Isolation of wheat bran-colonizing and metabolizing species from the human fecal microbiota

Kim De Paepe[1], Joran Verspreet[2*], Mohammad Naser Rezaei[2], Silvia Hidalgo Martinez[3], Filip Meysman[3,4], Davy Van de Walle[5], Koen Dewettinck[5], Jeroen Raes[6,7], Christophe M. Courtin[2] and Tom Van de Wiele[1]

[1] Center for Microbial Ecology and Technology (CMET), Department of Biotechnology, Faculty of Bioscience Engineering, Universiteit Gent, Ghent, Belgium

[2] Laboratory of Food Chemistry and Biochemistry, Leuven Food Science and Nutrition Research Centre (LFoRCe), Faculty of Bioscience Engineering, KU Leuven, Heverlee, Belgium

[3] Ecosystem Management Research Group (ECOBE), Department of Biology, Faculty of Sciences, Universiteit Antwerpen, Antwerp, Belgium

[4] Department of Biotechnology, Delft University of Technology, Delft, The Netherlands.

[5] Laboratory of Food Technology and Engineering (FTE), Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Universiteit Gent, Ghent, Belgium

[6] Department of Microbiology and Immunology, Rega Institute, KU Leuven, Leuven, Belgium

[7] Center for Microbiology, VIB, Leuven, Belgium

* Current affiliation: Flemish Institute for Technological Research (VITO), Mol, Belgium

Corresponding Author:
Tom Van de Wiele[1]
Coupure Links 653, 9000 Ghent, Belgium
Tom.VandeWiele@UGent.be

ABSTRACT

Undigestible, insoluble food particles, such as wheat bran, are important dietary constituents that serve as a fermentation substrate for the human gut microbiota. The first step in wheat bran fermentation involves the poorly studied solubilization of fibers from the complex insoluble wheat bran structure. Attachment of bacteria has been suggested to promote the efficient hydrolysis of insoluble substrates, but the mechanisms and drivers of this microbial attachment and colonization, as well as subsequent fermentation remain to be elucidated. We have previously shown that an individually dependent subset of gut bacteria is able to colonize the wheat bran residue. Here, we isolated these bran-attached microorganisms, which can then be used to gain mechanistic insights in future pure culture experiments. Four healthy fecal donors were screened to account for inter-individual differences in gut microbiota composition. A combination of a direct plating and enrichment method resulted in the isolation of a phylogenetically diverse set of species, belonging to the *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria* phyla. A comparison with 16S rRNA gene sequences that were found enriched on wheat bran particles in previous studies, however, showed that the isolates do not yet cover the entire diversity of wheat-bran colonizing species, comprising among others a broad range of *Prevotella*, *Bacteroides* and *Clostridium* cluster XIVa species. We, therefore, suggest several modifications to the experiment set-up to further expand the array of isolated species.

INTRODUCTION

Gut microbiome research has recently started to focus on the microbial composition and functionality of distinct gut environments, such as the mucus layer (Belzer & de Vos 2012; Bhat et al. 1980; Bollinger et al. 2007a; Bollinger et al. 2007b; Macfarlane & Dillon 2007; Nava et al. 2011; Probert & Gibson 2002; Swidsinski et al. 2008; Van den Abbeele et al. 2013; Van den Abbeele et al. 2012). The importance of undigested, insoluble food particles as microbial colonization sites and their impact on functionality, however, has been poorly studied (Macfarlane et al. 2011). Results obtained by Walker *et al.* (2008) and Macfarlane *et al.* (1997, 2006) are inconclusive with respect to the existence of a distinct microbial community associated with the particulate matter in fecal samples (Macfarlane & Macfarlane 2006; Macfarlane et al. 1997; Walker et al. 2008). Leitch *et al*. (2007) previously pinpointed a specific colonization pattern of insoluble substrates in an anaerobic fermentor system, with wheat bran colonization being dominated by members of *Clostridium* cluster XIVa and *Bacteroides* species (Leitch et al. 2007). In an attempt to shed more light on the specific colonization of plant polysaccharides present in the human diet, we have previously performed a series of experiments using wheat bran as an insoluble model substrate. Static batch incubations and experiments in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) confirmed the colonization of wheat bran by a specific subset of gut bacteria, comprising *Prevotella copri*, *Bacteroides ovatus/cellulosilyticus/stercoris/eggerthii/xylanisolvens*, *Roseburia faecis*, *Eubacterium rectale*, *Coprococcus eutactus*, *Hungatella hathewayi*, *Dialister succinatiphilus/propionicifaciens*, *Bifidobacterium faecale/adolescentis*, *Lactobacillus*, *Pediococcus*, *Fusobacterium* and *Enterobacteriaceae* species (De Paepe et al. 2017; De Paepe et al. 2018).

68 An essential step in resolving the driving force and mechanisms behind this specific substrate

69 attachment entails the study of pure cultures. To this end, species deposited in culture collections

70 can be used, offering the advantage of working with fully characterized bacteria, from which

71 genome information is available. Alternatively, bacteria can be isolated, permitting the discovery

72 of novel strains and ensuring the use of relevant strains (Greub 2012). Recent technological

73 advancements have revived interest in bacterial culturing. The so-called culturomics approach,

74 which involves high-throughput microbial culturing using different conditions and media, has

75 shown that a large fraction of the gut microbial community is culturable (Browne et al. 2016;

76 Hugon et al. 2015; Lagier et al. 2012; Lagier et al. 2016). While the automated picking and

77 identification of millions of colonies is a promising strategy to capture microbial diversity in the

78 gut, it is not a standard analysis that requires specialized equipment. As we are specifically

79 interested in a subset of bacteria, capable of colonizing and metabolizing the wheat bran residue,

80 we can considerably bring down our isolation efforts by targeted enrichment prior to isolation. To

81 this end, in the work presented here, the fecal microbial communities derived from four healthy

82 individuals were cultured using wheat bran as the sole nutrient source. The washed wheat bran

83 residue with the attached microbiota, was subcultured four times in fresh medium to selectively

84 enrich the wheat bran-colonizing and metabolizing species. Additionally, the same fecal samples

85 were directly plated on wheat bran agar, selecting only for wheat bran-metabolizing species.

86

87

MATERIALS AND METHODS

Bacterial strains capable of metabolizing wheat bran as a sole nutrient source were isolated from a fecal slurry by a combination of direct plating and enrichment (Fig. 1). In order to account for inter-individual variability, the fecal sample of four different individuals was evaluated. Research incubation work with fecal microbiota from human origin was approved by the ethical committee of the Ghent University hospital under registration number B670201214538. Written informed consent was obtained from all participants. A fecal slurry was prepared according to De Boever *et al.* (2000) and consisted of a 20% (w/v) fecal sample, suspended and homogenized in 0.1 M phosphate buffer pH 6.8, supplemented with 1 g L^{-1} sodium thioglycolate (Sigma-Aldrich, St. Louis, MO, US), henceforth referred to as 'anaerobic phosphate buffer' (De Boever et al. 2000). All isolation work was subsequently carried out in an anaerobic workstation (GP-Campus, Jacomex, TCPS NV, Rotselaar, Belgium).

Solutions and growth media

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, US). The isolation medium used in the direct plating and enrichment method, contained pre-digested particle size reduced wheat bran (50 g L^{-1}), with chemical composition determined as described in the supplementary information and displayed in Fig. 1. The medium was reinforced with vitamin and hemin stock solutions (Table S1 and S2) and buffered at pH 5.8 and 6.8 respectively, to mimic proximal and distal colon pH using a 0.1 M phosphate buffer (KH_2PO_4/Na_2HPO_4) (Cummings et al. 1987; Duncan et al. 2009). The amount of wheat bran was reduced to 10 g L^{-1} for donors 3 and 4.

For the direct plating method, the particle size reduced wheat bran was incorporated in the buffered autoclaved molten agar (15 g L^{-1}; Thermo Fisher Scientific, San José, CA, US) medium prior to

112   its solidification. Plates were poured in a laminar flow cabinet after addition of the filter sterilized

113   heat labile stock solution (Table S2) and the wheat bran and stored at 4°C, for maximum two

114   weeks.

115   The enrichment was performed in a buffered liquid broth. Besides the vitamin solutions, resazurin

116   (1 mg L^{-1}) was added as a redox indicator. After verifying the pH, the broth was heated with

117   intermittent shaking to remove dissolved oxygen until boiling, after which it was sparged with N_2

118   gas (30 min) while the medium was cooling down in ice water. The medium was subsequently

119   dispensed in Hungate tubes (Glasgeratebau Ochs Gmbh, Bovenden-Lenglern, Finland) (8 mL per

120   tube) under a continuous gas flow and tubes were quickly sealed with butyl rubber stoppers and

121   aluminum crimp seals to limit oxygen exposure. The headspace was flushed with N_2 for 30 cycles

122   using a gas exchange apparatus (Plugge 2005). The Hungate tubes were autoclaved. The filter

123   sterilized heat labile stock solution (Table S2) and wheat bran were added in the anaerobic

124   workstation, alongside 0.5 mL reducing reagent stock solution at the targeted pH (Table S3).

125   Purified, isolated bacterial colonies were further characterized in a defined general nutritional

126   YCFAG medium (Browne et al. 2016; Duncan et al. 2002). The medium was modified by adding

127   0.1 M KH_2PO_4/Na_2HPO_4 and correcting the pH to 6.8 and 5.8 using 10 M NaOH and by replacing

128   the cysteine-HCl and bicarbonate by the reducing reagent stock solution shown in Table S3. As

129   for the isolation medium, this reducing reagent stock solution was added to the YCFAG broth right

130   before use. The heat labile vitamins were added after autoclaving. YCFAG plates were poured in

131   the laminar flow cabinet and stored at 4°C. Anaerobic YCFAG liquid broth was prepared by

132   boiling, sparging and flushing the medium as described before, except that 10 mL of the medium

133   was distributed per tube. After subculturing to confirm purity, the obtained isolates in YCFAG

134   medium were stored in cryovials at -80°C in the presence of a cryoprotective agent (Table S4).

135

Direct plating

137 In the anaerobic workstation, tenfold serial dilutions (10^{-1} to 10^{-6}) of the 20% (w/v) fecal slurry

138 were prepared in 0.1 M anaerobic phosphate buffer at pH 6.8. The -3 to -6 dilutions were spread

139 (100 µL) on the surface of a solid agar plate (both at pH 5.8 and 6.8) containing wheat bran as the

140 sole nutrient source, using disposable sterile cell spreaders (VWR, Radnor, PA, US). Plates were

141 transferred to the anaerobic workstation at least 12 h before inoculation. After inoculation, plates

142 were incubated in the anaerobic workstation at 37°C. To check for contamination, two plates, one

143 for each pH, were inoculated with the anaerobic phosphate buffer used to prepare the serial

144 dilutions, without the addition of a bacterial mix derived from the fecal slurry. Plates were daily

145 inspected for growth. In case of perceivable growth, for each pH, ten discrete colonies were picked

146 with an inoculating loop and streak plates were made on solid YCFAG agar medium. Plates were

147 incubated in the anaerobic workstation at 37°C. Again, YCFAG plates without bacterial

148 suspension were included as a control. Growth on the YCFAG plates was monitored and if pure

149 colonies were observed, a single colony was transferred to YCFAG broth in a Hungate tube, which

150 was consequently incubated at 37°C on an orbital shaker (90 rpm) in a 45° tilted position outside

151 of the anaerobic working station. When visual growth occurred, 1 mL suspension was sampled

152 and stored at -80°C in the presence of a cryoprotective agent (1 mL) (Table S4) and as a control

153 to confirm purity, an inoculating loop of suspension was subcultured on YCFAG plates, to visually

154 assess conformity of the colony appearance.

155

156   **Enrichment**

157   In the anaerobic workstation (GP-Campus, Jacomex, TCPS NV, Rotselaar, Belgium), 2 mL of the

158   20% (w/v) fecal slurry was inoculated in an enrichment tube, one for each pH, containing 8 mL

159   isolation medium with wheat bran as the sole nutrient source. Two tubes without fecal inoculum

160   were included as a control. The Hungate tubes were capped with butyl rubber stoppers and

161   aluminum crimp seals and removed from the anaerobic workstation for incubation at 37°C on an

162   orbital shaker (90 rpm) in a 45° tilted position. After 24 h the Hungate tubes, including the controls,

163   were transferred to the anaerobic workstation. The wheat bran residue was harvested on an

164   autoclaved filter paper inserted in an autoclaved glass funnel and rinsed three times with anaerobic

165   phosphate buffer to remove loosely attached luminal bacteria. The wheat bran residue was sampled

166   with a disposable inoculating loop and five (donors 1 and 2) or two (donors 3 and 4) loops were

167   transferred to a new Hungate tube with fresh isolation medium. This procedure was repeated three

168   times. The supernatant of the last transfer (referred to as 'luminal suspension') was serially diluted

169   (10^{-1} to 10^{-6}) in anaerobic phosphate buffer in a 96-well plate and the -2 to -6 dilutions were plated

170   onto solid YCFAG agar medium. Single colonies (10 per pH) were isolated and pure cultures were

171   obtained as described for the direct plating method.

