Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize pectin, uronic acids and host-derived substrates for growth

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ABSTRACT

Faecalibacterium prausnitzii is one of the most abundant commensal bacteria in the healthy human large intestine, but information on genetic diversity and substrate utilization is limited. Here, we examine the phylogeny, phenotypic characteristics and influence of gut environmental factors on growth of *F. prausnitzii* strains isolated from healthy subjects. Phylogenetic analysis based on the 16S rRNA sequences indicated that the cultured strains were representative of *F. prausnitzii* sequences detected by direct analysis of fecal DNA, and separated the available isolates into two phylogroups. Most *F. prausnitzii* strains tested grew well under anaerobic conditions on apple pectin. Furthermore *F. prausnitzii* strains competed successfully in co-culture with two other abundant pectin-utilizing species, *Bacteroides thetaiotaomicron* and *Eubacterium eligens,* with apple pectin as substrate, suggesting that this species makes a contribution to pectin fermentation in the colon. Many *F. prausnitzii* isolates were able to utilise uronic acids for growth, an ability previously thought to be confined to *Bacteroides* spp. among human colonic anaerobes. Most strains grew on N-acetyl glucosamine, demonstrating an ability to utilise host-derived substrates. All strains tested were bile-sensitive, showing at least 80% growth inhibition in the presence of 0.5 µg/ml bile salts, while inhibition at mildly acidic pH was strain dependent. These attributes help to explain the abundance of *F. prausnitzii* in the colonic community, but also suggest factors in the gut environment that may limit its distribution.

INTRODUCTION

Faecalibacterium (formerly *Fusobacterium*) *prausnitzii* (11) is one of the three most abundant species detected in human feces by anaerobic cultivation (32) and by 16S rRNA-based molecular analyses (21, 51, 52, 57). Following its first isolation (4, 20) this species received little attention, partly because of its oxygen sensitivity (14), until new isolates became available from studies on the dominant butyrate-producing bacteria from the human colon (2) that allowed the definition of the new genus *Faecalibacterium* (11). Interest in this bacterium has increased recently with reports that the relative abundance of *F. prausnitzii* among the human colonic microbiota, as estimated by 16S rRNA-based culture-independent methods, is reduced in certain forms of inflammatory bowel disease (IBD). Crohn's disease (CD) patients, mainly those with ileal involvement, have been reported to exhibit diminished prevalence of *Firmicutes* often with a concomitant increase in *Proteobacteria* (15, 30, 60). Molecular analysis of both fecal and biopsy samples has revealed that the depletion in the former is due in part to decreased abundance of the *F. prausnitzii* group (6, 45, 47, 50, 60) . Reduced *F. prausnitzii* abundance has also been reported in colorectal cancer (1) and in the frail elderly (29, 56) leading to the suggestion that this bacterium could provide an indicator of a healthy gut microbiota. *F. prausnitzii* is one of the main sources of butyrate in the colon (27, 37) and the multiple effects of butyrate as the preferred energy source for the colonocytes and upon apoptosis, inflammation and oxidative stress are generally considered to be beneficial to intestinal health (18, 37, 40).

74 *F. prausnitzii* is also thought to have additional anti-inflammatory properties that are
75 suggested by cellular studies and TNBS colitis models in mice (49).

76 In view of the proposed role of *F. prausnitzii* in intestinal health it is important
77 to gain a better understanding of the microbial ecology of this species. It is currently
78 unclear what major substrates, of dietary or host origin, are likely to support growth
79 and what factors in the gut environment may influence its distribution in the intestine.
80 It is also important to establish how much genetic and phenotypic variation occurs
81 within this species, and the extent to which available cultured strains represent the
82 diversity present *in vivo*. This study addresses these questions by examining the
83 characteristics of the available cultured strains, including new isolates from healthy
84 humans.

85

86 **MATERIALS AND METHODS**

87 **Bacterial strains and growth conditions.** The *F. prausnitzii* isolates listed in Table 1
88 were from stocks held by the authors (S.H. Duncan, Rowett Institute of Nutrition and
89 Health, Aberdeen, UK and H. J. M. Harmsen, Department of Medical Microbiology,
90 University of Groningen, Groningen, The Netherlands) and all are of human fecal
91 origin (Table 1). *F. prausnitzii* related isolates were obtained from the highest
92 countable dilution of human fecal samples in roll tubes of anaerobic M2GSC medium
93 (31), as described previously (2). Anaerobic culture methods were those of Bryant,
94 1972 (3) using Hungate culture tubes, sealed with butyl rubber septa (Bellco Glass).
95 Additional *F. prausnitzii* strains designated as HTF isolates were isolated from freshly

