *infantis* strains, which predominantly metabolize low-DP AXOS rich in xylose. Taking into account that AX showed higher arabinose contents than AXOS (Supplementary material Table S3), its preferential metabolization by *B. longum* subsp. *longum* strains might be linked to a most prevalent representation of activities associated to arabinose metabolism in this subspecies as compared to *B. longum* subsp. *infantis* strains, as previously suggested by other works (Blanco et al., 2020) and by the *in silico* analysis previously described.

3.4. Formulation of solid medium to discriminate AX and AXOS metabolization capacity in bifidobacteria

In view of the differential ability of *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* strains to grow and metabolise AX and AXOS, as supported by growth profiles, sugar consumption and *in silico* genome analyses, we subsequently aimed at formulating a medium to aid rapidly discriminating bifidobacterial isolates based on its AX metabolism. For this purpose, the growth of selected strains of both subspecies was evaluated on CFM solid media supplemented with AXOS and/or AX as the only carbon sources. This analysis confirmed that, while *B. longum* subsp. *longum* was capable to grow and form colonies in all the media tested, *B. longum* subsp. *infantis* did only grow with AXOS, not being able to form any colonies on AX (Supplementary material Table S5 and Supplementary Fig. S2). Besides, it was very characteristic the appearance of a halo of precipitation surrounding the colonies of *B. longum*

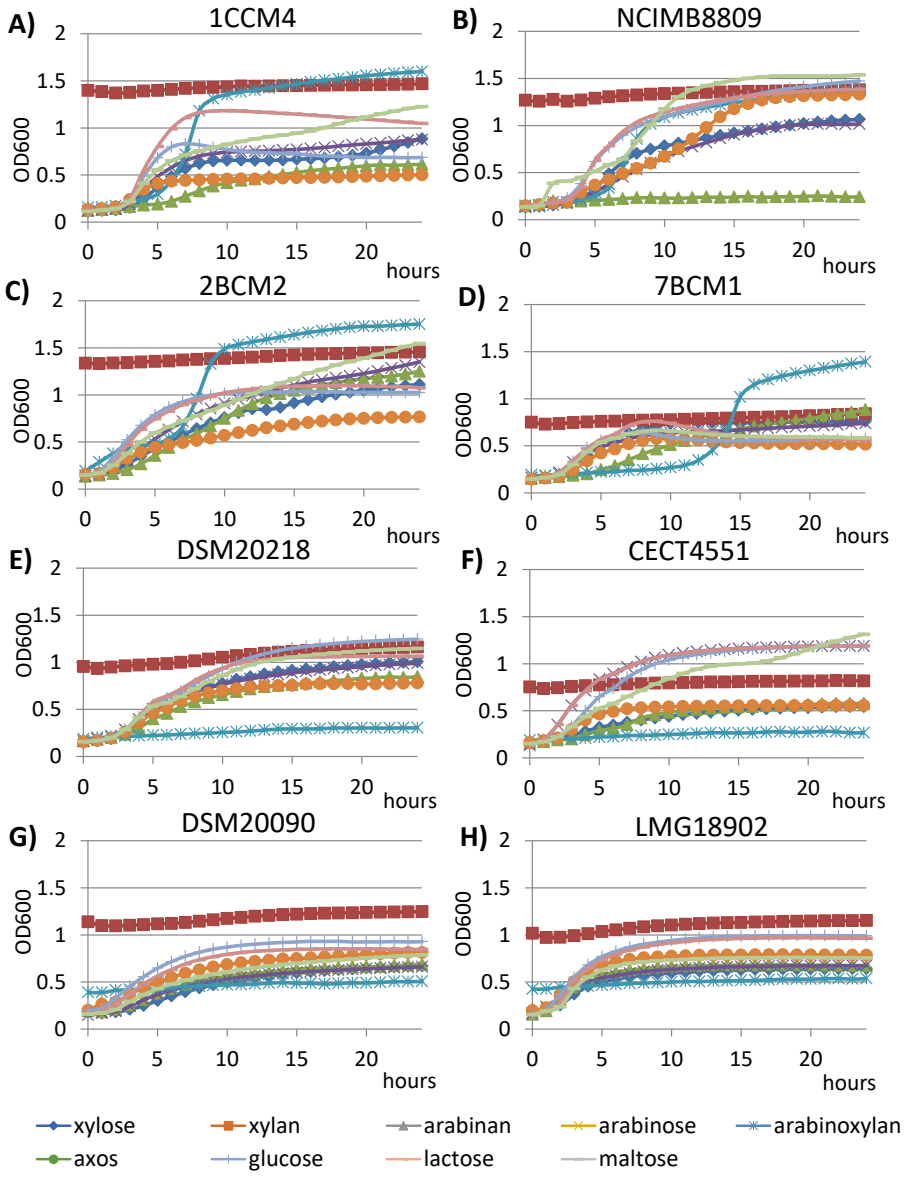

Fig. 3. Growth curves of four strains of *Bifidobacterium longum* subsp. *longum* (1CCM4, **A**; NCIMB8809, **B**; 2BCM2, **C**; 7BCM1, **D**) and *Bifidobacterium longum* subsp. *infantis* (DSM20218, **E**; CECT4551, **F**; DSM20090, **G**; LMG18902, **H**). Strains were grown in carbohydrate-free medium (**CFM**) with different sugars; xylan, xylose, arabinose, arabinan, arabinoxylan, arabinoxylo-oligosaccharides (**AXOS**), glucose, lactose and maltose during 24 h. Average of independent biological triplicates are represented.