172

173   **Sampling and analysis**

174   The fecal slurry was aliquoted for the purpose of Short Chain Fatty Acids (SCFA) analysis and

175   DNA extraction, followed by next-generation 16S rRNA gene amplicon sequencing. Colonies on

176   the surface of the wheat bran containing solid agar plates were enumerated. The wheat bran residue

177   and liquid broth in the enrichment Hungate tubes were sampled after each transfer for DNA

178   extraction and SCFA analysis. Samples after the first and final enrichment from the low and high

179   pH incubation for each donor were sent for next-generation 16S rRNA gene amplicon sequencing.

180   For one donor, the complete sequence of enrichments was analyzed. The pure cultures in the

181   YCFAG medium resulting from both approaches were identified by 16S rRNA gene Sanger

182   sequencing after DNA extraction and metabolically characterized by SCFA measurement. All data

183   visualization and processing was performed in R version 3.4.2 (2017-09-28) (R Core Team 2016),

184   unless stated otherwise. The R code is provided in Supplementary Data S1 and S2 under the form

185   of an Rmarkdown file and the knitted pdf version. Raw SCFA and 16S rRNA gene amplicon

186   sequencing data is included in the Supplementary data files S3-S9. 16S rRNA gene Sanger

187   sequences of the isolates are supplied as a compressed folder (Sanger_isolates.zip). Additionally,

188   DataS10-22 comprise i) user defined functions, ii) Mothur reports with the closest 16S rRNA gene

189   Sanger reference for each OTU obtained by 16S rRNA gene amplicon sequencing, iii) OTU

190   sequences obtained by 16S rRNA gene next-generation amplicon sequencing in fasta format and

191   iv) RDP taxonomic annotation of the 16S rRNA gene Sanger sequences of the isolates, which are

192   all imported in the Rmarkdown file.

193   All samples for functional analysis and for DNA extraction (the pellet obtained after centrifuging

194   250 μL sample at 5000g for 10 min or 0.250 g washed bran residue) were stored at -20°C. Samples

195   for SCFA analysis of the enrichment tubes and fecal slurry were 1:2 diluted in demineralized water

196   prior to analysis.

197   SCFA analysis and a phenol-chloroform based DNA purification, following DNA extraction

198   through chemical and mechanical lysis by multidirectional beating were performed according to

199   De Paepe et al. (2017). The DNA quality was verified by electrophoresis on a 1.5% (w/v) agarose

200   gel and the DNA concentration was measured using the QuantiFluor ® dsDNA kit (Promega,

201   Madison, WI, US) and Glomax ®-Multi+ system (Promega, Madison, WI, US).

202   The 16S rRNA gene from the pure cultures was amplified by PCR with the 63F

203   (5'CAGGCCTAACACACATGCAAGTC3')   -   1378R

204   (5'CGGTGTGTACAAGGCCCGGGAACG3') primer pair in a BioRad T100™ Thermal Cycler

205   (Applied Biosystems, Foster City, CA, US) (Lane 1991). Primers were synthesized by Biolegio

206   (Nijmegen, The Netherlands) and added in a final concentration of 0.2 µM in sterile nuclease-free

207   water (Sigma-Aldrich, St. Louis, MO, US), containing 0.1 µL Taq buffer μL^{-1} PCR-mix, 0.025

208   units Recombinant Taq DNA polymerase μL^{-1} PCR-mix, 0.2 µM dNTP Mix, 1.5 µM $MgCl_2$

209   (Fermentas Molecular Biology Tools, Waltham, MA, US), 0.75 µM BSA (Roche Applied Science,

210   Penzberg, Germany) and 0.04 µL DNA extract μL^{-1} PCR-mix. The PCR amplification was

211   initiated by a pre-denaturation step (5 min at 94°C), followed by repeated denaturation (1 min at

212   95°C), annealing (1 min at 53°C) and extension (2 min at 72°C) for 30 cycles, followed by 10 min

213   at 72°C. PCR-products were purified with the innuPREP PCRpure Kit (Analytik Jena, Jena,

214   Germany) and sent for molecular identification by bi-directional Sanger sequencing

215   (LGCGenomics, Teddington, Middlesex, UK). Forward and reverse 16S rRNA gene Sanger reads

216   were classified through the RDP web interface using the RDP SeqMatch tool, restricting the

217   database search to type strains with only near-full-length good quality sequences, and blasted in

218   NCBI against the 16S rRNA gene sequences, selecting only type material, with optimization of

219   the BLAST algorithm for highly similar sequences (accession date: June 2017) (Altschul et al.

220   1990; Cole et al. 2014; Wang et al. 2007). Results were manually compared and yielded a good

221   correspondence. Bioedit was used to assess sequence quality, by manual inspection of the sequence

222   traces in the chromatograms (Hall 1999). Short reads or reads with a lot of ambiguous base calls

223   were precluded from the analysis.

224 Next-generation 16S rRNA gene amplicon sequencing of the V4 region (515F-806R) was

225 performed on an Illumina MiSeq platform (Illumina, Hayward, CA, US) using Illumina MiSeq v2

226 chemistry at the VIB Nucleomics core (VIB, Gasthuisberg Campus, Leuven, Belgium). Positive

227 and negative controls were taken along as discussed in De Paepe et al. (2018).

228 The mothur software package (v.1.39.5) and guidelines were used to process the amplicon data as

229 described in detail in De Paepe et al. (2018) (Kozich et al. 2013). An OTU is hereinafter defined

230 as a collection of sequences with a length between 220 and 253 nucleotides that are found to be

231 more than 97% similar to one another in the V4 region of their 16S rRNA gene after applying

232 OptiClust clustering (Chen et al. 2013; Schloss & Westcott 2011; Schloss et al. 2009; Wang et al.

233 2012). Taxonomy was assigned using the RDP version 16 and silva.nr_v123 database (Cole et al.

234 2014; Quast et al. 2013; Wang et al. 2007). The resulting OTU table and taxonomy file were loaded

235 in R (R Core Team 2016). All samples from donor 4, except for the final enrichment step, were

236 discarded due to an insufficient number of reads (<100). The outcome of the enrichment procedure

237 was assessed by computing richness (Chao1 Richness estimator) and diversity (Shannon, Simpson,

238 inverse Simpson and Fisher alpha) estimators using vegan_2.4-4 (Oksanen et al. 2016). The

239 proportional community composition was displayed in bar graphs. For this purpose, the OTU table

240 was filtered according to the arbitrary cutoff's described by McMurdie and Holmes (2014),

241 whereby OTUs observed in less than 5% of the samples and with read counts below 0.5 times the

242 number of samples were removed (McMurdie & Holmes 2014). At genus level RDP version 16

243 taxonomy is displayed. To arrive at a species level classification, OTUs were manually annotated

244 using the RDP web interface using the RDP SeqMatch tool, restricting the database search to type

245 strains with only near-full-length good quality sequences, and blasted in NCBI against the 16S

246 rRNA gene sequences, selecting only type material, with optimization of the BLAST algorithm

247   for highly similar sequences (accession date: June 2017) (Altschul et al., 1990; Wang et al.,

248   2007;Cole et al., 2014). Inconsistent species level taxonomy assignments were not reported. The

249   sequence data has been submitted to the NCBI database under accession number SRP091975.

250   Finally, the resulting OTUs were compared to the 16S rRNA gene Sanger reads of the obtained

251   isolates by means of a phylogenetic placement analysis. Forward and reverse Sanger sequences

252   for each donor were grouped into separate files and reverse complements and summary statistics

253   were obtained using the mothur software package (v.1.39.5) (Schloss et al. 2009). The 515F-806R

254   primer pair, used for Illumina MiSeq 16S rRNA gene amplicon sequencing was located in the

255   Sanger reads (forward and reverse compliment). In case both primers were not present on one and

256   the same read (either forward, or reverse), consensus sequences (contigs) were generated using the

257   sangeranalyseR package (version 0.1.0) (Lanfear 2015). Contigs with more than 100 degenerated

258   positions, indicative of a poor quality alignment, were omitted. A reference alignment was built

259   from the Sanger reads (for each donor separately) applying the sina alinger (Pruesse et al. 2012).

260   OTUs were aligned to this reference Sanger alignment in mothur (align.seqs), yielding a report

261   with the closest Sanger reference for each OTU based on kmer searching (Schloss et al. 2009).

262   This report was loaded into R (R Core Team 2016). For each isolate in the report, the top two

263   OTUs with the highest SearchScores were selected. A fasta file was constructed containing these

264   OTUs. In order to compare the OTUs spanning the V4 region of the 16S rRNA gene with the near

265   full-length Sanger reads, the RAxML implementation of the evolutionary placement algorithm of

266   short reads, as introduced by Berger et al. (2011), was used (Stamatakis 2014). The bootstrap

267   supported maximum likelihood (ML) phylogenetic reference tree was also constructed using

268   RAxML, selecting the General Time Reversible model of nucleotide substitution under the

269   Gamma model of rate heterogeneity (GTRGAMMA) with the parsimony random seed set to

270   12345. The rapid bootstrap analysis was conducted starting from N=1000 distinct randomized

271   maximum parsimony trees and was followed by a search for the best-scoring ML tree with rapid

272   bootstrap random number seed 123 (Stamatakis 2014). The best scoring ML tree with the OTU

273   short read insertions was visualized in iTOL (Letunic & Bork 2016). The proportional abundance

274   of the OTUs in the fecal slurry and in the luminal suspension after the last passage at pH 5.8 and

275   6.8 were integrated in the tree as a multi-value bar chart.

276   **SEM and cryo-SEM Visualization**

277   Native, pre-digested and fermented wheat bran samples were visualized using cryo-SEM and

278   desktop SEM. For the purpose of SEM microscopy, the bran samples were chemically dried with

279   hexamethyldisilazane (HMDS) as described by Araujo et al. (2003). After complete evaporation

280   of the HMDS, samples were mounted on an aluminum pin (diameter: 12 mm) using double sided

281   carbon tape and subsequently gold sputtered for 45 sec at 30 mA (Agar Sputter Coater B7340,

282   Agar Scientific, UK). Images were collected using a Phenom Pro X SEM microscope (Phenom-

283   World B. V., the Netherlands) with a beam intensity of 10 keV.

284   As an alternative to SEM microscopy, samples were also visualized via cryo-SEM using a Jeol

285   JSM 7100F scanning electron microscope (JEOL Ltd, Tokyo, Japan). A small amount of wheat

286   bran was placed on a sticky carbon surface mounted on an aluminium stub, vitrified in a nitrogen

287   slush and transferred under vacuum conditions into the cryo-preparation chamber (PP3010T

288   Cryo-SEM Preparation System, Quorum Technologies, UK) conditioned at -140°C.

289   Subsequently, the sample was sublimated for 20 min at -70°C to remove frost artefacts, sputter-

290   coated with platinum using argon gas, transferred to the SEM stage at -140°C and electron beam

291   targeted at 3 keV.

292

293

RESULTS & DISCUSSION

In an attempt to isolate human fecal bacteria capable of growing on and attaching to wheat bran, two different approaches were adopted: direct plating on a wheat bran based solid agar medium and plating the cultures after a series of enrichment steps. Inter-individual variability and pH were previously shown to determine the outcome of wheat bran colonization (Cummings et al. 1987; Duncan et al. 2009) and were accounted for in the present study by examining four different donors at two pH values representative for proximal (pH 5.8) and distal (pH 6.8) colon conditions. As wheat bran was not autoclaved to avoid structural modifications, a control wheat bran sample was incubated under both pH conditions. Bacterial growth was observed in the control, despite the pre-incubation at low pH in the presence of digestive enzymes, mimicking the gastro-intestinal transit. 16S rRNA gene sequencing of the V4 region, however, revealed that one OTU accounted for 100% of the reads. This *Enterobacteriaceae* OTU could not be unambiguously classified and was not recovered in any of the incubations inoculated with a fecal sample, nor did anything grow on the YCFAG plates after enrichment of the control.