96 voided human stools, by plating 1 µl of the fecal material with a loop as a lawn
97 directly on YCFAG-medium (see below). After 12 h to 16 h incubation at 37 ^{o}C in an
98 anaerobic tent (80 % N_2, 12 % CO_2, and 8 % H_2) 500 translucent colonies per sample
99 were selected, and sub-cultured on fresh plates (50 per plate in a grid-like fashion).
100 After growth, the colonies were presumptively identified based on morphology,
101 eliminating 95 % of the colonies. The remaining colonies were further purified and
102 Gram stained. Up to 5 colonies per sample were finally identified by 16S rRNA-gene
103 sequencing. The isolates were routinely maintained by growing for 16-18 h at 37 °C
104 in 7.5 ml aliquots of M2GSC medium (31) and maintained anaerobically using O_2-
105 free CO_2. The low % G+ C Gram-positive Firmicutes strains screened for pectin
106 utilization in this study (see Table 3) were also from stocks held by the authors
107 (Rowett Institute of Nutrition and Health, Aberdeen, UK) and several came from
108 previous studies (2, 26). *Roseburia intestinalis* L1-82 (DSM 14610^{T}), *Roseburia*
109 *hominis* A2-183 (DSM 16839^{T}), *Roseburia inulinivorans* strains A2-194 (DSM
110 16841^{T}) and L1-83, *Roseburia faecis* M72/1 (DSM 16841^{T}) and M88/1 and
111 *Eubacterium rectale* A1-86 (DSM 17629), M104/1 and L2-21 with type strains
112 deposited with Deutsche Sammlung von Mikrooganismen und Zellkulturen (DSMZ).
113 Other Firmicutes tested in the study included *Butyrivibrio fibrisolvens* 16/4 which was
114 isolated as a butyrate producing wheat bran degrader (41). *Eubacterium siraeum* 70/3
115 (8) and V10Sc8a are also isolates from human fecal samples. *Eubacterium eligens*
116 DSM 3376 was from DSMZ and *Bacteroides thetaiotaomicron* B5482 was a gift from
117 A. Salyers and both strains were included in the co-culture studies.

Growth medium. YCFA medium consists of (per 100ml): casitone (1.0 g), yeast extract (0.25 g), $NaHCO_3$ (0.4 g), cysteine (0.1 g), K_2HPO_4 (0.045 g), KH_2PO_4 (0.045 g) NaCl, (0.09 g) $(NH_4)_2SO_4$ (0.09 g), $MgSO_4 \cdot 7H_2O$ (0.009 g), $CaCl_2$, (0.009 g) resazurin (0.1 mg), haemin (1 mg), biotin (1 µg), cobalamin (1 µg), p-aminobenzoic acid (3 µg), folic acid (5 µg), pyridoxamine (15 µg). In addition the following SCFA are included (final concentrations): acetate (33mM), propionate (9 mM), iso-butyrate, iso-valerate and valerate (each 1 mM). Cysteine is added to the medium following boiling and dispensed into Hungate tubes while the tubes are flushed with CO_2. After autoclaving, filter sterilized solutions of thiamine and riboflavin are added to give final concentrations of 0.05 µg ml^{-1} of each. For some experiments the casitone content was decreased to 0.2%; this modified medium is referred to as YcFA. Carbohydrate or other energy sources were added as indicated and the final pH of the medium was adjusted to 6.8±0.1.

DNA extraction, PCR amplification and DGGE fingerprinting. DNA was extracted and purified from 18 h-old cultures of *F. prausnitzii* strains grown on M2GSC medium by using the Wizard™ Genomic Purification Kit (Promega Corporation, Madison, WI, USA). 16S rRNA sequences were amplified using universal bacterial primers GC-357F (33) and 907R (34) to give an approximately *ca.* 580-bp product flanking variable regions V3 to V5. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) were carried out as previously reported (30).

16S rRNA gene amplification and sequencing. 16S rRNA genes were amplified using the universal bacterial primers 7F and 1510R (23) as described previously (12). PCR products were cleaned with the Wizard PCR product purification kit (Promega, Southampton, UK) and used to obtain bidirectional partial 16S rRNA gene sequences by using primers 7F, 519F, 519R, 916F, 916R and 1510R (16, 23) on a Beckman capillary sequencer. All primers were obtained from Eurofins MWG.

16S rRNA gene sequence full length construction and phylogenetic analysis. Sequences from cultured isolates were manually inspected in order to assess quality. Sequences edition and assembling was carried out using the BioEdit Sequence Alignment Editor version 7.0.9.0. (17). Sequences were then aligned in Mothur [http://www.mothur.org; (46)] using the SILVA bacteria database as reference alignment, available at the same source. Alignment was then imported into the ARB software package (28) loaded with the SILVA 16S rRNA-ARB-compatible database [SSURef-100, August 2009, available through the SILVA rRNA database project at http://www.arb-silva.de/, (36)]. For the detection of chimeric sequences each sequence was checked manually in the alignment and phylogenetic trees were screened for sequences with unrealistic long branches or unique branching sites. Cultured representatives from *Ruminococcaceae* were included as reference and *Eubacterium desmolans* was used to root the tree. Phylogenetic analyses of the 16S rRNA gene sequences were conducted using the ARB-software package, using the neighbour-joining method (42) and Jukes-Cantor algorithm for distance analysis. Tree topologies were evaluated using maximum parsimony and maximum likelihood

161 methods. No filters or masks were used when constructing the trees. Bootstrapping
162 analysis (1000 replicates) was done to test the robustness of the NJ-JC tree using
163 PHYLIP (13).
164 To assess which *F. prausnitzii* phylogroups were represented by the isolates,
165 representative sequences of 16S rRNA genes directly amplified from fecal DNA were
166 included (boldfaced in Fig. 2). These uncultured sequences were aligned and
167 processed as described above and then added to the isolates-based tree using the
168 *parsimony quick add marked tool* already implemented in ARB software package,
169 thereby maintaining the overall tree topology.
170 The 16S rRNA gene full length sequences of isolates S3L/3, S4L/4, HTF-A, HTF-B,
171 HTF-C, HTF-E, HTF-F, HTF-I, HTF-60C, HTF-75H, L2-15, L2-39 and L2-61 were
172 deposited to the GenBank/EMBL/DDBJ database under the accession numbers
173 HQ457025-HQ457033, and JN037415-JN037417 respectively.
174 **RAPD-PCR**. Isolates were screened by random amplified polymorphic DNA-PCR
175 (RAPD-PCR) using the primer 1254, following a previously described method (59).
176 RAPD-PCR profiles were compared using the GelComparII software (Applied
177 Maths, Belgium). The UPGMA method was used to build the dendrogram (Fig. S1)
178 and clusters were defined at a similarity score of >93.5%.
179 **Carbohydrate utilization and assessment of bacterial growth.** Substrate utilization
180 was determined by adding a final concentration of 0.5% wt./vol. sugar to YCFA
181 medium. Where possible, growth was measured spectrophotometrically as OD_{650} for
182 triplicate cultures at regular intervals up to stationary phase. For insoluble xylan,