subsp. *longum* strains when grown in CFM-AX (Supplementary Fig. S2), which was not observed for any of the strains in CFM-AXOS. In view of these differences, we subsequently aimed at formulating a CFM-based medium, which could support the growth of both subspecies simultaneously, while enabling to discriminate AX metabolizing colonies by macroscopic inspection. In this regards, a CFM media supplemented with a mixture of AX and AXOS, CFM-AX-AXOS, was demonstrated effective to recover and discriminate by visual inspection AX-metabolizing *B. longum* strains from complex samples (fecal samples artificially enriched in one or other subpsecies), as *B. longum* subsp. *longum* form larger colonies surrounded by a precipitation halo, whereas *B. longum* subsp. *infantis* form much smaller colonies with no precipitation halo (Fig. 5). The appearance of this precipitation may be attributed to an incomplete metabolization of AX by *B. longum* subsp. *longum,* and may correspond to non-degraded and/or partially degraded AX chains, and allows discriminating strains co-existing in the same sample and harboring differential AX metabolization capabilities. The subspecies identity of a random selection of colonies obtained from the sample containing both subspecies was confirmed through colony recovery and partial 16S rDNA sequencing (data not shown).

CFM-AX-AXOS media performance to isolate and discriminate bifidobacterial isolates, based on their AX-metabolizing capacity, was also evaluated on fecal samples freshly collected from lactating infants (n = 8). Macroscopic visualization of the colonies and isolates identity confirmation through partial 16S rRNA sequencing, verified that a number of bifidobacterial species different from *B. longum* can grow in CFM-AX-AXOSm. These include *B. adolescentis*, *B. bifidum, B. breve, B. dentium, B. pseudocatenulatum* and *B. suillum* (Fig. 6). Remarkably, 100% of the colonies displaying the characteristic precipitation halo were further identified as *B. longum* subsp. *longum*, based on 16S rRNA sequencing, while all of the non-halo formers were presumptively identified as belonging to *B. longum* subsp. *infantis,* based on 16S rRNA sequencing and on the detection of the urease gene, or to bifidobacterial species other than *B. longum* (Fig. 6 and Supplementary material Table S6). This corroborates CFM-AX-AXOS is a valuable media to rapidly detect AX-metabolising bifidobacterial species including *B. longum* subsp. *longum*, as well as the key role of this subspecies as primary degrader of complex AX. Besides, the pattern of bifidobacterial species recovered from the samples is highly consistent with the species detected through bifidobacterial ITS-sequencing in the corresponding samples (Supplementary Fig. S3). Thus these results confirm that CFM-AX-AXOS media enable to recover a wide range of bifidobacterial