Enrichment of wheat bran-attached bacteria

Incubation of fecal microbiota with wheat bran as a sole nutrient source resulted in short chain fatty acid (SCFA) production, concomitant with a marked acidification, confirming the microbial growth that was visually observed as an increased optical density (Fig. 2). During the consecutive enrichment steps, the microbial richness and diversity decreased, as illustrated in the case of donor 1 (Fig. 3), confirming the effectiveness of the enrichment procedure. After the first 24 h incubation of the fecal sample of donor 1 in the presence of wheat bran, the bran-attached community was clearly enriched in *Bifidobacterium* OTU1 (most similar to *B. faecale/adolescentis*) at pH 5.8 and

317   *Dialister* OTU12 at pH 6.8 (Fig. 4). This wheat bran residue served as an inoculum for the next 24

318   h incubation, a procedure which was repeated three times in total. The enrichment of bifidobacteria

319   at low pH persisted during successive passages and lactobacilli appeared from the second

320   enrichment step onwards. Acetate was the main metabolic end product detected, but it must be

321   noted that lactate production was not measured. In the pH 6.8 condition, butyrate was formed next

322   to acetate, which can be linked to the enrichment of *Faecalibacterium prausnitzii* OTU5 besides

323   *Bifidobacterium* OTU1. In the other two donors, next to *Lactobacillus*, bifidobacteria and *F.*

324   *prausnitzii*, an enrichment of *Pediococcus*, *Enterobacteriaceae*, *Escherichia/Shigella* and

325   *Fusobacterium* species was observed (Fig. 5 and 6). The successful colonization of bifidobacteria,

326   *Lactobacillus* and *Pediococcus* on wheat bran has been observed in the proximal colon

327   compartment of the Dietary Particle Mucus Simulator of the Human Intestinal Microbial

328   Ecosystem (DP-M-SHIME) (De Paepe et al. 2018). The efficient wheat bran colonization could

329   possibly be attributed to extensive adhesive properties and might involve the expression of pili or

330   the production of EPS (Foroni et al. 2011; Hidalgo-Cantabrana et al. 2014; Johansson et al. 2011;

331   Kankainen et al. 2009; Lebeer et al. 2012; Roos & Jonsson 2002; Ruas-Madiedo et al. 2007; Van

332   den Abbeele et al. 2013; Van den Abbeele et al. 2009; Van den Abbeele et al. 2012). Their

333   preference for the proximal colon compartment was suggested to reflect their acid tolerance

334   (Chung et al. 2016; De Paepe et al. 2018; Duncan et al. 2009; Tannock 2004; Van den Abbeele et

335   al. 2012; Walker et al. 2005). In line with this, *Lactobacillu*s and *Pediococcus* were confined to

336   the pH 5.8 enrichments in donors 1 and 2, which were characterized by a large pH drop by more

337   than one pH unit (Fig. 2). In order to reduce the degree of acidification and associated enrichment

338   of pH tolerant species, the wheat bran concentration and seeding amount were lowered from 50 to

339   10 g L^{-1} and from five to two inoculating loops respectively in donors 3 and 4. This resulted in a

340   more moderate pH decrease to 6.36 ± 0.15 and pH 5.18 ± 0.19 and reduced the share of lactobacilli

341   in the enrichments. Donor 3 and 4, were instead characterized by large *Escherichia/Shigella*

342   proportions, both at pH 5.8 and 6.8. Adhesive structures are well documented in

343   *Enterobacteriaceae* species and colonization of the wheat bran residue might bestow resilience to

344   a suboptimal lower pH (Duncan et al. 2009; Gaastra et al. 2014; Lawley & Walker 2013; The et

345   al. 2016). *Enterobacteriaceae* were less present on the bran in the DP-M-SHIME and mostly

346   resided in the mucus layer. In the absence of a mucus micro-environment in static batch

347   incubations, however, we previously found *Enterobacteriaceae* to dominate the early stages of

348   wheat bran colonization (De Paepe et al. 2018, submitted to ISME Journal). The latter was also

349   observed for *Fusobacteria*, which were abundant in the final enrichment stage of donor 2 in the

350   present experiment. Finally, the establishment of *F. prausnitzii* in donors 1, 3 and 4 is surprising

351   and was not perceived in any of the previous experiments. *F. prausnitzii* is positioned in the mucus

352   layer, close to the gut epithelium and is able to adhere to mucins, but not to epithelial cells (Khan

353   et al. 2012; Martin et al. 2017; Marzorati et al. 2014). Moreover *F. prausnitzii* was shown to adhere

354   to wheat bran in a synbiotic formulation (Khan et al. 2014). Little is known regarding its

355   mechanism of adhesion.

356   Besides the capacity to adhere, the enriched bacteria depended on wheat bran as the sole nutrient

357   source. In general, micro-organisms need a carbon source as building blocks for organic matter, a

358   nitrogen/phosphorous/sulphur source for protein and nucleic acid synthesis, some trace elements

359   such as iron, magnesium, cobalt and manganese as cofactors for enzymes, and, in case of

360   auxotrophic growth, some vitamins and amino acids (Madigan & Brock 2011). Wheat bran is a

361   versatile substrate, containing all of the above compounds (Hemdane et al. 2016). The compounds

362   are, however, part of a complex macro-molecular configuration. Carbohydrates in wheat bran

363   mainly consist of non-starch cell wall polysaccharides (NSP), comprising arabinoxylans, β-glucan,

364   cellulose and fructan, which are physically intertwined with lignin (Hemdane et al. 2016). Some

365   residual starch can be present in the attached endosperm resulting from the crude milling process

366   (Fig. 7-8) (Hemdane et al. 2016; United States Department of Agriculture (USDA) 2016). The

367   residual endosperm fraction also contains some proteins (Chick et al. 1947; Shewry 2009). The

368   major part of the endosperm components is expected to disappear during a pre-digestion with

369   gastric and pancreatic enzymes (Fig. 7) (Amrein et al. 2003; Chick et al. 1947). Wheat bran

370   proteins, however, are also located in aleurone cells, which are more recalcitrant to digestion

371   (Amrein et al. 2003; Arte et al. 2015). This complex wheat bran structure and its insoluble nature

372   limits fermentability. In that sense, the retrieved enriched bacteria are unexpected as none of the

373   species is considered to be a wheat bran primary degrader, judged by the fact that they are not

374   equipped with the enzymatic complement required to solubilize and degrade cellulose and

375   arabinoxylan polymers.

376   Bifidobacteria generally prefer arabinoxylan oligosaccharides (AXOS) as a substrate (van den

377   Broek et al. 2008). *Bifidobacterium longum* and *Bifidobacterium adolescentis*, however, are able

378   to grow on arabinoxylans (Savard & Roy 2009; van den Broek et al. 2008). Genomic studies

379   confirmed the presence of arabinofuranosidases and xylosidases in these species. *Bifidobacterium*

380   *longum* also possesses a multi-domain enzyme with a putative endo-xylanase (GH43) flanked by

381   two carbohydrate-binding modules (CBM) that might interact with xylans. (van den Broek et al.

382   2008). Arabinoxylan or AXOS consumption by lactic acid bacteria (LAB) received less attention,

383   so far (Michlmayr et al. 2013). Lactobacilli have been reported to respond to AXOS treatment in

384   the SHIME model but *Lactobacillus brevis* is the only species for which arabinofuranosidase and

385   xylosidase activity is evidenced (Michlmayr et al. 2013; Sanchez et al. 2009). Based on functional

386   predictions, it is suggested that the AXOS degrading capacity might be shared by *Pediococcus* and

387   *Enterococcus* spp. (Michlmayr et al. 2013). Interestingly, despite this apparently limited wheat

388   bran degrading capacity, our findings are consistent with *Enterococcus* spp., *Lactobacillus* spp.

389   and *Pediococcus pentosaceus* being the main species recovered from spontaneous bran

390   fermentations and the fact that wheat bran is often used as a substrate in industrial fermentations

391   with lactobacilli (Arte et al. 2015; Katina et al. 2012; Prückler et al. 2015). In this context, native

392   wheat bran is used, containing more residual starch. Moreover, the activity of endogenous wheat

393   bran endoxylanases and cinnamoyl esterases, solubilizes the arabinoxylans and increases the

394   availability of arabinose and xylose monomers, which can be used by lactobacilli (Katina et al.

395   2012; Prückler et al. 2015). Besides endogenous xylanases, wheat kernels can contain xylanases

396   from microbial origin (Dornez et al. 2006). The fate of these wheat bran associated xylanases and

397   other endogenous wheat bran polymer degrading enzymes during *in vivo* gastro-intestinal

398   digestion is unknown. But, a reduced, yet, preserved activity of those enzymes has been observed

399   after *in vitro* pre-digestion (unpublished results De Paepe et al.), which could explain the isolation

400   of species lacking these first-line enzymes. *Enterobacteriaceae* and *Fusobacterium* species are

401   capable of saccharolytic fermentation, but are also lacking xylan and cellulose degrading enzymes

402   (Brady et al. 2009; Cantarel et al. 2009; Gao et al. 2015; Lombard et al. 2014; Marzorati et al.

403   2017; Mazur & Zimmer 2011; Richardson et al. 2013; Robrish et al. 1991; Salamanca-Cardona et

404   al. 2014; Thompson et al. 1997). *F. prausnitzii* is shown to degrade wheat bran in pure cultures to

405   a small extent (~ 4% substrate loss) (Duncan et al. 2016). The required enzymatic activity is not

406   experimentally characterized but genomic predictions have identified several glycosyl hydrolases

407   which can display β-xylosidase (EC 3.2.1.37), α-L-arabinofuranosidase (EC 3.2.1.55), endo-1,4-

408   β-xylanase   (EC   3.2.1.8)   and   feruloyl   esterase   (EC   3.1.1.73   )   activity

409 (GH1,GH3,GH31,GH43,CE1), as well as a possible xylan binding domain (CBM13) (Cantarel et

410 al. 2009; Lombard et al. 2014). The release of ferulic acid from wheat bran by a pure culture of *F.*

411 *prausnitzii* has been demonstrated. Additionally, *F. prausnitzii* can benefit from the high acetate

412 concentrations through cross-feeding, supporting our finding of *F. prausnitzii* to be one of the

413 isolates (Duncan et al. 2002; Rios-Covian et al. 2015).

414 Besides the carbohydrate degrading capacity, growth on wheat bran as the sole nutrient source

415 requires proteolytic activity. The breakdown of proteins is essential for the assimilation of peptides

416 and amino-acids into new microbial biomass and can sustain fermentation leading to the

417 production of branched SCFA and ammonium (Macfarlane et al. 1986). Ammonium was not

418 measured in this study, and branched SCFA concentrations were negligible, despite the proteolytic

419 fermentation capacity of some of the enriched species (*Enterobacteriaceae, Fusobacterium*)

420 (Marzorati et al. 2017; Richardson et al. 2013; Robrish et al. 1991). The breakdown of wheat bran

421 proteins by gut bacteria is poorly studied. Wheat bran fermentation by LAB starter cultures has

422 indicated that enzymes from microbial origin contribute to the size reduction of oligopeptides and

423 the generation of free amino acids, while the release of oligopeptides depended on the endogenous

424 wheat bran proteases (Arte et al. 2015). Isolation experiments with gluten proteins revealed that

425 *Enterococcus*, *Bifidobacterium*, *Pediococcus*, *Lactobacillus* and *Bacteroides fragilis* are capable

426 of hydrolyzing gluten proteins and derived peptides (Caminero et al. 2014). Interestingly, *F.*

427 *prausnitzii* and *Bacteroides dorei* were recovered from liquid incubations with gluten proteins as

428 the main nitrogen source, but could not be isolated from solid agar media (Caminero et al. 2014).

429 **Isolation of wheat bran degrading and attached bacteria**

430 The luminal suspension obtained after the final enrichment step was plated onto nutritional

431 medium and alternatively, the fecal sample was directly plated on wheat bran based solid agar

432   plates. Control wheat bran agar plates, which were inoculated with anaerobic phosphate buffer,

433   without the addition of a fecal sample showed no growth. From both strategies, at pH 5.8 and 6.8,

434   ten single colony isolates were picked. Isolates were identified through 16S rRNA gene Sanger

435   sequencing (Table 1).

436   Plating of the enrichment suspension resulted in the isolation of *Lactobacillus* and *Collinsella*

437   species in donor 1, *Escherichia/Shigella*, *Pediococcus*, *Bifidobacterium* and *Enterococcus* species

438   in donor 2, *Escherichia/Shigella*, *Enterococcus* and *Klebsiella* species in donor 3 and *Collinsella*,

439   *Escherichia/Shigella, Eubacterium, Hungatella* and *Bifidobacterium* species in donor 4 (Fig. S1-

440   S4, Table 1). The isolated species after enrichment correspond to the microbial community

441   composition determined by Illumina sequencing, with the exception of *F. prausnitzii* and

442   *Fusobacterium mortiferum*, which were enriched but could not be isolated on solid agar plates. *F.*

443   *prausnitzii* is difficult to isolate due to its oxygen sensitivity (Duncan et al. 2002; Khan et al. 2014;

444   Lopez-Siles et al. 2017). All isolation work was carried out in an anaerobic workstation and the

445   YCFAG medium has been shown to support *F. prausnitzii* growth (Duncan et al. 2002; Khan et

446   al. 2012). The solid agar media were, however, not pre-reduced in this experiment, as opposed to

447   the liquid broth, which explains why *F. prausnitzii* could not be cultivated on YCFAG plates

448   (Holdeman et al. 1977). The direct plating method resulted in the additional isolation of

449   *Bacteroides*, *Bifidobacterium* and *Streptococcus* species (Table 1). This is in agreement with the

450   results from the enrichment series of donor 2, showing a higher diversity, including *Bacteroides*

451   species, after the first 24 h of incubation.

452   **Discussion of the experimental set-up**

453   The enrichment and isolation of predominantly LAB, bifidobacteria and *Enterobacteriaceae* at

454   first sight seems to contest the previous reports of wheat bran colonization by a subset of

455   *Prevotella*, *Bacteroides* and *Clostridium* cluster XIVa organisms (De Paepe et al. 2017; Duncan

456   et al. 2016; Leitch et al. 2007). There are, however, some important differences in experimental

457   set-up that might account for the observed disparity and need to be addressed in future isolation

458   procedures.