however, fermentation was monitored by final pH measurement. To study competition for pectin, *F. prausnitzii* strains S3L/3 and A2-165 were inoculated individually and together with the known pectin-utilizing species *B. thetaiotaomicron* B5482 and *E. eligens* 3376 in co-cultures and tri-cultures (see Table S2). These experiments used YcFA medium supplemented with 0.5% apple pectin (BDH Chemicals) that had been pre-adjusted to three different initial pH values (6.12, 6.45, 6.79). Samples were collected at 0 h and 24 h to estimate bacterial numbers by FISH, total sugar analysis and SCFA concentrations. SCFA were analysed by gas chromatography following conversion to *t*-butyldimethylsilyl derivatives (39). Total sugars were determined using the colorimetric phenol sulphuric assay (10).

Influence of initial pH and bile salts on bacterial growth. Each strain was inoculated into YCFA medium supplemented with 10 mM glucose (YCFAG) that had been adjusted to the three different initial pH values (6.7, 6.2 and 5.75) as described previously (12). Growth was followed for 24 h by measuring absorbance at 650 nm for triplicate cultures and specific growth rates (h^{-1}) calculated in exponential phase. The influence of bile salts (Sigma B8631) was assessed by inoculating into YCFAG medium containing 0% (control), 0.1%, 0.25% or 0.5% bile salts (wt./vol.), in triplicate. Growth was measured spectrophotometrically up to 24 h using absorbance at 650 nm wavelength. The pH of the medium was also monitored at the beginning and at the end of each experiment.

Enumeration of *F. prausnitzii* by FISH analysis. Cultures were prepared for analysis as described previously (19). Cell suspensions were applied to gelatin-coated

205 slides. Dried slides were hybridised with 10 µl of the Fprau645 oligonucleotide probe
206 (52) (50 ng/µl stock solution) and washed. Between 25 and 30 fields were counted
207 per well using an epifluorescence microscope (Olympus) and image analysis software
208 (Olympus Cell F digital imaging software) or manual counting for numbers less than
209 10 fluorescent cells per field.
210 **Statistical analysis.** Quantitative parameters, such as growth rates and relative OD_{650}
211 values were compared by one-way ANOVA. Bonferroni post-hoc test was applied for
212 multi-comparisons of those variables with more than two subgroups of samples.
213 Prior, data normality was assessed by Shapiro-Wilks test and Leven test was
214 conducted to assess for homoscedasticity. Kruskal-Wallis non parametric tests was
215 performed when required. All statistical analyses were conducted via SPSS 15.0
216 (SPSS Inc., Chicago, IL, USA).
217
218 **RESULTS AND DISCUSSION**
219 **Phylogenetic diversity of *Faecalibacterium prausnitzii*.** Near full-length
220 16S rRNA gene sequences were determined for the first time here for 13 recent
221 isolates of *Faecalibacterium prausnitzii* (Table 1, Fig. 1). The 16S rRNA sequences
222 define two branches within the *Ruminococcaceae*, within which sequences share
223 >97% sequence identity; these also include five sequences reported previously for the
224 isolates M21/2, ATCC27766, ATCC27768^T (phylogroup I), A2-165 and L2-6
225 (phylogroup II). The 18 isolates shown in Fig.1 originated from 10 healthy
226 individuals. Each of these 16S rRNA sequences is unique and came from a different

227 colony, although there was a tendency for sequences to group by isolation and
228 individual. This was also suggested by RAPD-PCR profiles for these strains (Figure
229 S1). Comparison was also made with *F. prausnitzii*-related OTUs defined by partial
230 16S rRNA gene sequences obtained in two recent human studies by direct
231 amplification from fecal DNA (53, 57) (Fig. 2). These represent an additional 23
232 individuals. Phylogroups I and II together account for 97.9% of these directly
233 amplified *F. prausnitzii*-related sequences, with phylogroup I more abundant in the
234 six subjects examined by Walker et al. (57) (62%) than in the 17 subjects examined
235 by Tap et al. (53) (8.3%).

236 DGGE analysis of PCR products amplified from phylogroup I isolates showed
237 a distinct band position compared with phylogroup II isolates (Fig. 3). These band
238 positions correspond to two dominant bands that have previously been associated
239 with *F. prausnitzii* in DGGE analyses of 16S rRNA sequences amplified from human
240 fecal and biopsy samples (22, 30). This previous work also suggested that there is a
241 differential reduction in phylotypes related to M21/2 (phylogroup I) compared with
242 A2-165 relatives (phylogroup II) in biopsies (30) and fecal samples (22) from CD
243 patients.