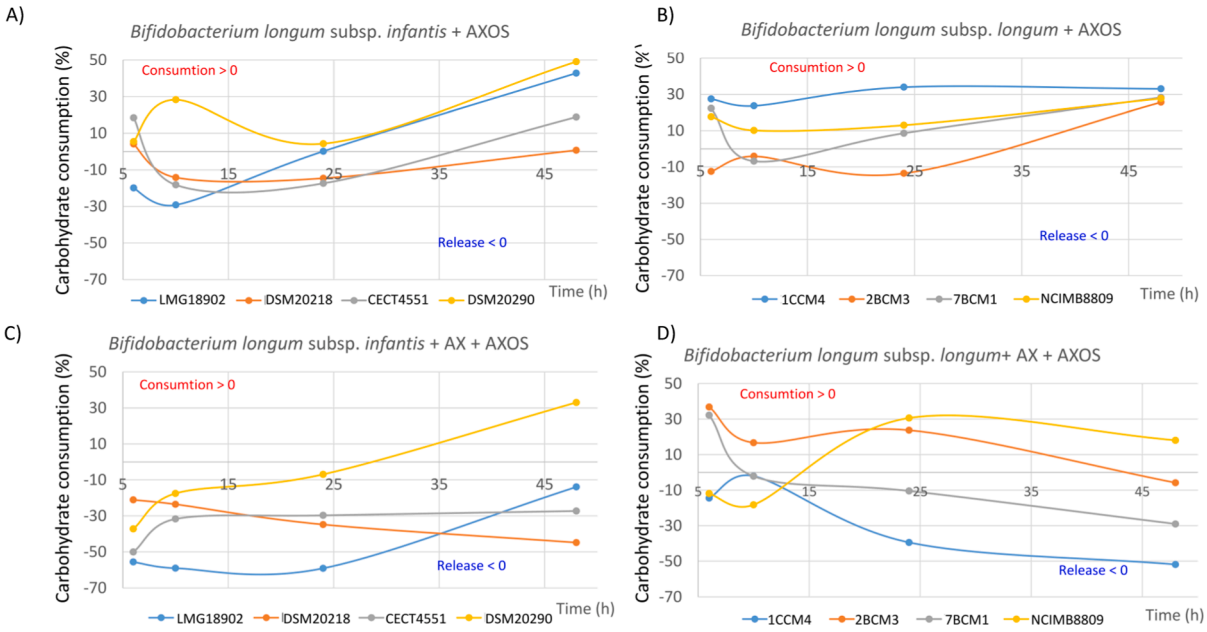

Fig. 4. Arabinoxylo-oligosaccharide (AXOS) consumption (%) determined for *Bifidobacterium longum* subsp. *infantis* (A, C) and *Bifidobacterium longum* subsp. *longum* (B, D) strains in culture media supplemented with AXOS (A, B) or AXOS and arabinoxylan (AX) mixtures (C, D). Results represent average of independent biological duplicates.

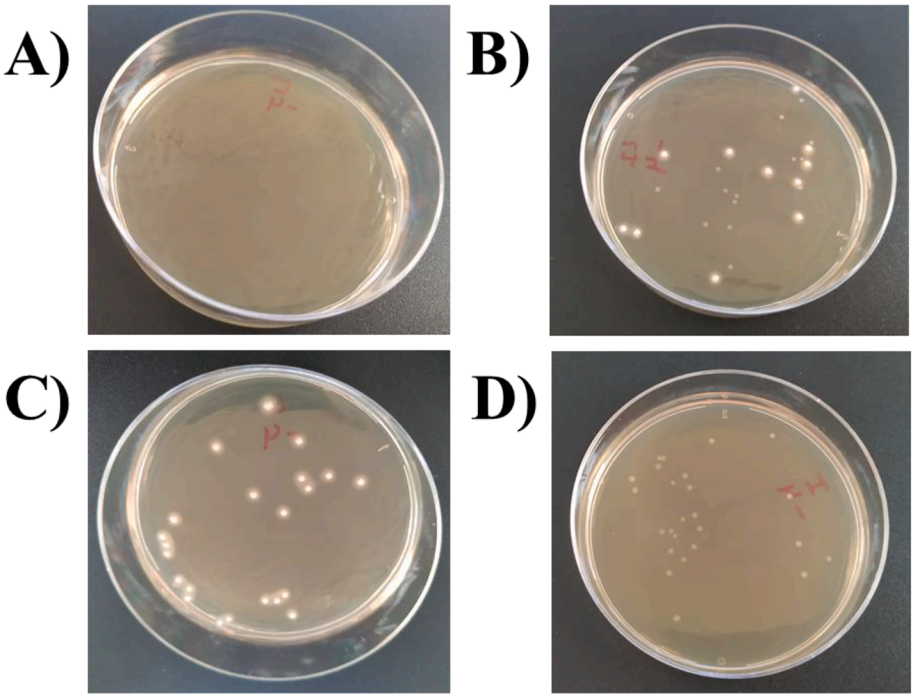

Fig. 5. Recovery of *Bifidobacterium longum* subsp. *infantis* (CECT4551) and *B. longum* subsp. *longum* (NCIMB8809) from a bifidobacterial enriched fecal sample in solid carbohydrate-free medium combining AX and AXOS (CFM-AX-AXOS). A) Control, microbiota without bifidobacteria. B) Microbiota supplemented with *B. longum* subsp. *infantis* and *B. longum* subsp. *longum* cultures. C) Microbiota supplemented with *B. longum* subsp. *longum* culture. D) Microbiota supplemented with *B. longum* subsp. *infantis* culture.

species from complex samples, yet they enable an easy and rapid discrimination with the naked eye of AX-metabolizing *B. longum* subsp. *longum* isolates.

4. Discussion

Bifidobacterium longum is commonly present in the human gut and several strains have interest as probiotics, with different subspecies exhibiting adaptation to carbohydrates characteristic in either the infant or adult diet (Russell et al., 2011). Among the latest, AX is a major constituent of dietary fiber from cereal grains that is mainly metabolised in the large intestine by bifidobacteria, what generally involves an initial arabinose cleavage, with or without subsequent xylose backbone utilisation (Liu et al., 2020; Lynch et al., 2021; Rivière et al., 2014; Saito et al., 2020). Besides, AX and particularly AXOS, are considered emergent prebiotics that stimulate the growth of bifidobacteria and the production of butyrate in the human colon (Rivière et al., 2015; Sabater et al., 2021). Their capacity to impact the gut microbiota has been confirmed through several clinical trials that revealed that *B. longum* was significantly more abundant in the microbiota of healthy, elderly and overweight volunteers following AXOS supplementation (Chung et al., 2020; Benítez-Páez et al., 2019; Cloetens et al., 2010). Therefore, AXOS