459   First of all, the comparable enrichment of LAB, bifidobacteria, *Streptococci* and

460   *Faecalibacterium*, in the current study and in an isolation experiment using gluten as the major

461   protein source, could indicate an inability of *Prevotella*, *Bacteroides* and *Clostridium* species to

462   compete for wheat bran proteins. This would explain their limited growth compared to previous

463   studies using a protein-rich medium containing peptone and yeast-extract (De Paepe et al. 2017;

464   De Paepe et al. 2018).

465   Secondly, the 24 h incubation period in between transfers in the present study might have

466   influenced the results. A detailed analysis of the time course of wheat bran colonization and

467   fermentation. (Submitted to ISME Journal) revealed a succession of bacterial taxa alternately

468   dominating the community over a 72 h timespan. Early stages were dominated by

469   *Enterobacteriaceae* and *Fusobacterium* species and characterized by a low butyrate production.

470   After 48 h, the butyrate ratio increased, corresponding to donor-dependent proportional increases

471   of *Bacteroides ovatus/stercoris*, *Prevotella copri* and *Firmicutes* species. We hypothesized that

472   depletion of the easily digestible compounds induced a shift towards carbohydrate degrading

473   specialists, possessing the enzymatic capacity to breakdown the complex molecules. This

474   hypothesis is also valid with wheat bran as the sole nutrient source. Apart from residual starch, the

475   more fermentable wheat bran components are located in the aleurone layer (Amrein et al. 2003;

476   Stevens & Selvendran 1988). Indeed, we observed a preferential microbial colonization and

477   degradation of aleurone through SEM (Fig. 8). This aleurone degradation might be aided by the

478   activity of wheat kernel associated endogenous or microbial enzymes, including xylanases. The

479   latter remains speculative, as these enzymes might be deactivated upon gastro-intestinal passage

480   and their activity can be inhibited by TAXI (*Triticum aestivum* xylanase inhibitor) and XIP

481   (xylanase-inhibiting protein) xylanase inhibitors, which are also present in wheat bran.

482   Finally, the large extent of acidification due to the high wheat bran concentration in the first two

483   donors (50 g L^{-1}) might be responsible for the unintentional selection for acidophilic or aciduric

484   LAB.

485   In order to investigate the above hypotheses, it would be interesting to repeat the enrichment

486   procedure with a medium containing an additional protein source, extending the incubation time

487   in between passages to 48 h and ensuring a sufficient buffering capacity. This might result in the

488   enrichment and isolation of a vastly different array of gut bacteria. In addition, for the direct plating

489   strategy, the medium should be pre-reduced.

490   **CONCLUSIONS**

491

492   Enrichment of the wheat bran-colonizing microbial community resulted in the isolation of a

493   diverse set of *Lactobacillus, Bifidobacterium*, *Collinsella*, *Escherichia/Shigella*, *Pediococcus*,

494   *Enterococcus, Klebsiella, Eubacterium and Hungatella* species. These isolated species were also

495   found to be enriched on the wheat bran residue by next-generation amplicon sequencing,

496   demonstrating that the proposed enrichment procedure is a sensible and efficient approach to

497   isolate wheat bran-colonizing species. As insoluble wheat bran presented the sole carbohydrate

498   and protein source, the isolated species should be capable of degrading and metabolizing some of

499   the wheat bran constituents. Based on the metabolic capacity documented in literature, the isolates

500   likely thrived on proteins and some residual easily fermentable starch in the current set-up, while

501   in a protein-rich medium, we have previously revealed that the wheat bran-colonizing species

502   possessed arabinoxylan and cellulose degrading enzymatic potential. Combining the new insights

503   from this study with previous observations suggests that adhesion is a rather common trait among

504   gut bacteria, that the outcome of wheat bran colonization is determined by species competition and

505   that external conditions such as pH and nutrient availability tip the balance in favor of the best-

506   adapted species (Macfarlane et al. 2011). It would, hence, be of interest to modify the experimental

507   conditions, as outlined above, to enrich arabinoxylan and cellulose degrading species. Pure culture

508   studies should then be performed to characterize the fermentation of wheat bran components by

509   the obtained isolates and to further our understanding of the mechanisms of wheat bran attachment

510   which could include Extracellular Polymeric Substances (EPS) or even a cellulosome enzyme

511   system, in case of *R. champanellensis* and common adhesive features such as pili and fimbriae.

512   Finally, the co-culturing of different isolates would be interesting to unravel cooperative and

513   competitive interactions during substrate fermentation.

514

515   **ACKNOWLEDGEMENTS**

516

517   We would like to acknowledge Veerle Rober for technical assistance and Jolien De Paepe for

518   reviewing this manuscript.

519

520

521 **References**

522 Altschul SF, Gish W, Miller W, Myers EW, and Lipman DJ. 1990. Basic Local Alignment Search Tool.
523   *Journal of Molecular Biology* 215:403-410. DOI 10.1006/jmbi.1990.9999
524 Amrein TM, Granicher P, Arrigoni E, and Amado R. 2003. *In vitro* digestibility and colonic fermentability
525   of aleurone isolated from wheat bran. *Lebensmittel-Wissenschaft Und-Technologie-Food Science*
526   *and Technology* 36:451-460. 10.1016/S0023-6438(03)00036-7
527 Araujo JC, Teran FC, Oliveira RA, Nour EAA, Montenegro MAP, Campos JR, and Vazoller RF. 2003.
528   Comparison of hexamethyidisilazane and critical point drying treatments for SEM analysis of
529   anaerobic biofilms and granular sludge. *Journal of Electron Microscopy* 52:429-433. DOI
530   10.1093/jmicro/52.4.429
531 Arte E, Rizzello CG, Verni M, Nordlund E, Katina K, and Coda R. 2015. Impact of Enzymatic and Microbial
532   Bioprocessing on Protein Modification and Nutritional Properties of Wheat Bran. *J Agric Food*
533   *Chem* 63:8685-8693. 10.1021/acs.jafc.5b03495
534 Belzer C, and de Vos WM. 2012. Microbes inside-from diversity to function: the case of Akkermansia.
535   *Isme Journal* 6:1449-1458. 10.1038/ismej.2012.6
536 Berger SA, Krompass D, and Stamatakis A. 2011. Performance, accuracy, and Web server for
537   evolutionary placement of short sequence reads under maximum likelihood. *Systematic Biology*
538   60:291-302. 10.1093/sysbio/syr010
539 Bhat P, Albert MJ, Rajan D, Ponniah J, Mathan VI, and Baker SJ. 1980. Bacterial-Flora of the Jejunum - a
540   Comparison of Luminal Aspirate and Mucosal Biopsy. *Journal of Medical Microbiology* 13:247-
541   256.
542 Bollinger RR, Barbas AS, Bush EL, Lin SS, Lin SS, and Parker W. 2007a. Biofilms in the normal human large
543   bowel: fact rather than fiction. *Gut* 56:1481-1482.
544 Bollinger RR, Barbas AS, Bush EL, Lin SS, and Parker W. 2007b. Biofilms in the large bowel suggest an
545   apparent function of the human vermiform appendix. *Journal of Theoretical Biology* 249:826-
546   831. 10.1016/j.jtbi.2007.08.032
547 Brady CL, Venter SN, Cleenwerck I, Engelbeen K, Vancanneyt M, Swings J, and Coutinho TA. 2009.
548   Pantoea vagans sp nov., Pantoea eucalypti sp nov., Pantoea deleyi sp nov and Pantoea
549   anthophila sp nov. *International Journal of Systematic and Evolutionary Microbiology* 59:2339-
550   2345. 10.1099/ijs.0.009241-0
551 Browne HP, Forster SC, Anonye BO, Kumar N, Neville BA, Stares MD, Goulding D, and Lawley TD. 2016.
552   Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation.
553   *Nature* 533:543-546. 10.1038/nature17645
554 Caminero A, Herran AR, Nistal E, Perez-Andres J, Vaquero L, Vivas S, de Morales JMGR, Albillos SM, and
555   Casqueiro J. 2014. Diversity of the cultivable human gut microbiome involved in gluten
556   metabolism: isolation of microorganisms with potential interest for coeliac disease. *FEMS*
557   *Microbiology Ecology* 88:309-319. 10.1111/1574-6941.12295
558 Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, and Henrissat B. 2009. The Carbohydrate-
559   Active EnZymes database (CAZy): an expert resource for Glycogenomics. *Nucleic Acids Research*
560   37:D233-D238. 10.1093/nar/gkn663
561 Chen W, Zhang CK, Cheng Y, Zhang S, and Zhao H. 2013. A comparison of methods for clustering 16S
562   rRNA sequences into OTUs. *PLoS One* 8:e70837. 10.1371/journal.pone.0070837
563 Chick H, Cutting ME, Martin C, and Slack E. 1947. Observations on the digestibility and nutritive value of
564   the nitrogenous constituents of wheat bran. *Br J Nutr* 1:161-182.
565 Chung WS, Walker AW, Louis P, Parkhill J, Vermeiren J, Bosscher D, Duncan SH, and Flint HJ. 2016.
566   Modulation of the human gut microbiota by dietary fibres occurs at the species level. *BMC Biol*
567   14:3. 10.1186/s12915-015-0224-3

568   Cole JR, Wang Q, Fish JA, Chai BL, McGarrell DM, Sun YN, Brown CT, Porras-Alfaro A, Kuske CR, and
569   Tiedje JM. 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis.
570   *Nucleic Acids Research* 42:D633-D642. 10.1093/nar/gkt1244
571   Cummings JH, Pomare EW, Branch WJ, Naylor CP, and Macfarlane GT. 1987. Short chain fatty acids in
572   human large intestine, portal, hepatic and venous blood. *Gut* 28:1221-1227.
573   De Boever P, Deplancke B, and Verstraete W. 2000. Fermentation by gut microbiota cultured in a
574   simulator of the human intestinal microbial ecosystem is improved by supplementing a soygerm
575   powder. *Journal of Nutrition* 130:2599-2606.
576   De Paepe K, Kerckhof F-M, Verspreet J, Courtin CM, and Van de Wiele T. 2017. Inter-individual
577   differences determine the outcome of wheat bran colonization by the human gut microbiome.
578   *Environmental Microbiology* 19:3251-3267. 10.1111/1462-2920.13819
579   De Paepe K, Verspreet J, Verbeke K, Raes J, Courtin CM, and Van de Wiele T. 2018. Introducing insoluble
580   wheat bran as a gut microbiota niche in an in vitro dynamic gut model stimulates propionate
581   and butyrate production and induces colon region specific shifts in the luminal and mucosal
582   microbial community. *Environmental Microbiology* 20:3406-3426. 10.1111/1462-2920.14381
583   Dornez E, Joye IJ, Gebruers K, Delcour JA, and Courtin CM. 2006. Wheat-kernel-associated
584   endoxylanases consist of a majority of microbial and a minority of wheat endogenous
585   endoxylanases. *Journal of Agricultural and Food Chemistry* 54:4028-4034. 10.1021/jf060129d
586   Duncan SH, Hold GL, Harmsen HJM, Stewart CS, and Flint HJ. 2002. Growth requirements and
587   fermentation products of Fusobacterium prausnitzii, and a proposal to reclassify it as
588   Faecalibacterium prausnitzii gen. nov., comb. nov. *International Journal of Systematic and
589   Evolutionary Microbiology* 52:2141-2146. 10.1099/ijs.0.02241-0
590   Duncan SH, Louis P, Thomson JM, and Flint HJ. 2009. The role of pH in determining the species
591   composition of the human colonic microbiota. *Environmental Microbiology* 11:2112-2122.
592   10.1111/j.1462-2920.2009.01931.x
593   Duncan SH, Russell WR, Quartieri A, Rossi M, Parkhill J, Walker AW, and Flint HJ. 2016. Wheat bran
594   promotes enrichment within the human colonic microbiota of butyrate-producing bacteria that
595   release ferulic acid. *Environmental Microbiology* 18:2214-2225. 10.1111/1462-2920.13158
596   Foroni E, Serafini F, Amidani D, Turroni F, He F, Bottacini F, Motherway MO, Viappiani A, Zhang ZD,
597   Rivetti C, van Sinderen D, and Ventura M. 2011. Genetic analysis and morphological
598   identification of pilus-like structures in members of the genus Bifidobacterium. *Microbial Cell
599   Factories* 10. 10.1186/1475-2859-10-S1-S16
600   Gaastra W, Kusters JG, Van Duijkeren E, and Lipman LJA. 2014. Escherichia fergusonii. *Veterinary
601   Microbiology* 172:7-12. 10.1016/j.vetmic.2014.04.016
602   Gao D, Luan Y, Wang Q, Liang Q, and Qi Q. 2015. Construction of cellulose-utilizing Escherichia coli based
603   on a secretable cellulase. *Microbial Cell Factories* 14:159. 10.1186/s12934-015-0349-7
604   Greub G. 2012. Culturomics: a new approach to study the human microbiome. *Clin Microbiol Infect*
605   18:1157-1159. 10.1111/1469-0691.12032
606   Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for
607   Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98. citeulike-article-id:691774
608   Hemdane S, Jacobs PJ, Dornez E, Verspreet J, Delcour JA, and Courtin CM. 2016. Wheat (Triticum
609   aestivum L.) Bran in Bread Making: A Critical Review. *Comprehensive Reviews in Food Science
610   and Food Safety* 15:28-42. 10.1111/1541-4337.12176
611   Hidalgo-Cantabrana C, Sanchez B, Milani C, Ventura M, Margolles A, and Ruas-Madiedo P. 2014.
612   Genomic Overview and Biological Functions of Exopolysaccharide Biosynthesis in
613   Bifidobacterium spp. *Applied and Environmental Microbiology* 80:9-18. 10.1128/Aem.02977-13
614   Holdeman LV, Cato EP, Moore WEC, and University VPIaS. 1977. *Anaerobe laboratory manual*.
615   Blacksburg, Va.: V.P.I. Anaerobe Laboratory.