244 **Substrate utilisation by *Faecalibacterium prausnitzii* isolates.** Growth on
245 carbohydrates of dietary and host origin by four phylogroup I and six phylogroup II
246 isolates is shown in Table 2. The basal YCFA medium (described in the Materials and
247 Methods) contained 30 mM acetate which is known to stimulate the growth of *F.*
248 *prausnitzii* strains (11). Growth was assessed where possible by the change in OD_{650},

249 but for insoluble substrates such as xylan it was necessary to rely on change in
250 medium pH as an indicator of substrate fermentation. The ability of *F. prausnitzii* to
251 utilise dietary polysaccharides was somewhat limited with no growth on
252 arabinogalactan, no fermentation of xylan, and little or no growth on soluble starch.
253 While two strains grew well on inulin, the remainder grew poorly. Stimulation of
254 *F. prausnitzii* 16S rRNA sequences by inulin has been reported *in vivo* in healthy
255 human volunteers (38) but it appears likely from the present work that this
256 stimulation may favour certain strains. Interestingly, most isolates grew on apple
257 pectin, although not on citrus pectin. Salyers *et al.* (43, 44) noted that the utilization
258 of uronic acids was unusual in genera from the human colon other than *Bacteroides*
259 species. In the present study several *F. prausnitzii* strains were able to utilise
260 galacturonic acid, which is an important constituent of pectin.

261 Growth was also detected for most *F. prausnitzii* strains on the host-derived
262 sugar N-acetyl glucosamine and for some strains on D-glucosamine, and D-glucuronic
263 acid, while β–glucuronidase activity has been reported previously in some *F.*
264 *prausnitzii* isolates (8). This suggests that *F. prausnitzii* has the ability to switch
265 between diet- and host-derived substrates in common with several other dominant
266 human colonic species (48). None of the carbohydrates tested allowed differentiation
267 between the two phylogroups.

268 Very little growth was observed when carbohydrates were omitted from the
269 medium, although the basal YCFA medium contains 1% casitone. This indicates that

F. prausnitzii strains have little or no ability to grow with peptides as their sole energy source. No evidence was found for fermentation of porcine gastric mucin.

Tolerance of *Faecalibacterium prausnitzii* isolates to the gut environment. Previous studies have reported that *F. prausnitzii* growth is inhibited by slightly acidic pH (12). The eight isolates tested showed growth rates at pH 5.75 ranging between 20% (for A2-165) and 80% (for HTF-F) of those at pH 6.7 (Fig. 4A). On average there was a 14% decrease at pH 6.2, but a 60% decrease at pH 5.75, compared with pH 6.7. Tolerance of bile salts, whose concentrations have been reported to increase in certain gut disorders (24, 35) is also considered to be an important factor for survival in the intestine. Bile salt tolerance differed among isolates particularly at the lowest concentration tested (0.1%) but all the strains tested were bile salt sensitive, showing on average 76%, 95% and 97% inhibition at 0.1%, 0.25% and 0.5% bile salts, respectively (Fig. 4B). By contrast, other species of intestinal bacteria such as *Bacteroides* spp. and *Enterococcus faecium* have been reported to be resistant up to 20% and 40% bile salt concentrations respectively (5). Bile acids are synthesised in the liver and released into the small intestine where it is estimated that 90-95% of secreted bile is absorbed. The concentration of bile in the healthy large intestine is approximately 0.05-0.3%. The sensitivity of all the *F. prausnitzii* isolates tested to bile salts suggests that this is a factor that may restrict populations of this species in regions of high bile concentration, eg. within the small intestine.

While these differences in sensitivity to bile salts and pH seem likely to influence the distribution of individual strains, there was no statistically significant evidence for consistent differences within phylogroups.

Potential role of *Faecalibacterium prausnitzii* in the fermentation of pectin in the colon. Pectin is extensively fermented in the human colon (7, 55) but the ability to utilize pectin for growth has been reported for relatively few groups of human colonic bacteria. Salyers et al. (43, 44) showed that pectin utilization was relatively common among *Bacteroides* spp. occurring in 47 % of 188 isolates surveyed and prompting subsequent studies on *B. thetaiotaomicron* (9, 54). In contrast, of the 154 strains of Gram-positive anaerobes tested, which included five strains reported as *Fusobacterium prausnitzii,* only *Eubacterium eligens* was previously found to utilize pectin or polygalacturonic acid (43) (Table 3). The present data, however, indicate that *F. prausnitzii* could have a major role in pectin utilization (Table 3).

In order to test this hypothesis further, we examined the ability of two *F. prausnitzii* strains (S3L/3 and A2-165) to compete for apple pectin with representatives of the two other known groups of pectin utilizing bacteria, *B. thetaiotaomicron* and *E. eligens.* As previous studies have shown that pH plays a critical role in determining the outcome of competition between *Bacteroides* spp. and *Firmicutes* (12, 58) incubations were performed at three initial pH values typical of the range seen in the distal colon (Fig.5, Tables S1, S2). In pure cultures, the major fermentation products produced from pectin were butyrate for *F. prausnitzii*, acetate

312 and succinate for *B. thetaiotaomicron,* and formate and acetate for *E. eligens* (Fig.
313 5A). As previously observed for growth on starch and glucose (12) the lowest pH
314 (6.12) curtailed fermentation of pectin by *B. thetaiotaomicron*. As expected (see
315 Fig.4A) both *F. prausnitzii* strains grew well at the lowest pH (Fig.5). Tri-cultures
316 including all three species showed large amounts of butyrate at all three pH values,
317 thus confirming the ability of *F. prausnitzii* to compete for this substrate with the
318 other two pectin utilizing species (Fig. 5B). Counts estimated by FISH for *F.*
319 *prausnitzii* after 24 h incubation indicated greater numbers in the triculture at the
320 lowest pH than at the highest pH (Fig. 5C). Butyrate concentration was less affected
321 by pH, indicating continued fermentative activity by *F. prausnitzii* in spite of
322 decreased cell growth at the highest pH. Data for two-membered co-cultures from this
323 experiment are shown in Tables S1 and S2. Pectin utilization (measured by decrease
324 in total sugar) was highest for cultures including *B. thetaiotaomicron* at pH 6.79
325 (Table S2).