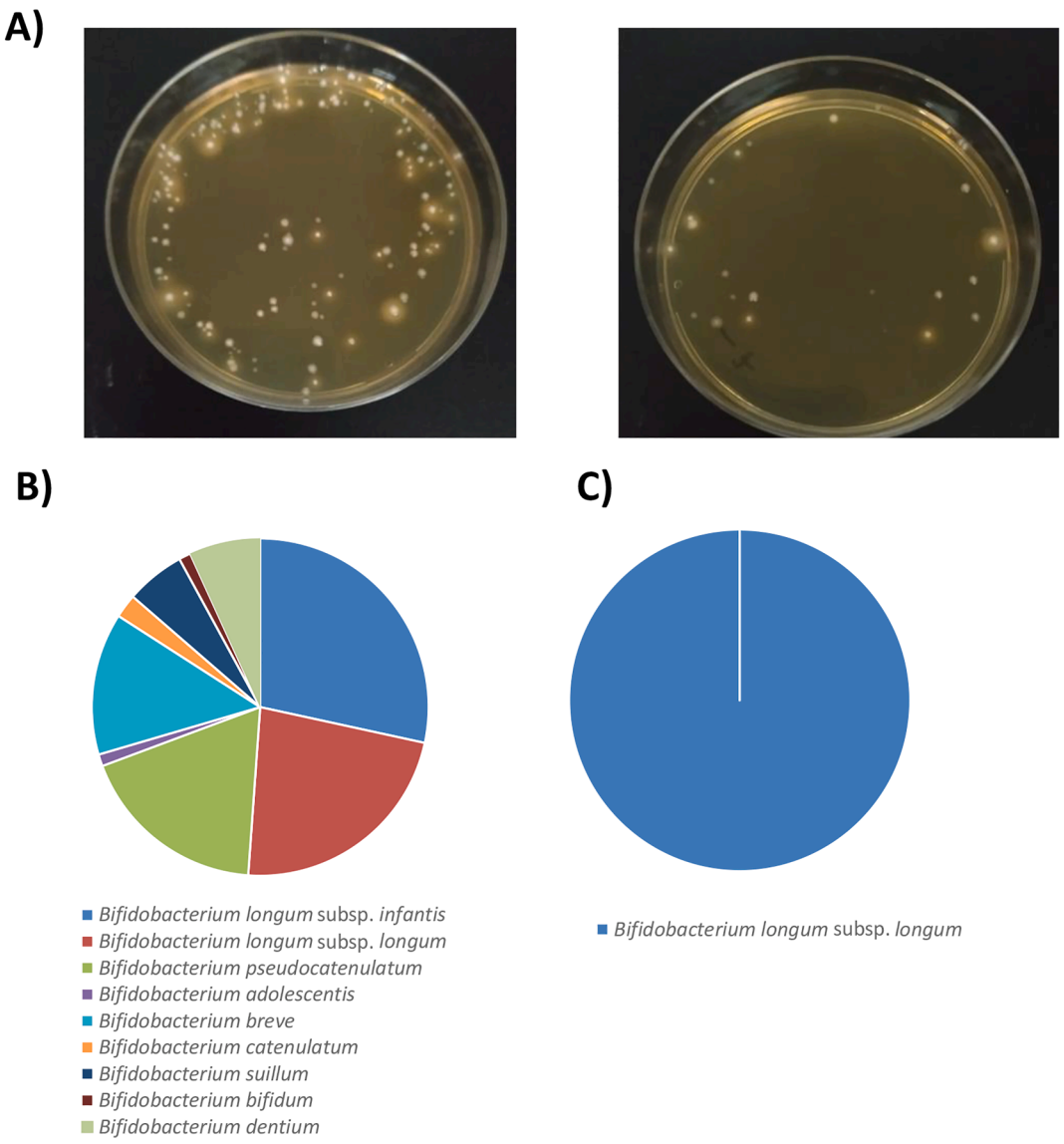

Fig. 6. Representative CFM-AX-AXOSm plates (carbohydrate-free medium (**CFM**) supplemented with 1% arabinoxylan (**AX**), 1% arabinoxylooligosaccharides (**AXOS**) and mupirocin) spread with an infant fecal sample, showing appearance of colonies surrounded by a precipitation halo as well as colonies lacking the precipitation halo **(A)**. Distribution of *Bifidobacterium* species among the isolates identified (n = 88) (including halo formers and non-halo formers) from infant fecal samples in CFM-AS-AXOSm. Species were identified through 16S rRNA sequencing and PCR detection of urease gene was used for identification of *B. longum* subsp. *infantis* **(B)**. Distribution of *Bifidobacterium* species exhibiting a halo of precipitation surrounding the corresponding colonies, as determined through 16S rRNA sequencing **(C).**

may be considered a viable prebiotic option. However, only particular bifidobacterial strains belonging to a few species, including *B. longum*, exhibit the required capabilities to achieve its metabolization (Song et al., 2020). For these reasons, understanding AX and AXOS metabolization by beneficial commensals and probiotics species, such as those belonging to *Bifidobacterium*, is of great interest to develop novel functional foods including AX-metabolising probiotic species, AX-based prebiotics or synbiotics.