616   Hugon P, Dufour JC, Colson P, Fournier PE, Sallah K, and Raoult D. 2015. A comprehensive repertoire of
617   prokaryotic species identified in human beings. *Lancet Infectious Diseases* 15:1211-1219.
618   10.1016/S1473-3099(15)00293-5
619   Johansson MEV, Ambort D, Pelaseyed T, Schutte A, Gustafsson JK, Ermund A, Subramani DB, Holmen-
620   Larsson JM, Thomsson KA, Bergstrom JH, van der Post S, Rodriguez-Pineiro AM, Sjovall H,
621   Backstrom M, and Hansson GC. 2011. Composition and functional role of the mucus layers in the
622   intestine. *Cellular and Molecular Life Sciences* 68:3635-3641. 10.1007/s00018-011-0822-3
623   Kankainen M, Paulin L, Tynkkynen S, von Ossowski I, Reunanen J, Partanen P, Satokari R, Vesterlund S,
624   Hendrickx APA, Lebeer S, De Keersmaecker SCJ, Vanderleyden J, Hamalainen T, Laukkanen S,
625   Salovuori N, Ritari J, Alatalo E, Korpela R, Mattila-Sandholm T, Lassig A, Hatakka K, Kinnunen KT,
626   Karjalainen H, Saxelin M, Laakso K, Surakka A, Palva A, Salusjarvi T, Auvinen P, and de Vos WM.
627   2009. Comparative genomic analysis of Lactobacillus rhamnosus GG reveals pili containing a
628   human-mucus binding protein. *Proceedings of the National Academy of Sciences of the United
629   States of America* 106:17193-17198. 10.1073/pnas.0908876106
630   Katina K, Juvonen R, Laitila A, Flander L, Nordlund E, Kariluoto S, Piironen V, and Poutanen K. 2012.
631   Fermented Wheat Bran as a Functional Ingredient in Baking. *Cereal Chemistry* 89:126-134.
632   10.1094/Cchem-08-11-0106
633   Khan MT, Duncan SH, Stams AJ, van Dijl JM, Flint HJ, and Harmsen HJ. 2012. The gut anaerobe
634   Faecalibacterium prausnitzii uses an extracellular electron shuttle to grow at oxic-anoxic
635   interphases. *Isme Journal* 6:1578-1585. 10.1038/ismej.2012.5
636   Khan MT, van Dijl JM, and Harmsen HJM. 2014. Antioxidants Keep the Potentially Probiotic but Highly
637   Oxygen-Sensitive Human Gut Bacterium Faecalibacterium prausnitzii Alive at Ambient Air. *PLoS
638   One* 9. 10.1371/journal.pone.0096097
639   Kozich JJ, Westcott SL, Baxter NT, Highlander SK, and Schloss PD. 2013. Development of a Dual-Index
640   Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq
641   Illumina Sequencing Platform. *Applied and Environmental Microbiology* 79:5112-5120.
642   10.1128/Aem.01043-13
643   Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G,
644   Maraninchi M, Trape JF, Koonin EV, La Scola B, and Raoult D. 2012. Microbial culturomics:
645   paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 18:1185-1193.
646   10.1111/1469-0691.12023
647   Lagier JC, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, Caputo A, Cadoret F, Traore SI, Seck EH,
648   Dubourg G, Durand G, Mourembou G, Guilhot E, Togo A, Bellali S, Bachar D, Cassir N, Bittar F,
649   Delerce J, Mailhe M, Ricaboni D, Bilen M, Nieko NPMD, Badiane NMD, Valles C, Mouelhi D, Diop
650   K, Million M, Musso D, Abrahao J, Azhar EI, Bibi F, Yasir M, Diallo A, Sokhna C, Djossou F, Vitton
651   V, Robert C, Rolain JM, La Scola B, Fournier PE, Levasseur A, and Raoult D. 2016. Culture of
652   previously uncultured members of the human gut microbiota by culturomics. *Nature
653   Microbiology* 1. 10.1038/Nmicrobiol.2016.203
654   Lane. 1991. 16S/23S rRNA sequencing. In: Stackebrandt E, and Goodfellow M, eds. *Nucleic acid
655   techniques in bacterial systematics*: Wiley, 115-175.
656   Lanfear R. 2015. sangeranalyseR: sangeranalyseR: a suite of functions for the analysis of Sanger
657   sequence data in R. R package version 0.1.0.
658   Lawley TD, and Walker AW. 2013. Intestinal colonization resistance. *Immunology* 138:1-11.
659   10.1111/j.1365-2567.2012.03616.x
660   Lebeer S, Claes I, Tytgat HLP, Verhoeven TLA, Marien E, von Ossowski I, Reunanen J, Palva A, de Vos
661   WM, De Keersmaecker SCJ, and Vanderleyden J. 2012. Functional Analysis of Lactobacillus
662   rhamnosus GG Pili in Relation to Adhesion and Immunomodulatory Interactions with Intestinal
663   Epithelial Cells. *Applied and Environmental Microbiology* 78:185-193. 10.1128/Aem.06192-11

664   Leitch ECM, Walker AW, Duncan SH, Holtrop G, and Flint HJ. 2007. Selective colonization of insoluble
665   substrates by human faecal bacteria. *Environmental Microbiology* 9:667-679. DOI
666   10.1111/j.1462-2920.2006.01186.x
667   Letunic I, and Bork P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation
668   of phylogenetic and other trees. *Nucleic Acids Research* 44:W242-W245. 10.1093/nar/gkw290
669   Lombard V, Ramulu HG, Drula E, Coutinho PM, and Henrissat B. 2014. The carbohydrate-active enzymes
670   database (CAZy) in 2013. *Nucleic Acids Research* 42:D490-D495. 10.1093/nar/gkt1178
671   Lopez-Siles M, Duncan SH, Garcia-Gil LJ, and Martinez-Medina M. 2017. Faecalibacterium prausnitzii:
672   from microbiology to diagnostics and prognostics. *Isme Journal* 11:841-852.
673   10.1038/ismej.2016.176
674   Macfarlane GT, Cummings JH, and Allison C. 1986. Protein-Degradation by Human Intestinal Bacteria.
675   *Journal of General Microbiology* 132:1647-1656.
676   Macfarlane S, and Dillon JF. 2007. Microbial biofilms in the human gastrointestinal tract. *Journal of
677   Applied Microbiology* 102:1187-1196. 10.1111/j.1365-2672.2007.03287.x
678   Macfarlane S, Hopkins MJ, and Macfarlane GT. 2011. Bacterial Growth and Metabolism on Surfaces in
679   the Large Intestine. *Microbial Ecology in Health and Disease* 12. 10.3402/mehd.v12i2.8103
680   Macfarlane S, and Macfarlane GT. 2006. Composition and metabolic activities of bacterial biofilms
681   colonizing food residues in the human gut. *Appl Environ Microbiol* 72:6204-6211.
682   10.1128/AEM.00754-06
683   Macfarlane S, McBain AJ, and Macfarlane GT. 1997. Consequences of biofilm and sessile growth in the
684   large intestine. *Advances in Dental Research* 11:59-68.
685   Madigan MT, and Brock TD. 2011. *Brock Biology of Microorganisms*: Pearson.
686   Martin R, Miquel S, Benevides L, Bridonneau C, Robert V, Hudault S, Chain F, Berteau O, Azevedo V,
687   Chatel JM, Sokol H, Bermudez-Humaran LG, Thomas M, and Langella P. 2017. Functional
688   Characterization of Novel Faecalibacterium prausnitzii Strains Isolated from Healthy Volunteers:
689   A Step Forward in the Use of F. prausnitzii as a Next-Generation Probiotic. *Frontiers in
690   Microbiology* 8. 10.3389/fmicb.2017.01226
691   Marzorati M, Vanhoecke B, De Ryck T, Sadabad MS, Pinheiro I, Possemiers S, Van den Abbeele P,
692   Derycke L, Bracke M, Pieters J, Hennebel T, Harmsen HJ, Verstraete W, and Van de Wiele T.
693   2014. The HMI (TM) module: a new tool to study the Host-Microbiota Interaction in the human
694   gastrointestinal tract *in vitro*. *Bmc Microbiology* 14. 10.1186/1471-2180-14-133
695   Marzorati M, Vilchez-Vargas R, Bussche JV, Truchado P, Jauregui R, El Hage RA, Pieper DH, Vanhaecke L,
696   and Van de Wiele T. 2017. High-fiber and high-protein diets shape different gut microbial
697   communities, which ecologically behave similarly under stress conditions, as shown in a
698   gastrointestinal simulator. *Molecular Nutrition & Food Research* 61. 10.1002/mnfr.201600150
699   Mazur O, and Zimmer J. 2011. Apo- and cellopentaose-bound structures of the bacterial cellulose
700   synthase subunit BcsZ. *Journal of Biological Chemistry* 286:17601-17606.
701   10.1074/jbc.M111.227660
702   McMurdie PJ, and Holmes S. 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is
703   Inadmissible. *Plos Computational Biology* 10. 10.1371/journal.pcbi.1003531
704   Michlmayr H, Hell J, Lorenz C, Bohmdorfer S, Rosenau T, and Kneifel W. 2013. Arabinoxylan
705   oligosaccharide hydrolysis by family 43 and 51 glycosidases from Lactobacillus brevis DSM
706   20054. *Appl Environ Microbiol* 79:6747-6754. 10.1128/AEM.02130-13
707   Nava GM, Friedrichsen HJ, and Stappenbeck TS. 2011. Spatial organization of intestinal microbiota in the
708   mouse ascending colon. *Isme Journal* 5:627-638. 10.1038/ismej.2010.161
709   Oksanen J, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL,
710   Solymos P, Stevens H, Szoecs E, and Wagner H. 2016. vegan: Community Ecology Package. R
711   package version 2.4-0. https://CRAN.R-project.org/package=vegan.

712 Plugge CM. 2005. Anoxic media design, preparation, and considerations. *Environmental Microbiology*
713   397:3-16. 10.1016/S0076-6879(05)97001-8
714 Probert HM, and Gibson GR. 2002. Bacterial biofilms in the human gastrointestinal tract. *Curr Issues*
715   *Intest Microbiol* 3:23-27.
716 Prückler M, Lorenz C, Endo A, Kraler M, Durrschmid K, Hendriks K, Soares da Silva F, Auterith E, Kneifel
717   W, and Michlmayr H. 2015. Comparison of homo- and heterofermentative lactic acid bacteria
718   for implementation of fermented wheat bran in bread. *Food Microbiol* 49:211-219.
719   10.1016/j.fm.2015.02.014
720 Pruesse E, Peplies J, and Glockner FO. 2012. SINA: accurate high-throughput multiple sequence
721   alignment of ribosomal RNA genes. *Bioinformatics* 28:1823-1829.
722   10.1093/bioinformatics/bts252
723 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, and Glöckner FO. 2013. The SILVA
724   ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic*
725   *Acids Research* 41:D590-D596. 10.1093/nar/gks1219
726 R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical
727   Computing, Vienna, Austria. https://www.R-project.org/.
728 Richardson AJ, McKain N, and Wallace RJ. 2013. Ammonia production by human faecal bacteria, and the
729   enumeration, isolation and characterization of bacteria capable of growth on peptides and
730   amino acids. *Bmc Microbiology* 13. 10.1186/1471-2180-13-6
731 Rios-Covian D, Gueimonde M, Duncan SH, Flint HJ, and de los Reyes-Gavilan CG. 2015. Enhanced
732   butyrate formation by cross-feeding between Faecalibacterium prausnitzii and Bifidobacterium
733   adolescentis. *Fems Microbiology Letters* 362. 10.1093/femsle/fnv176
734 Robrish SA, Oliver C, and Thompson J. 1991. Sugar Metabolism by Fusobacteria - Regulation of
735   Transport, Phosphorylation, and Polymer Formation by Fusobacterium-Mortiferum Atcc-25557.
736   *Infection and Immunity* 59:4547-4554.
737 Roos S, and Jonsson H. 2002. A high-molecular-mass cell-surface protein from Lactobacillus reuteri 1063
738   adheres to mucus components. *Microbiology-Sgm* 148:433-442.
739 Ruas-Madiedo P, Moreno JA, Salazar N, Delgado S, Mayo B, Margolles A, and de los Reyes-Gavilan CG.
740   2007. Screening of exopolysaccharide-producing Lactobacillus and Bifidobacterium strains
741   isolated from the human intestinal microbiota. *Applied and Environmental Microbiology*
742   73:4385-4388. 10.1128/Aem.02470-06
743 Salamanca-Cardona L, Ashe CS, Stipanovic AJ, and Nomura CT. 2014. Enhanced production of
744   polyhydroxyalkanoates (PHAs) from beechwood xylan by recombinant Escherichia coli. *Appl*
745   *Microbiol Biotechnol* 98:831-842. 10.1007/s00253-013-5398-4
746 Sanchez JI, Marzorati M, Grootaert C, Baran M, Van Craeyveld V, Courtin CM, Broekaert WF, Delcour JA,
747   Verstraete W, and Van de Wiele T. 2009. Arabinoxylan-oligosaccharides (AXOS) affect the
748   protein/carbohydrate fermentation balance and microbial population dynamics of the Simulator
749   of Human Intestinal Microbial Ecosystem. *Microbial Biotechnology* 2:101-113. 10.1111/j.1751-
750   7915.2008.00064.x
751 Savard P, and Roy D. 2009. Determination of Differentially Expressed Genes Involved in Arabinoxylan
752   Degradation by Bifidobacterium longum NCC2705 Using Real-Time RT-PCR. *Probiotics and*
753   *Antimicrobial Proteins* 1:121-129. 10.1007/s12602-009-9015-x
754 Schloss PD, and Westcott SL. 2011. Assessing and Improving Methods Used in Operational Taxonomic
755   Unit-Based Approaches for 16S rRNA Gene Sequence Analysis. *Applied and Environmental*
756   *Microbiology* 77:3219-3226. 10.1128/Aem.02810-10
757 Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks
758   DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, and Weber CF. 2009. Introducing
759   mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing

760   and Comparing Microbial Communities. *Applied and Environmental Microbiology* 75:7537-7541.
761   10.1128/Aem.01541-09
762   Shewry PR. 2009. Wheat. *Journal of Experimental Botany* 60:1537-1553. 10.1093/jxb/erp058
763   Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large
764   phylogenies. *Bioinformatics* 30:1312-1313. 10.1093/bioinformatics/btu033
765   Stevens BJ, and Selvendran RR. 1988. Changes in composition and structure of wheat bran resulting
766   from the action of human faecal bacteria *in vitro*. *Carbohydr Res* 183:311-319.
767   Swidsinski A, Loening-Baucke V, Verstraelen H, Osowska S, and Doerffel Y. 2008. Biostructure of fecal
768   microbiota in healthy subjects and patients with chronic idiopathic diarrhea. *Gastroenterology*
769   135:568-579. 10.1053/j.gastro.2008.04.017
770   Tannock GW. 2004. A special fondness for lactobacilli. *Appl Environ Microbiol* 70:3189-3194.
771   10.1128/AEM.70.6.3189-3194.2004
772   The HC, Thanh DP, Holt KE, Thomson NR, and Baker S. 2016. The genomic signatures of Shigella
773   evolution, adaptation and geographical spread. *Nature Reviews Microbiology* 14:235-250.
774   10.1038/nrmicro.2016.10
775   Thompson J, Robrish SA, Bouma CL, Freedberg DI, and Folk JE. 1997. Phospho-beta-glucosidase from
776   Fusobacterium mortiferum: purification, cloning, and inactivation by 6-phosphoglucono-delta-
777   lactone. *Journal of Bacteriology* 179:1636-1645.
778   United States Department of Agriculture (USDA). 2016. National Nutrient Database for Standard
779   Reference Release 28.
780   Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, De Weirdt R, Kerckhof
781   FM, and Van de Wiele T. 2013. Butyrate-producing Clostridium cluster XIVa species specifically
782   colonize mucins in an *in vitro* gut model. *Isme Journal* 7:949-961. 10.1038/ismej.2012.158
783   Van den Abbeele P, Grootaert C, Possemiers S, Verstraete W, Verbeken K, and Van de Wiele T. 2009. *In*
784   *vitro* model to study the modulation of the mucin-adhered bacterial community. *Applied*
785   *Microbiology and Biotechnology* 83:349-359. 10.1007/s00253-009-1947-2
786   Van den Abbeele P, Roos S, Eeckhaut V, MacKenzie DA, Derde M, Verstraete W, Marzorati M,
787   Possemiers S, Vanhoecke B, Van Immerseel F, and Van de Wiele T. 2012. Incorporating a
788   mucosal environment in a dynamic gut model results in a more representative colonization by
789   lactobacilli. *Microbial Biotechnology* 5:106-115. 10.1111/j.1751-7915.2011.00308.x
790   van den Broek LAM, Hinz SWA, Beldman G, Vincken JP, and Voragen AGJ. 2008. Bifidobacterium
791   carbohydrases-their role in breakdown and synthesis of (potential) prebiotics. *Molecular*
792   *Nutrition & Food Research* 52:146-163. 10.1002/mnfr.200700121
793   Walker AW, Duncan SH, Harmsen HJM, Holtrop G, Welling GW, and Flint HJ. 2008. The species
794   composition of the human intestinal microbiota differs between particle-associated and liquid
795   phase communities. *Environmental Microbiology* 10:3275-3283. DOI 10.1111/j.1462-
796   2920.2008.01717.x
797   Walker AW, Duncan SH, McWilliam Leitch EC, Child MW, and Flint HJ. 2005. pH and peptide supply can
798   radically alter bacterial populations and short-chain fatty acid ratios within microbial
799   communities from the human colon. *Appl Environ Microbiol* 71:3692-3700.
800   10.1128/AEM.71.7.3692-3700.2005
801   Wang Q, Garrity GM, Tiedje JM, and Cole JR. 2007. Naive Bayesian classifier for rapid assignment of
802   rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*
803   73:5261-5267. 10.1128/Aem.00062-07
804   Wang XY, Cai YP, Sun YJ, Knight R, and Mai V. 2012. Secondary structure information does not improve
805   OTU assignment for partial 16s rRNA sequences. *Isme Journal* 6:1277-1280.
806   10.1038/ismej.2011.187

807

RDP Seqmatch and NCBI BLAST results from the single colony isolates.

The best hits, with the highest percentage identity (NCBI) and similarity score (S_ab in RDP) are shown. Species obtained by direct plating are indicated in boldface.

Isolate	Best NCBI/RDP match (>98% identity)
	Donor 1
83,95	*Bacillus anthracis/toyonensis/cereus/thuringiensis/ pseudomycoides/mycoides/weihenstephanensis/ marcorestinctum/bingmayongensis/manliponensis/ gaemokensis/cytotoxicus*
78	*Bacteroides eggerthii*
84,87	*Bacteroides dorei*
79,85	*Bacteroides fragilis*
86	*Bacteroides thetaiotaomicron/faecis*
76	*Bifidobacterium bifidum*
80,88,90	*Bifidobacterium adolescentis/faecale/ruminantium*
82,91,92,93,94,96,97,99	*Collinsella aerofaciens*
81	*Dorea formicigenerans*
103,105,106,107,108,109,110	*Lactobacillus delbrueckii*
101,102,104	*Lactobacillus fermentum*
	Donor 2
19,25,28	*Bifidobacterium faecale/adolescentis*
21	*Bifidobacterium bifidum*
26	*Bifidobacterium pseudocatenulatum*
34	*Bifidobacterium faecale/adolescentis/ruminantium*
10,27,30,31,32,35,37	*Escherichia coli/fergusonii/marmotae/vulneris/albertii* *Shigella sonnei/flexneri/dysenteriae/boydii*

29	*Enterococcus durans*
36,40,41,42,43,44,45,46	*Pediococcus pentosaceus*
13,15,18	*Streptococcus pasteurianus/macedonicus/equinus/ gallolyticus/lutetiensis/loxodontisalivarius/ infantarius/saliviloxodontae*

Donor 3	
153	*Enterococcus faecium*
131,138,146,151,152,153,154 ,156,157,158,159,160,161	*Enterococcus faecium/lactis/durans/villorum/hirae/ thailandicus/mundtii/dispar/canintestini/ratti/raffinosus/ pseudoavium/ casseliflavus/avium/viikkiensis/gilvus/ malodoratus/devriesei/gallinarum/ xiangfangensis/canis/ asini/massiliensis/pallens*
143,144,149	*Shigella sonnei/flexneri Escherichia fergusonii*
128,129,141,145,147	*Klebsiella michiganensis/oxytoca/pneumoniae/ quasipneumoniae, Enterobacter cloacae/bugandensis/ cancerogenus/asburiae/xiangfangensis Escherichia vulneris, Yokenella regensburgei Kluyvera cryocrescens Raoultella ornithinolytica/terrigena*
133,137,140	*Streptococcus lutetiensis/infantarius/equinus pasteurianus/macedonicus*
136,155	*Pediococcus pentosaceus/stilesii/ claussenii*

	Donor 4
184,185	*Bacteroides ovatus* (90%,96% similarity)
186	*Bacteroides ovatus*
206	*Bifidobacterium adolescentis/faecale*
181,182,195,196,201,202	*Collinsella aerofaciens*
200	*Eubacterium rectale*
187,188,189,190,191,192,193,194	*Escherichia coli/fergusonii/vulneris/albertii/marmotae*
	Shigella flexneri/sonnei/boydii/dysenteriae
	Brenneria alni
198	*Hungatella effluvii*

Figure 1

Overview of the experimental set-up.

In this study, wheat bran-metabolizing and colonizing species were isolated from human fecal microbiota obtained from four healthy individuals. To this end, insoluble wheat bran particles with a characterized chemical composition were incorporated in solid agar plates used in a direct plating method on the one hand and added to a liquid broth used in an enrichment procedure on the other hand. As described in details in the materials and methods section of the manuscript, the fecal sample is directly plated onto the solid wheat bran agar plates to isolate wheat bran metabolizing species. Whereas, the enrichment method selects for wheat bran-attaching and metabolizing species by subculturing the wheat bran residue during three consecutive transfers.
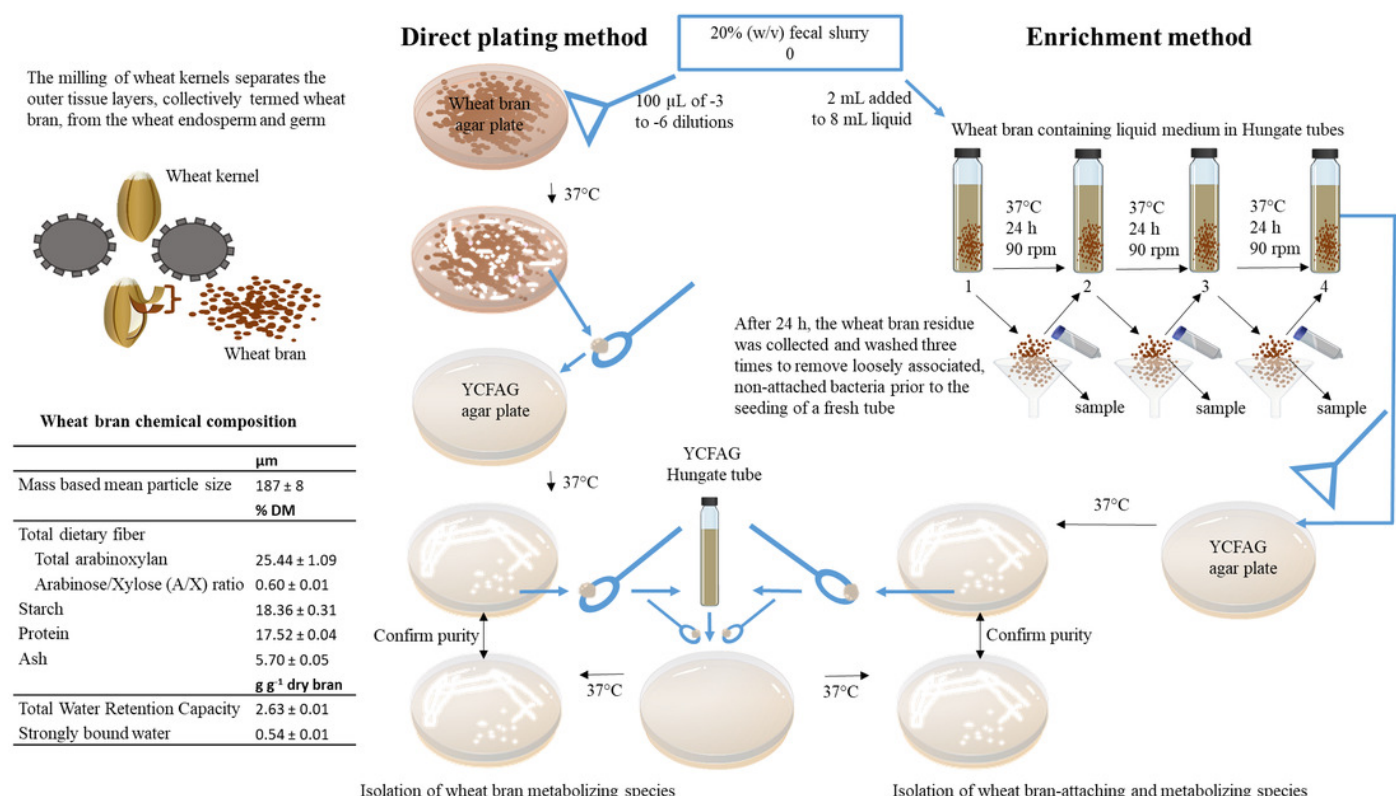
Figure 2

Short Chain Fatty Acid (SCFA) production by the fecal microbiota derived from four different donors during enrichment with wheat bran as the sole nutrient source.