326 **Conclusions.** *F. prausnitzii* is one of the three most abundant bacterial species
327 found in the healthy adult human large intestine, but its ecology has remained largely
328 unknown. This study has substantially increased the number of cultured,
329 characterized *F. prausnitzii* isolates of human origin, and has begun to provide a
330 better understanding of the diversity and microbial ecology of this species in the
331 colon. Based on their 16S rRNA sequences, the available cultured isolates define two
332 broad phylogroups that also include 97 % of *F. prausnitzii* 16S rRNA sequences that
333 are detected by direct amplification from human fecal DNA. Our analysis of

334 phylogroup I and II strains from healthy individuals did not reveal systematic
335 differences between the phylogroups with respect to substrate utilization, pH
336 tolerance or bile sensitivity. Nevertheless, molecular surveys indicate that
337 representatives of both phylogroups often coexist among the dominant microbiota of
338 individuals (53, 57). There is evidence for reduced representation of *F. prausnitzii* in
339 active ileal Crohn's disease (50) and it would be of interest in the future to compare
340 the characteristics, including potential interactions with the immune system, of *F.*
341 *prausnitzii* isolated from Crohn's patients with those from healthy subjects.

342 Based on our analysis of substrate utilization in 10 cultured strains from seven
343 healthy individuals, most *F. prausnitzii* strains have the ability to utilise apple pectin
344 for growth. The previous report that *F. prausnitzii* strains failed to use pectin is most
345 likely to reflect the use of citrus pectin in that study (43). We have shown that *F.*
346 *prausnitzii* strains are able to compete for apple pectin as a substrate in the presence of
347 two other known pectin-utilizing species, *B. thetaiotaomicron* and *E. eligens,*
348 suggesting that they make a contribution to pectin fermentation in the colon. Our
349 results suggest that this may apply especially at mildly acidic pH values when
350 competition from *Bacteroides* spp. is reduced (12, 58). The possibility is also raised
351 that certain pectin-rich substrates might be used to develop prebiotic approaches for
352 stimulating *F. prausnitzii* numbers; interestingly apple pectin has been shown to
353 promote certain Firmicutes in a recent study with rats (25). Another notable attribute of
354 some *F. prausnitzii* strains is the utilization of uronic acids for growth, an ability
355 previously thought to be limited to *Bacteroides* spp. among human gut anaerobes.

Further analysis of substrate utilization in this species will undoubtedly be aided by the availability of draft genomes for several of the *F. prausnitzii* strains studied here. In conclusion, the present findings demonstrate a broad capacity to utilize both diet- and host-derived growth substrates that helps to explain the remarkable abundance of this species within the human colonic microbiota.

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586 **FIGURE LEGENDS**

587

588 **Figure 1. Phylogenetic relationship of *F. prausnitzii* isolates to other members of**
589 ***Clostridium* cluster IV (*Ruminococcaceae*) based on 16S rRNA gene sequences.** The tree
590 was constructed using ARB software package using the neighbour-joining method for
591 distance analysis (Jukes-Cantor algorithm) with 1533 informative positions considered (61 to
592 1442 *E. coli* 16S rRNA gene numbering). Bootstrap values above 80% (expressed as a
593 percentage of 1000 replications), are shown at branching points. Solid circles (●) indicate
594 branches that were consistent with calculations obtained by maximum-parsimony method.
595 Empty circles (○) represent those branches consistent with the maximum-likelihood. The
596 scale bar indicates the number of substitutions per site. *F. prausnitzii* isolates incorporated in
597 this study are highlighted in bold. Sequences accession numbers are shown in brackets. The
598 database sequence for ATCC27766 was included, but this strain was not studied here and it
599 is not listed in Table 1.

600

601 **Figure 2. Neighbour-Joining phylogenetic tree showing the relationship between**
602 **cultured *F. prausnitzii* strains and directly-amplified partial 16S rRNA gene sequences**
603 **from human fecal samples.** 16S rRNA sequences accession numbers are given in
604 parentheses. Squares indicate OTU representative sequences from two recent studies on gut
605 microbiota of healthy subjects (shown in boldface): Tap et al., 2009 (53) study (1,443
606 *F. prausnitzii* sequences out of 10,456 clones from 17 healthy adults of both sexes); Walker
607 et al., 2010 (57) study (534 *F. prausnitzii* sequences out of 5,915 total sequences from six