In silico analysis on genome sequences from *Bifidobacterium* species commonly found in the human gut, confirm a broader metabolic capacity to metabolise AX, AXOS and XOS of the species *B. longum* and, specifically, of *B. longum* subsp. *longum* as compared to *B. longum* subsp. *infantis*. These results agree with results from *in vitro* tests herein performed with a selection of strains and corroborate a better ability of the subspecies *longum* to metabolise the more structurally complex AX. Some studies have suggested that AX can be primarily metabolized by *B. longum*, which in this way may establish symbiotic relationships with other bifidobacteria like *B. adolescentis* that preferentially metabolise AXOS (Pastell et al., 2009). Indeed, some *B. longum* strains appear to prefer AX or AXOS with relatively high molecular weight and could grow well with high molecular weight AX as the sole carbon source (Song et al., 2020). This pattern has also been observed in our work, since only *B. longum* subsp. *longum* strains tested were capable to grow on AX, whereas *B. longum* subsp. *infantis* were not able to metabolise and grow on the high molecular weight AX (Fig. 3 and Supplementary Table S5). Interestingly, bioinformatic analysis performed in this work revealed that, across the analysed species, *B. longum* subsp. *longum* showed the highest number of glycosidases that may hydrolyse xylose backbones, although domains comprising arabinofuranosidases and xylosidases acting on AXOS have also been found in *B. pseudocatenulatum* genomes in agreement with other authors (Saito et al., 2020).

Differential glycosidase profiles may be useful to provide a theoretical background for metabolic cooperation between bifidobacteria; as

well as to assist the rational design of novel probiotics, prebiotics and synbiotic combinations. Interestingly, some *B. longum* subsp. *longum* strains have been suggested to establish symbiotic relationships with intestinal commensals other than bifidobacteria, such as *Eubacterium rectale* or *Bacteroides* species, leading to beneficial short-chain fatty acids production and overall modification of the fecal microbiota profiles (Fernandez-Julia, Commane, van Sinderen, & Munoz-Munoz, 2022; Liu et al., 2020; Moens, Weckx, & De Vuyst, 2016; Rivière, Gagnon, Weckx, Roy, & De Vuyst, 2015; Rivière, Selak, Geirnaert, Van den Abbeele, & De Vuyst, 2018). Hence, bifidobacterial strains with capacity to primary degrade complex AX-based prebiotics have great interest due to its potential to beneficially impact the gut microbiota ecosystem upon AX consumption, promoting the metabolism of key commensal species other than bifidobacteria. Results from this work show that *B. longum* subsp. *longum* strains showing several arabinoxylan-degrading domains are able to efficiently metabolise AX chains from media containing AX as the only carbon source; whilst *B. longum* subsp. *infantis* strains exhibiting a much-reduced distribution of xylan-degrading domains on their genomes, were not able to grow on AX-containing media but on AXOS-containing media. This might be attributed to a preference of *B. longum* subsp. *infantis* for low molecular weight AXOS, or for oligo- and polysaccharides with low arabinose contents.

Based on these observations, an AX and AXOS containing media, CFM-AX-AXOSm, was demonstrated effective to facilitate isolation and discrimination of potential probiotic bifidobacteria with AX metabolizing capacity. CFM-AX-AXOSm enabled selective isolation of bifidobacteria, and rapid discrimination of AX metabolizing bifidobacterial isolates as it led to a characteristic precipitation formation around the colonies when grown in solid AX-containing media. Its usefulness to efficiently isolate bifidobacteria and to discriminate AX utilizing bifidobacterial isolates was further validated on infant fecal microbiota samples. Precise identification of the specific genes/mechanisms responsible for the differential pattern of AX metabolization among *B. longum* isolates and the differential macroscopic colony aspect on AX-containing media would require comparative genome analyses and gene-trait matching analysis on a larger number of bifidobacterial isolates.

In **conclusion**, in this work, we have deepened into AX and AXOS metabolism in bifidobacteria and, particularly, in the most prevalent *B. longum* subspecies, *B. longum* subsp. *longum* and *B. longum* subsp. *infantis*, through a combination of *in silico* and *in vitro* analyses. We have also taken advantage on the macroscopic appearance of AX-metabolising bifidobacteria on AX-containing media to formulate a media that enables to recover a wide range of bifidobacterial species from complex samples, while facilitating rapid macroscopic discrimination of AX-metabolising isolates. The AX and AXOS-supplemented culture medium herein described could represent an attractive alternative to isolate novel probiotics and to rapidly discriminate their capacity to metabolize structurally complex AX-derived prebiotics. This can be convenient to assist formulation of novel functional foods, including bifidobacterial species with capacity to metabolize AX-derived prebiotic ingredients.

CRediT authorship contribution statement

Ines Calvete-Torre: Investigation, Formal analysis, Visualization, Writing – original draft. **Carlos Sabater:** Formal analysis, Visualization, Writing – original draft. **Susana Delgado:** Resources, Writing – review & editing. **Patricia Ruas-Madiedo:** Resources, Writing – review & editing. **Alicia Rupérez-García:** Investigation. **Antonia Montilla:** Investigation, Formal analysis, Writing – review & editing. **F. Javier Moreno:** Investigation, Writing – review & editing. **Abelardo Margolles:** Conceptualization, Supervision, Resources, Funding acquisition, Writing – review & editing. **Lorena Ruiz:** Conceptualization, Supervision, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Sequence data is deposited in SRA archive.