The fecal sample (enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4). The pH is indicated on top of the stacked bars and decreased considerably compared to the starting pH (5.8 and 6.8).
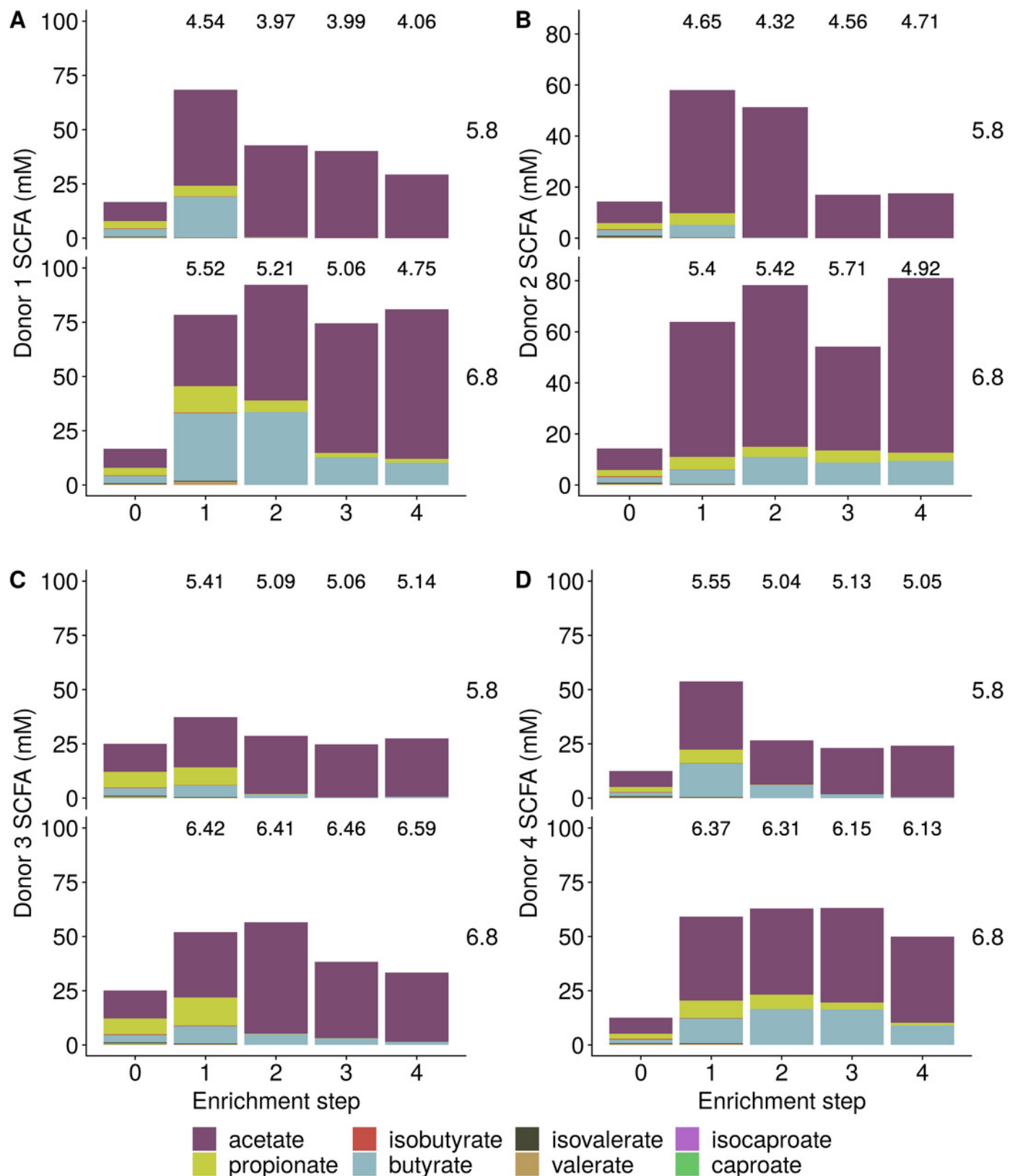
Figure 3

Reduction in the microbial community richness (Chao 1 richness estimator) and diversity (Shannon diversity index) during consecutive enrichment steps with wheat bran as the sole nutrient source, as shown for donor 1.

The fecal sample (enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4).
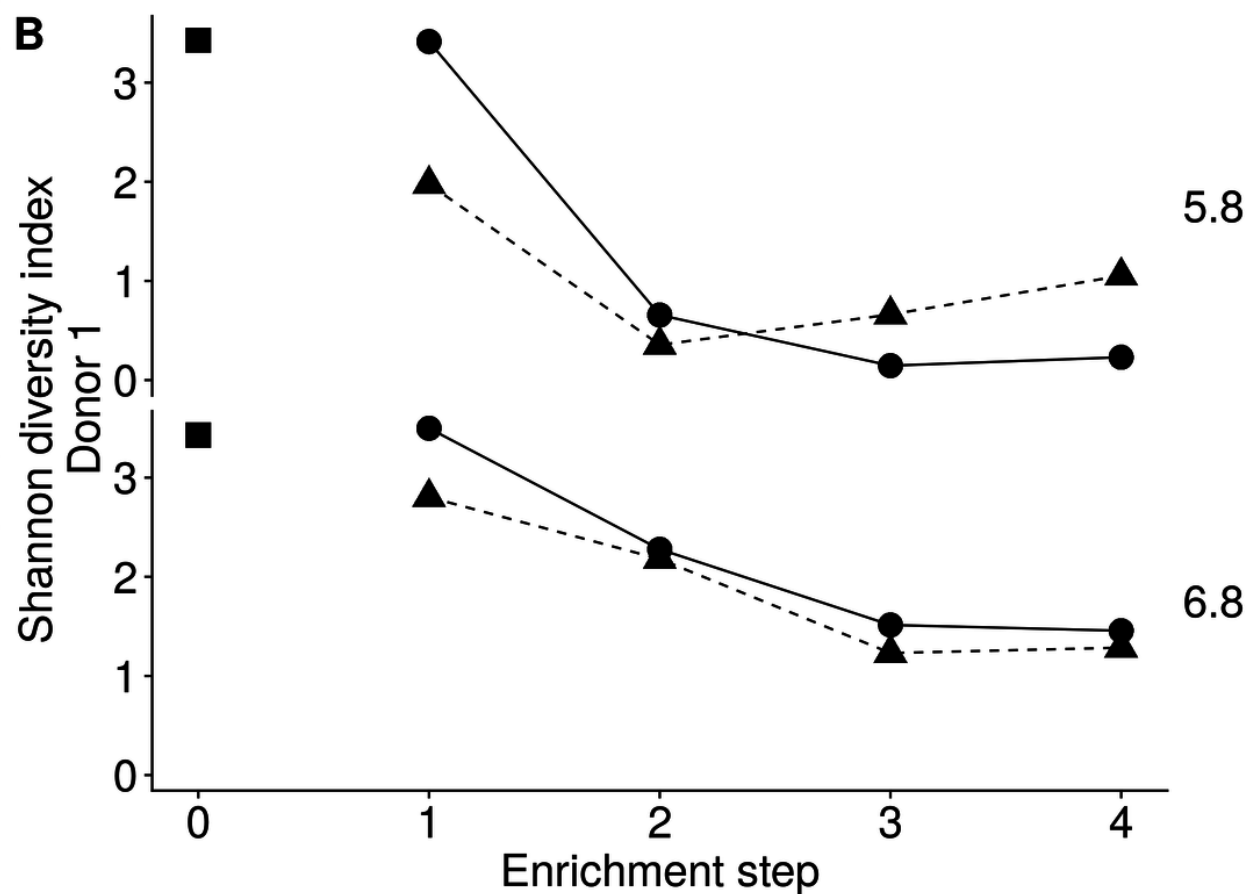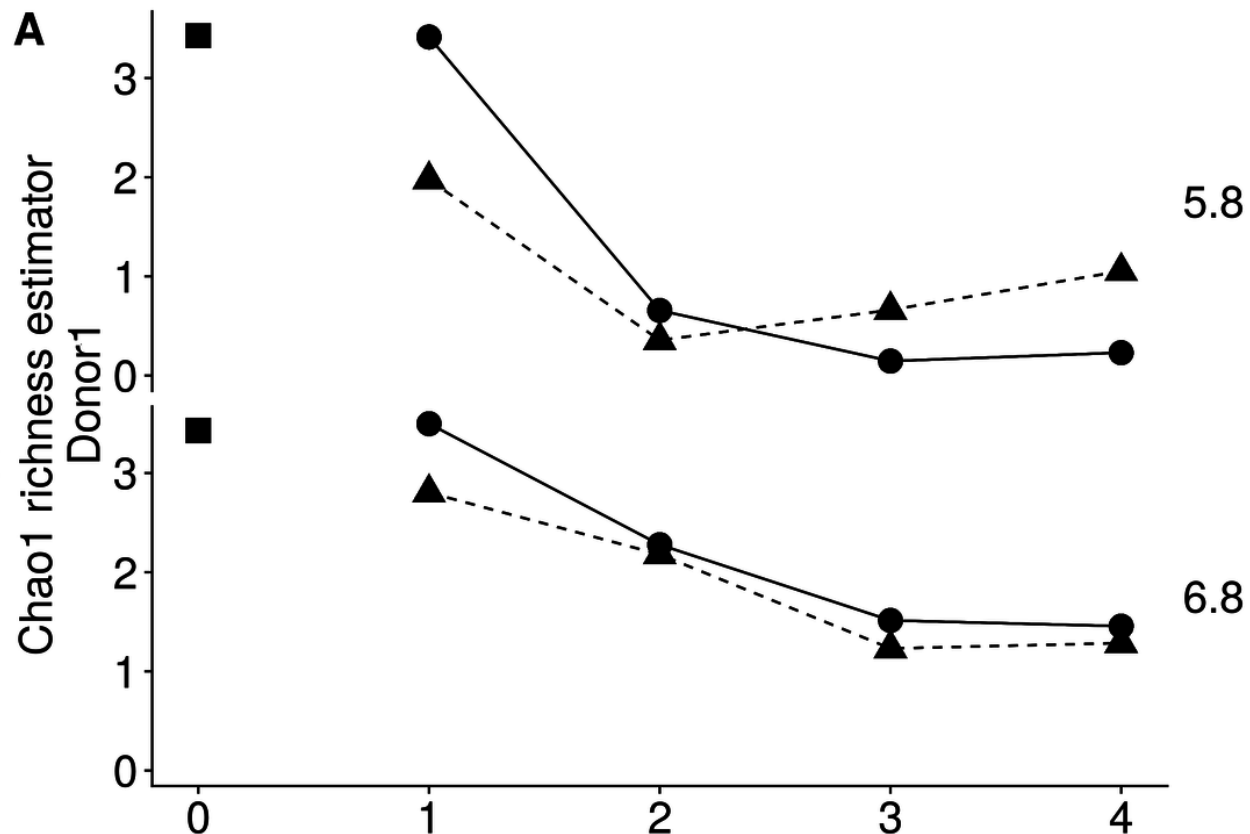
Figure 4

Shifts in genus (A) and species (B) level microbial community composition of donor 1 during consecutive enrichment steps with wheat bran as the sole nutrient source.

The fecal sample (FS; enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4). Family level taxa appearing in the genus level plots should be interpreted as unclassified genus belonging to the respective family.
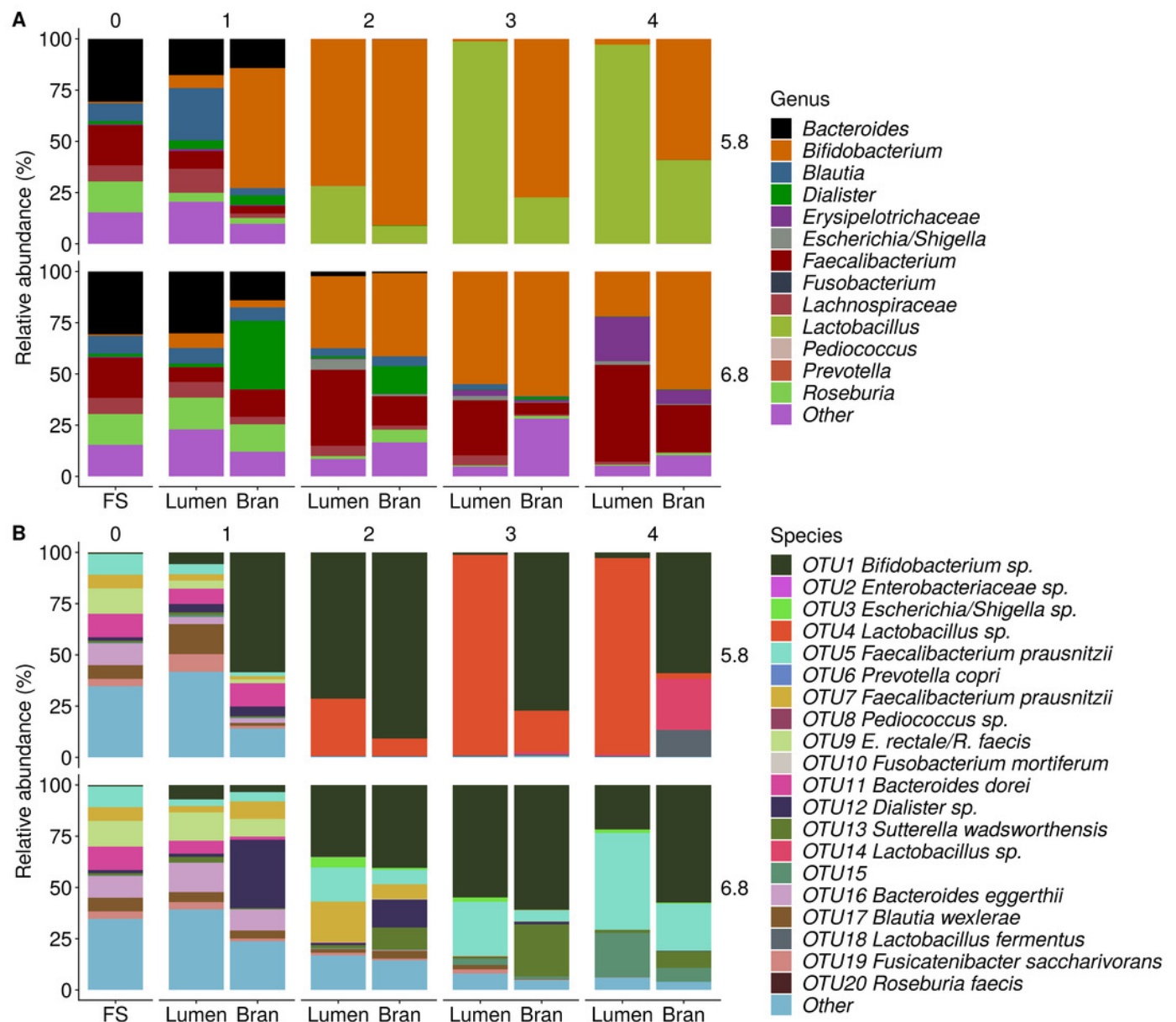
Figure 5