608 obese males. The % of all clones represented by each OTU in each of these studies is shown
609 on the right.
610
611 **Figure 3. PCR-DGGE fingerprints from *F. prausnitzii* isolates.** Isolates are
612 distributed in two separate bands that correlate with phylogroup designation (▷
613 phylogroup I; ○ phylogroup II). Asterisk points the ladder lane (made by 16S rRNA
614 gene fragments of *Mucor* sp. and *Pseudomonas fluorescens* and *Micrococcus luteus*,
615 respectively from the top to the bottom).
616
617 **Figure 4. Tolerance of *F. prausnitzii* isolates to changes in initial medium pH**
618 **values and bile salt concentrations. A.** Relative growth rates (h^{-1}) of *F. prausnitzii*
619 strains on YCFAG medium at three initial pH values (6.7, 6.2 and 5.75) have been
620 represented. For comparison, the growth rate determined for each strain at pH 6.7 is
621 taken as 1.0. **B.** Relative OD_{650} after 24h of *F. prausnitzii* isolates at four bile salt
622 concentrations (0%, 0.1%, 0.25% and 0.5%) on YCFAG medium. For comparison,
623 the OD_{650} after 24h of incubation determined for each isolate in medium without bile
624 salt has been taken as 1.0. Mean growth rates at pH 6.7, and mean OD_{650} in the
625 absence of bile salts for each strain (±SD) were as follows: (●) ATCC 27768
626 (0.17±0.02; 0.33±0.05); (▼) M21/2 (0.32±0.07, 1.39±0.05); (■) S3L/3 (0.16±0.02,
627 0.52±0.07); (◆) S4L/4 (0.20±0.02, 0.63±0.06); (○) A2-165 (0.55±0.04, 0.77±0.02);
628 (△) L2-6 (0.19±0.01, 0.47±0.02); (□) HTF-75H (0.15±0.01, 0.386±0.046);

629 (◇) HTF-F (0.18±0.01, 0.826±0.089). Phylogroup I isolates have been represented in
630 black while phylogroup II isolates are shown in white.
631
632 **Figure 5. Competition for apple pectin. A.** Change in acidic product concentrations
633 in the growth medium after 24 h fermentation of 0.5% apple pectin by monocultures
634 and co-cultures of isolated pectin-utilizing bacteria. F1 = *F. prausnitzii* SL3/3, F2 = *F.*
635 *prausnitzii* A2-165, E = *Eubacterium eligens* 3376, B= *B. thetaiotaomicron* 5482
636 (monocultures); **B.** F1+E+B and F2+E+B were tri-cultures of the three strains
637 indicated. Negative values for acetate reflect the net consumption of acetate initially
638 present in the medium by *F. prausnitzii* strains. Each strain or strain combination was
639 inoculated into media adjusted to three different initial pH values (6.12, 6.45. 6.79).
640 Final medium pH (measured in all cases and detailed in Table S2) had decreased after
641 24 h by up to 0.3 unit for *F. prausnitzii* monocultures, up to 0.7 unit for *B.*
642 *thetaiotaomicron* and up to 0.9 unit for *E. eligens*. The final pH in the tri-cultures was:
643 6.16 (F1+E+B) and 6.07 (F2+E+B) from initial pH 6.79; 5.79 (F1+E+B) and 5.64
644 (F2+E+B) from initial pH 6.45; 5.47 (F1+E+B) and 5.33 (F2+E+B) from initial pH
645 6.12. Data for two-membered co-cultures and on overall sugar utilization from the
646 same experiment are given in Tables S1 and S2. **C.** Numbers of *F. prausnitzii* cells
647 detected by fluorescent in situ hybridization in cultures and co-cultures. Counts
648 immediately after inoculation (t=0) were: S3L/3 ($0.91\pm0.05 \times 10^7$); A2-165 (1.31 ± 0.01
649 $\times 10^7$).
650

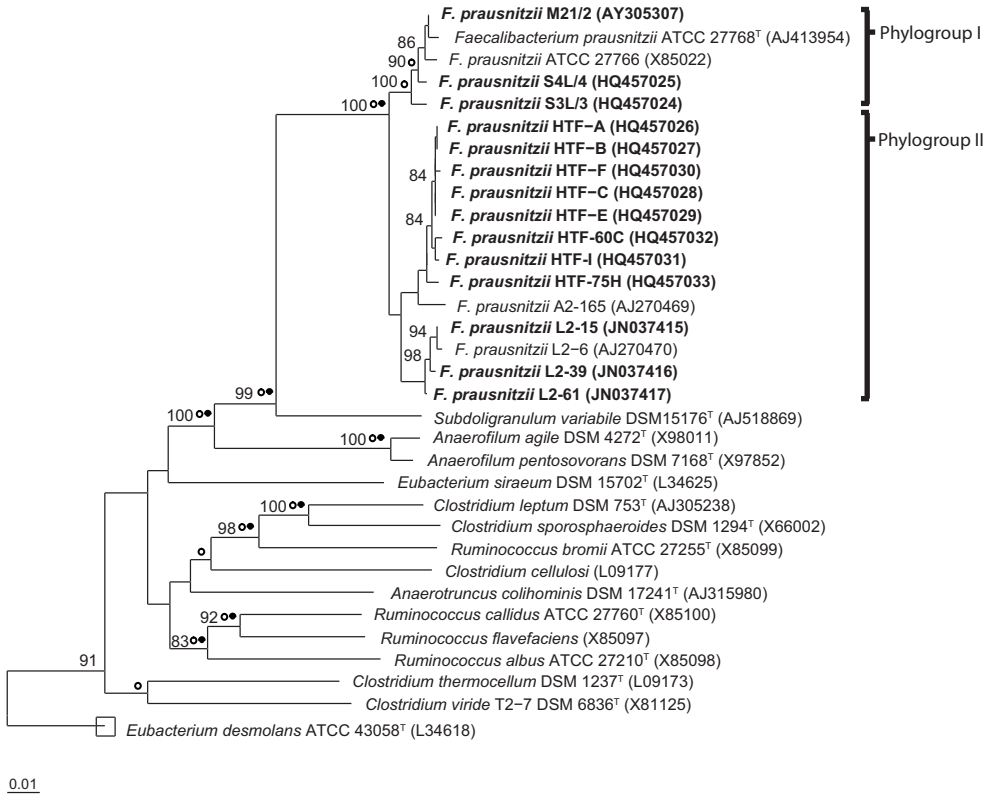

Figure 1. Lopez-Siles et al.