Acknowledgements

The work in our research groups was funded by the grants from the Spanish State Research Agency RTI2018-095021-J-I00, AGL2016-78311-R and AGL 2017-84614-C2-1-R (funded by MCIN/AEI/10.13039/501100011033/ and by "ERDF A way of making Europe"), PID2019-104546RB-100 (funded by MCIN/ AEI /10.13039/501100011033), and TED2021-131541B-100 funded by MCIN/AEI/10.13039/501100011033 and Europe Union "NextGenerationEU"/PRTR. We also thank the grant AYUD-2021-50910 from the autonomic Government of Principado de Asturias (FICYT, supported by FEDER), CSIC for funding this research with the grant PIE-201970E019, and grant 101060130, funded by HORIZON-WIDERA-2021-ACCESS-02. CS is a postdoctoral researcher supported by the Juan de la Cierva-Formación Postdoctoral Trainee Program from MICINN, the Spanish Ministry of Science and Innovation (FJC2019-042125-I). The authors wish to acknowledge Cargill (Minneapolis, MN, USA) for kindly providing a sample of AXOS powder and the Servicio Interdepartamental de Investigación (SIdI-UAM) of Madrid for technical support.

Ethical Statement.

Participants were recruited under the project PID2019-104546RB-100, and the study was approved by the Regional Ethics Committee for Clinical Research of Principality of Asturias (Ref. 343/19).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112711.

References

Aachary, A. A., & Prapulla, S. G. (2011). Xylooligosaccharides (XOS) as an emerging prebiotic: Microbial synthesis, utilization, structural characterization, bioactive properties, and applications. *Comprehensive Reviews in Food Science and Food Safety, 10*(1), 2–16. https://doi.org/10.1111/j.1541-4337.2010.00135.x

Arboleya, S., Bottacini, F., O'Connell-Motherway, M., Ryan, C. A., Ross, R. P., van Sinderen, D., et al. (2018). Gene-trait matching across the *Bifidobacterium longum* pan-genome reveals considerably diversity in carbohydrate catabolism among human infant strains. *BMC Genomics, 19*(1), 33. https://doi.org/10.1186/s12864-017-4388-

Benítez-Páez, A., Kjølbæk, L., Gómez del Pulgar, E. M., Brahe, L. K., Astrup, A., Matysik, S., et al. (2019). A multi-omics approach to unraveling the microbiome-mediated effects of arabinoxylan oligosaccharides in overweight humans. *Msystems, 4*(4), e00209–e00219. https://doi.org/10.1128/mSystems.00209-19

Blanco, G., Ruiz, L., Tamés, H., Ruas-Madiedo, P., Fdez-Riverola, F., Sánchez, B., et al. (2020). Revisiting the metabolic capabilities of *Bifidobacterium longum* susbp. *longum* and *Bifidobacterium longum* subsp. *infantis* from a glycoside hydrolase perspective. *Microorganisms, 8*(5), 723. https://doi.org/10.3390/microorganisms8050723

Broekaert, W. F., Courtin, C. M., Verbeke, K., van de Wiele, T., Verstraete, W., & Delcour, J. A. (2011). Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Critical Reviews in Food Science and Nutrition, 51*(2), 178–194. https://doi.org/10.1080/10408390903044768

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knigth, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Chung, W. S. F., Walker, A. W., Bosscher, D., Garcia-Campayo, V., Wagner, J., Parkhill, J., et al. (2020). Relative abundance of the *Prevotella* genus within the human gut microbiota of elderly volunteers determines the inter-individual responses to dietary supplementation with wheat bran arabinoxylan-oligosaccharides. *BMC microbiology, 20*(1), 1–14. https://doi.org/10.1186/s12866-020-01968-4

Cloetens, L., Broekaert, W. F., Delaedt, Y., Ollevier, F., Courtin, C. M., Delcour, J. A., et al. (2010). Tolerance of arabinoxylan-oligosaccharides and their prebiotic activity in healthy subjects: A randomised, placebo-controlled cross-over study. *British Journal of Nutrition, 103*(5), 703–713. https://doi.org/10.1017/S0007114509992248

Díaz, R., Torres-Miranda, A., Orellana, G., Garrido, G. (2021). Comparative genomic analysis of novel *Bifidobacterium longum* subsp. *longum* strains reveals functional divergence in the human gut microbiota. *Microorganisms* 9(9), 1906. 10.3390. microorganisms9091906.

Fernandez-Julia, P., Commane, D. M., van Sinderen, D., & Munoz-Munoz, J. (2022). Cross-feeding interactions between human gut commensals belonging to the *Bacteroides* and *Bifidobacterium* genera when grown on dietary glycans. *Microbiome Research Reports, 2022*(1), 12. https://doi.org/10.20517/mrr.2021.05.

Ferreira-Lazarte, A., Moreno, F. J., & Villamiel, M. (2020). Bringing the digestibility of prebiotics into focus: Update of carbohydrate digestion models. *Critical Reviews in Food Science and Nutrition*, 1–12. https://doi.org/10.1080/10408398.2020.1798344. ahead of print.