Shifts in genus (A) and species (B) level microbial community composition of donor 2 and 3 after four enrichment steps with wheat bran as the sole nutrient source.

The fecal sample (FS; enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4). Only the final enrichment step and FS are shown in this plot. Family level taxa appearing in the genus level plots should be interpreted as unclassified genus belonging to the respective family.
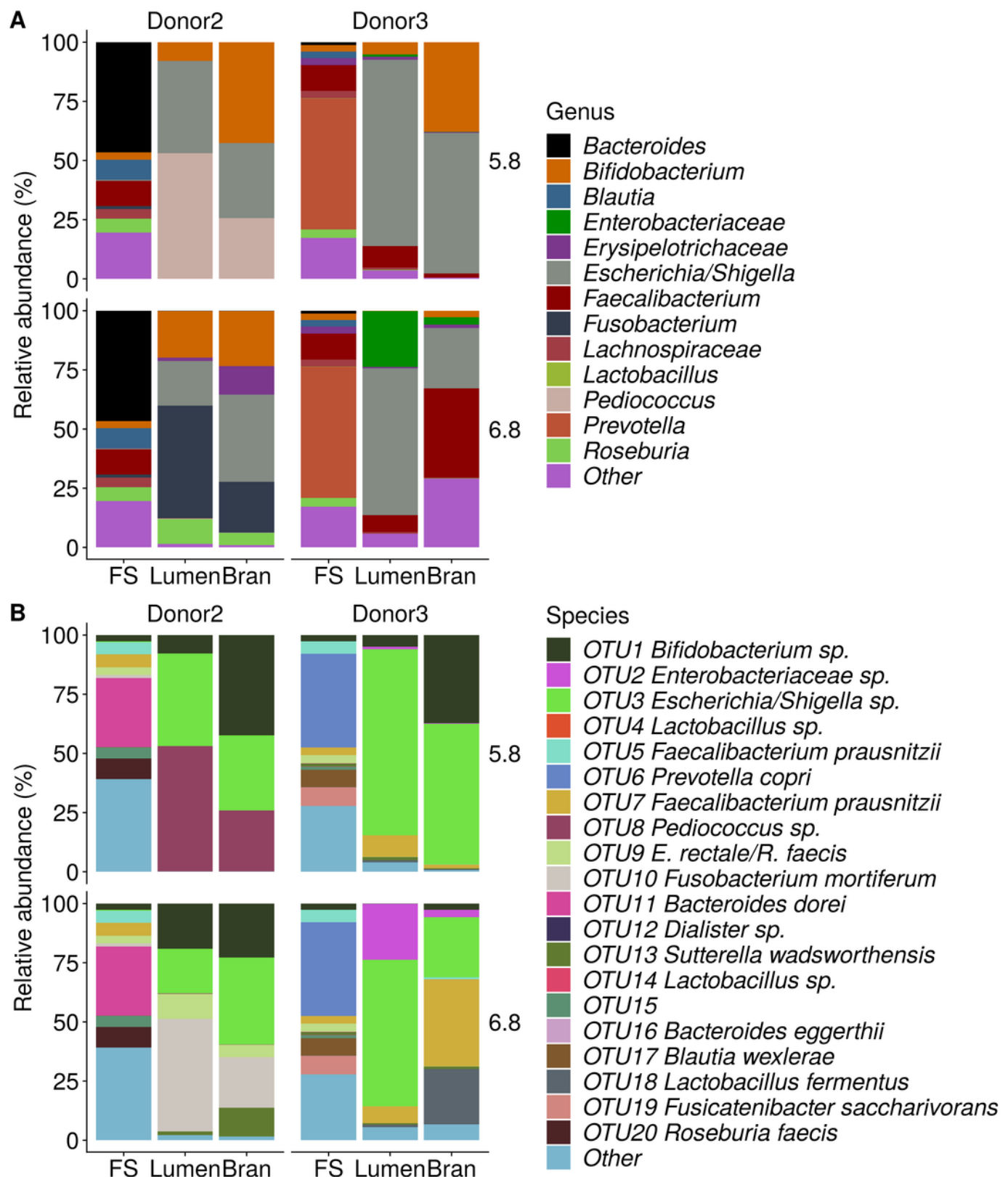
Figure 6

Genus (A) and species (B) level microbial community composition of donor 4 after the final enrichment step with wheat bran as the sole nutrient source.

The fecal sample (FS; enrichment step 0) was incubated with wheat bran for 24 h (enrichment step 1), after which the wheat bran residue was washed to remove loosely attached bacteria and used to seed a new incubation (enrichment step 2). This procedure was repeated two more times (enrichment step 3 and 4). Only the final enrichment step was shown in this plot, due to a low read number (<100 reads) in the fecal sample of donor 4. Family level taxa appearing in the genus level plots should be interpreted as unclassified genus belonging to the respective family.
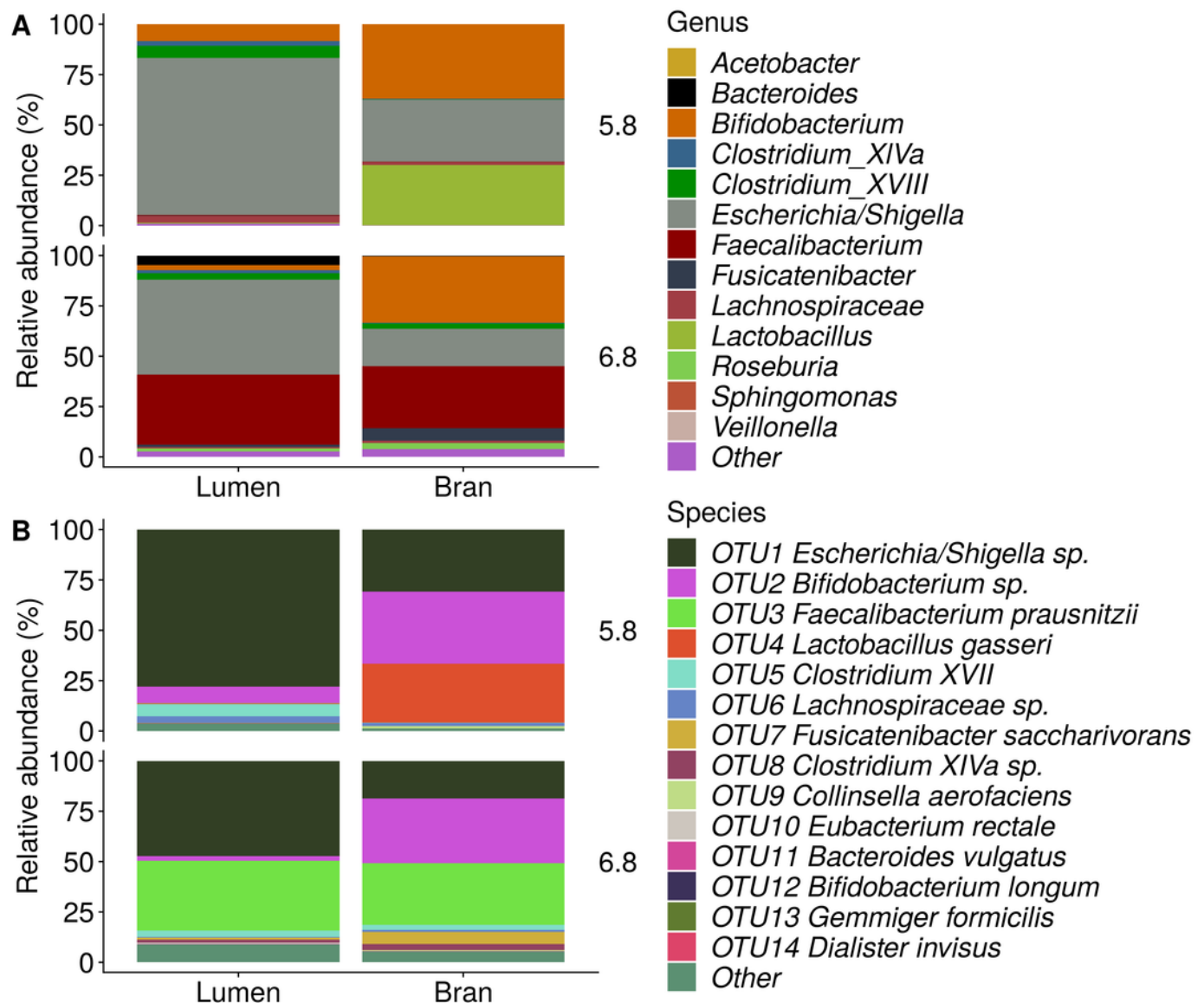
Figure 7

Removal of starch during pre-digestion.

'(A) Cryo-SEM image of an unmodified wheat bran particle with endosperm starch granules, resulting from the crude milling process, covering the surface of aleurone cells. (B) SEM image of a micronized wheat bran fragment after pre-digestion, without attached starch granules.'
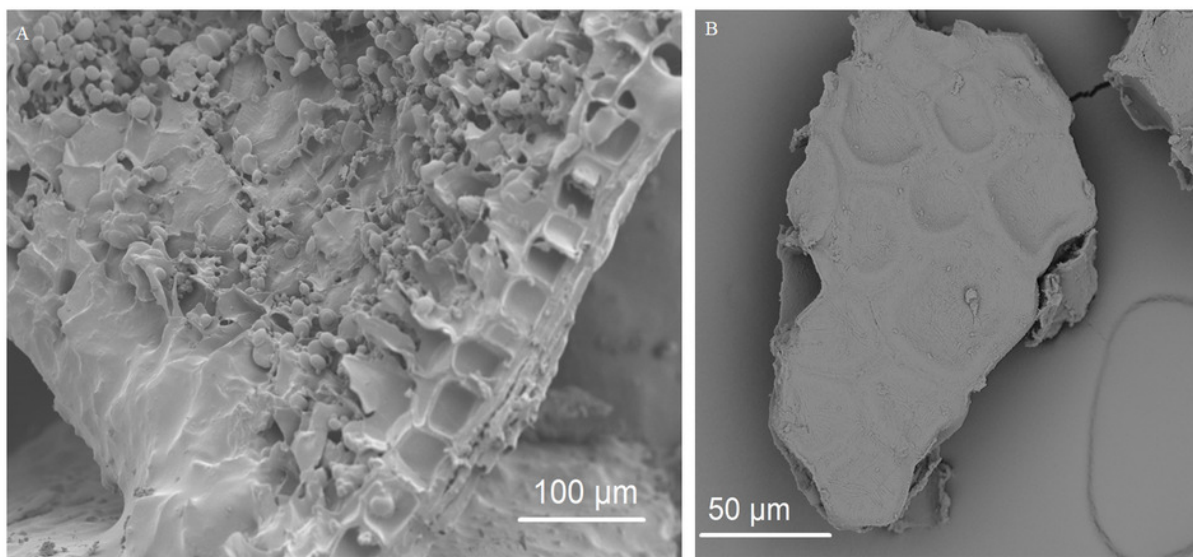
Figure 8

SEM images of the fermented wheat bran residue after 24 h.

'(A) Uncolonized pericarp tissue. (B) Residual starch granule. (C) Bacteria inside a wheat aleurone cell. (D) Bacteria on aleurone fragment. (E) Partial degradation of aleurone cell. (F) Ligature at the junction of aleurone cells.'

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.