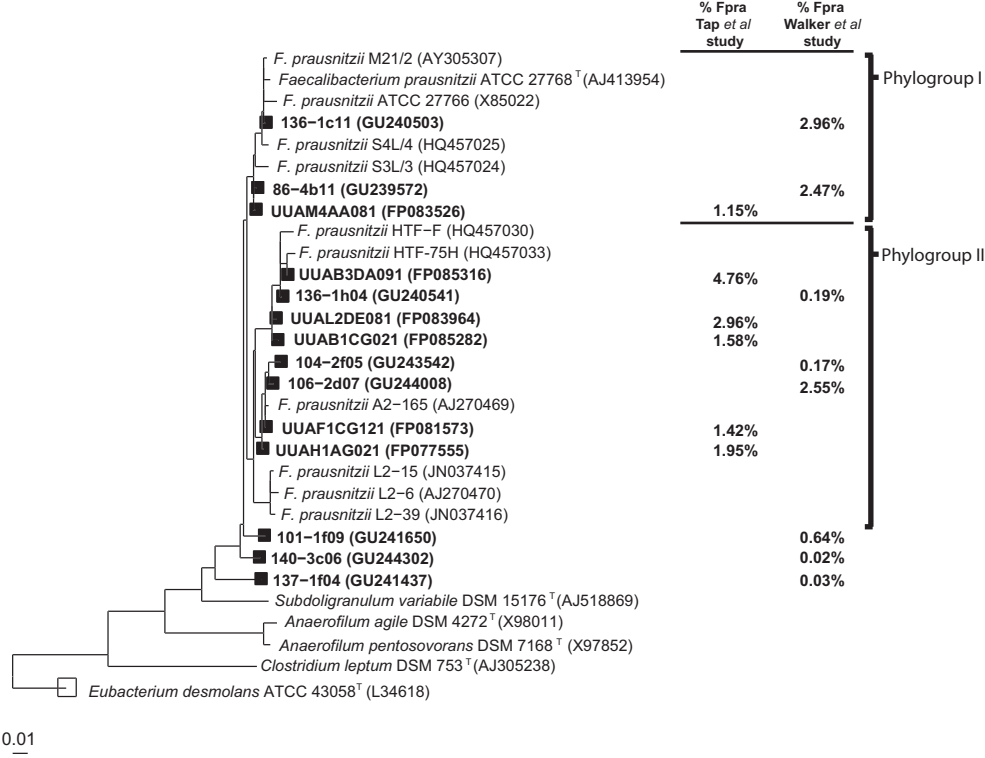

Figure 2. Lopez-Siles et al.

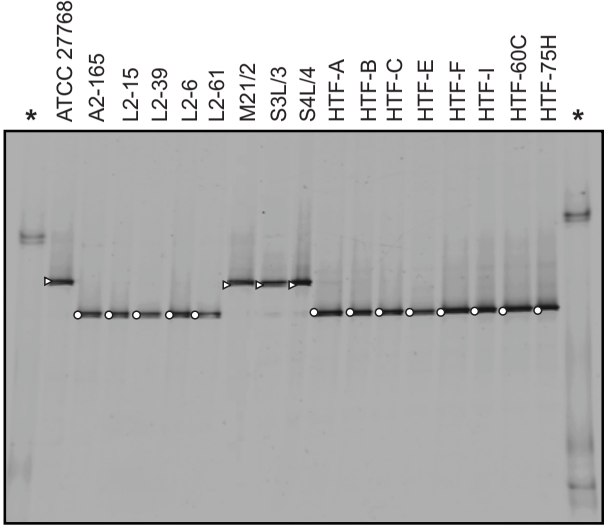

Figure 3. Lopez-Siles et al.