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., & Olsen, G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and Environmental Microbiology, 74*, 2461–2470. https://doi.org/10.1128/AEM.02272-07

Gibson, G. R., Hutkins, R., Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., et al. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology, 14*(8), 491–502. https://doi.org/10.1038/nrgastro.2017.75

Hevia, A., Milani, C., López, P., Donado, C. D., Cuervo, A., González, A., et al. (2016). Allergic patients with long-term asthma display low levels of *Bifidobacterium adolescentis*. *PLoS One, 11*(2), e0147809.

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*, 506–514. https://doi.org/10.1038/nrgastro.2014.66

Ivashkin, V., Fomin, V., Moiseev, S., Brovko, M., Maslennikov, R., Ulyanin, A., Sholomova, V., Vasilyeva, M., Trush, E., Shifrin, O., & Poluektova, E. (2021). Efficacy of a probiotic ponsisting of *Lacticaseibacillus rhamnosus* PDV 1705, *Bifidobacterium bifidum* PDV 0903, *Bifidobacterium longum* subsp. *infantis* PDV 1911, and *Bifidobacterium longum* subsp. *longum* PDV 2301 in the treatment of hospitalized 'patients with COVID-19: a randomized controlled trial. *Probiotics and Antimicrobial Proteins* 1–9. https://doi.org/10.1007/s12602-021-09858-5.

Komeno, M., Hayamizu, H., Fujita, K., & Ashida, H. (2019). Two Novel α- <scp>l</scp> -Arabinofuranosidases from *Bifidobacterium longum* subsp. *longum* belonging to glycoside hydrolase family 43 cooperatively degrade arabinan. *Applied and Environmental Microbiology, 85*(6). https://doi.org/10.1128/AEM.02582-18

Liu, Y., Heath, A. L., Galland, B., Rehrer, N., Drummond, L., Wu, X. Y., et al. (2020). Substrate use prioritization by a coculture of five species of gut bacteria fed mixtures of arabinoxylan, xyloglucan, β-glucan, and pectin. *Applied and Environmental Microbiology, 86*(2), e01905–e01919. https://doi.org/10.1128/AEM.01905-19

Lugli, G. A., Milani, C., Mancabelli, L., van Sinderen, D., & Ventura, M. (2016). MEGAnnotator: A user-friendly pipeline for microbial genomes assembly and annotation. *FEMS Microbiology Letters, 363*(7). https://doi.org/10.1093/femsle/fnw049

Lynch, K. M., Strain, C. R., Johnson, C., Patangia, D., Stanton, C., Koc, F., et al. (2021). Extraction and characterisation of arabinoxylan from brewers spent grain and investigation of microbiome modulation potential. *European Journal of Nutrition, 60*(8), 4393–4411. https://doi.org/10.1007/s00394-021-02570-8

Margolles, A., Ruiz, L. (2021). Methods for the isolation and recovery of bifidobacteria. In: "Bifidobacterial: methods and Protocols", Methods in Molecular Biology, van Sinderen and Ventura (Eds.) Humana Press. 2021. ISBN 978-1-0716-1273-6. Pp 1-12.

Mathew, S., Aronsson, A., Karlsson, E. N., & Adlercreutz, P. (2018). Xylo-and arabinoxylooligosaccharides from wheat bran by endoxylanases, utilisation by probiotic bacteria, and structural studies of the enzymes. *Applied Microbiology and Biotechnology, 102*(7), 3105–3120. https://doi.org/10.1007/s00253-018-8823-x

Milani, C., Hevia, A., Foroni, E., Duranti, S., Turroni, F., Lugli, G. A., et al. (2013). Assessing the fecal microbiota: An optimized ion torrent 16S rRNA gene-based analysis protocol. *PLoS One, 8*, e68739.

Milani, C., Lugli, G. A., Turroni, F., Mancabelli, L., Duranti, S., Viappiani, A., et al. (2014). Evaluation of bifidobacterial community composition in the human gut by means of a targeted amplicon sequencing (ITS) protocol. *FEMS Microbiology Ecology, 90*(2), 493–503. https://doi.org/10.1111/1574-6941

Moens, F., Weckx, S., & De Vuyst, L. (2016). Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*. *International Journal of Food Microbiology, 231*, 76–85. https://doi.org/10.1016/j.ijfoodmicro.2016.05.015

Moreno, F. J., Olano, A., Santa-Maria, C., & Corzo, N. (1999). Determination of maltodextrins in enteral formulations by three different chromatographic methods. *Chromatographia, 50*(11–12), 705–710. https://doi.org/10.1007/BF02497308

Odamaki, T., Bottacini, F., Kato, K., Mitsuyama, E., Yoshida, K., Horigome, A., et al. (2018). Genomic diversity and distribution of *Bifidobacterium longum* subsp. *longum* across the human lifespan. *Scientific Reports, 8*, 85. https://doi.org/10.1038/s41598-017-18391-x

Pastell, H., Westermann, P., Meyer, A. S., Tuomainen, P., & Tenkanen, M. (2009). In Vitro fermentation of arabinoxylan-derived carbohydrates by bifidobacteria and mixed fecal microbiota. *Journal of Agricultural and Food Chemistry, 57*(18), 8598–8606. https://doi.org/10.1021/jf901397b

Rivière, A., Moens, F., Selak, M., Maes, D., Weckx, S., & de Vuyst, L. (2014). The ability of bifidobacteria to degrade arabinoxylan oligosaccharide constituents and derived oligosaccharides is strain dependent. *Applied and Environmental Microbiology, 80*(1), 204–217. https://doi.org/10.1128/AEM.02853-13

Rivière, A., Gagnon, M., Weckx, S., Roy, D., & De Vuyst, L. (2015). Mutual cross-feeding interactions between *Bifidobacterium longum* subsp. *longum* NCC2705 and *Eubacterium rectale* ATCC 33656 explain the bifidogenic and butyrogenic effects of arabinoxylan oligosaccharides. *Applied and Environmental Microbiology, 81*(22), 7767–7781. https://doi.org/10.1128/AEM.02089-15

Rivière, A., Selak, M., Geirnaert, A., Van den Abbeele, P., & De Vuyst, L. (2018). Complementary mechanisms for degradation of inulin-type fructans and arabinoxylan oligosaccharides among bifidobacterial strains suggest bacterial cooperation. *Applied and Environmental Microbiology, 84*(9), e02893–e02917. https://doi.org/10.1128/AEM.02893-17

Ruiz-Barba, Maldonado, A., Jiménez-Díaz, R. (2005). Small-scale total DNA extraction from bacteria and yeast for PCR applications. *Analytical Biochemistry*, 347(2), 333–335. https://doi.org/j.ab.2005.09.028.

Russell, D. A., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2011). Metabolic activities and probiotic potential of bifidobacteria. *International Journal of Food Microbiology, 149*, 88–105. https://doi.org/10.1016/j.ijfoodmicro.2011.06.003

Sabater, C., Corzo, N., Olano, A., & Montilla, A. (2018). Enzymatic extraction of pectin from artichoke (*Cynara scolymus* L.) by-products using Celluclast®1.5L. *Carbohydrate Polymers, 190*, 43–49. https://doi.org/10.1016/j.carbpol.2018.02.055

Sabater, C., Calvete-Torre, I., Villamiel, M., Moreno, F. J., Margolles, A., & Ruiz, L. (2021). Vegetable waste and by-products to feed a healthy gut microbiota: Current evidence, machine learning and computational tools to design novel microbiome-targeted foods. *Trends in Food Science & Technology, 118*, 399–417. https://doi.org/10.1016/j.tifs.2021.10.002

Saito, Y., Shigehisa, A., Watanabe, Y., Tsukuda, N., Moriyama-Ohara, K., Hara, T., et al. (2020). Multiple transporters and glycoside hydrolases are involved in arabinoxylan-derived oligosaccharide utilization in *Bifidobacterium pseudocatenulatum*. *Applied and Environmental Microbiology, 86*(24), e01782–e01820. https://doi.org/10.1128/AEM.01782-20

Sarkar, A., & Mandal, S. (2016). Bifidobacteria- insight into clinical outcomes and mechanisms of its probiotic action. *Microbiological Research, 192*, 159–171. https://doi.org/10.1016/j.micres.2016.07.001

Schimmel, P., Kleinjans, L., Bongers, R., Knol, J., & Belzer, C. (2021). Breast milk urea as a nitrogen source for urease positive *Bifidobacterium infantis*. *FEMS Microbiology Ecology, 97(3):fiab019*. https://doi.org/10.1093/femsec/fiab019

Sela, D. A., Chapman, J., Adeuya, A., Kim, J. H., Chen, F., Whitehead, T. R., Lapidus, A., Rokhsar, D. S., Lebrilla, C. B., German, J. B., Price, N. P., Richardson, P. M., & Mills, D. A. (2008). The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. *Proceedings of the National Academy of Sciences*, *105*(48), 18964–18969. https://doi.org/10.1073/pnas.0809584105.

Song, A. X., Li, L. Q., Yin, J. Y., Chiou, J. C., & Wu, J. Y. (2020). Mechanistic insights into the structure-dependant and strain-specific utilization of wheat arabinoxylan by *Bifidobacterium longum*. *Carbohydrate Polymers, 249*, Article 116886. https://doi.org/10.1016/j.carbpol.2020.116886

Suriano, F., Bindels, L. B., Verspreet, J., Courtin, C. M., Verbeke, K., Cani, P. D., et al. (2017). Fat binding capacity and modulation of the gut microbiota both determine the effect of wheat bran fractions on adiposity. *Scientific Reports, 7*(1), 5621. https://doi.org/10.1038/s41598-017-05698-y

van den Abbeele, P., Taminiau, B., Pinheiro, I., Duysburgh, C., Jacobs, H., Pijls, L., et al. (2018). Arabinoxylo-oligosaccharides and inulin impact inter-individual variation on microbial metabolism and composition, which immunomodulates human cells. *Journal of Agricultural and Food Chemistry, 66*(5), 1121–1130. https://doi.org/10.1021/acs.jafc.7b04611

Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., Yang, Z., et al. (2018). dbCAN2: A meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Research, 46*(W1), W95–W101. https://doi.org/10.1093/nar/gky418