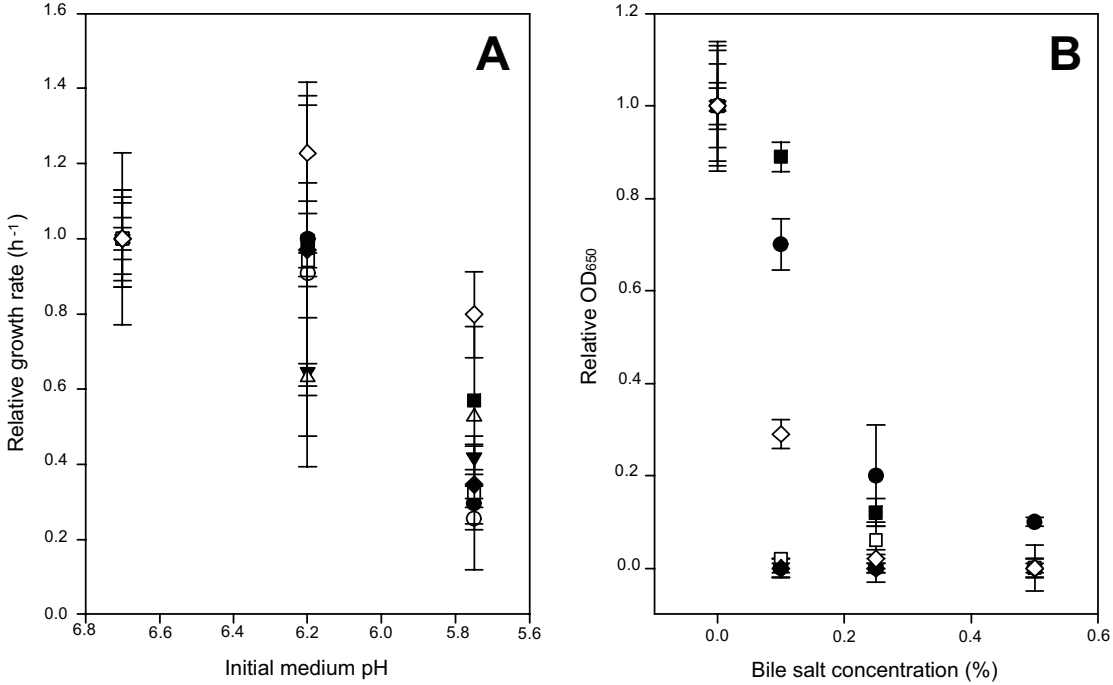

Figure 4. Lopez-Siles et al.

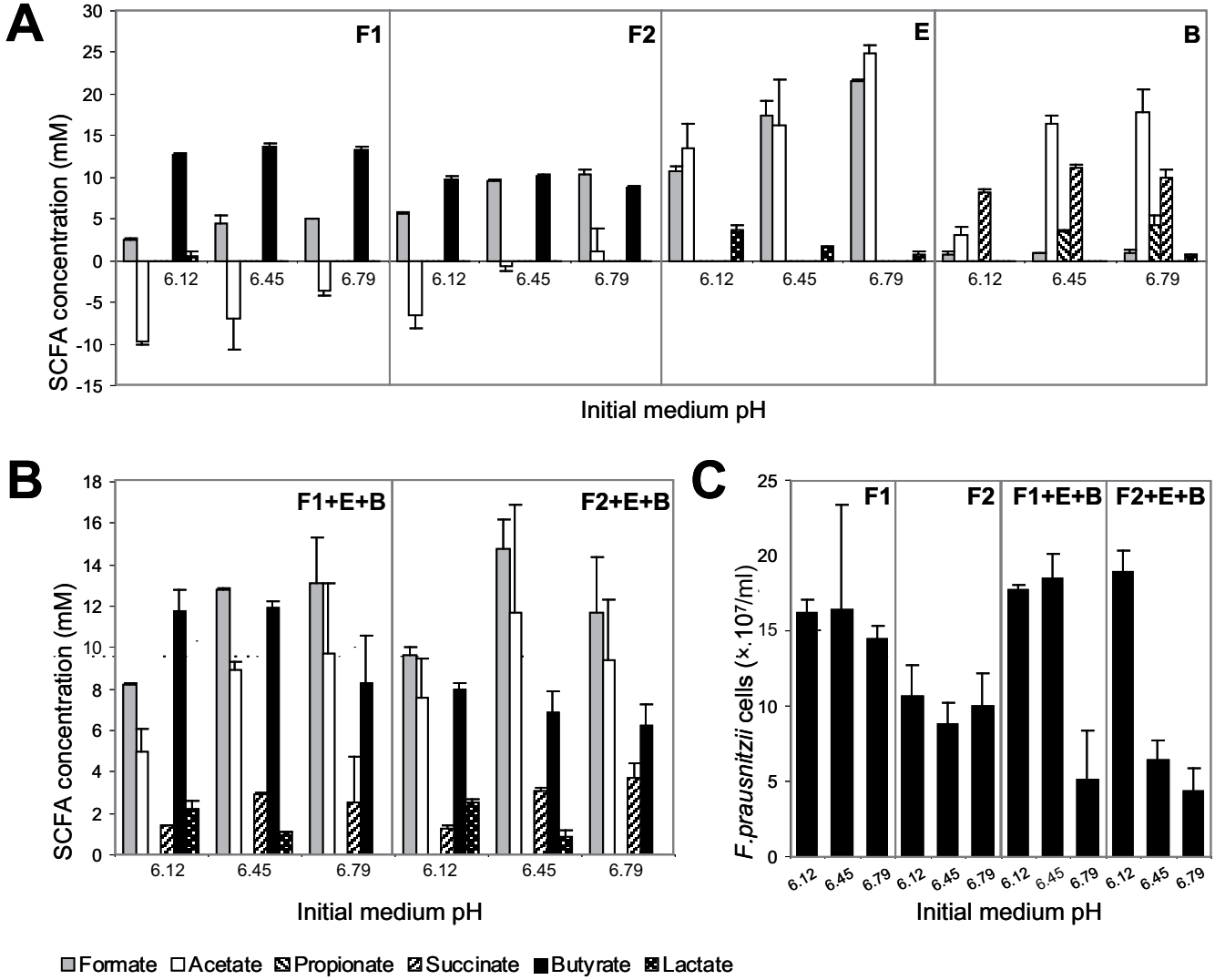

Figure 5. Lopez-Siles et al.

TABLES

Table 1. Details of *F. prausnitzii* strains included in this study. All the isolates were obtained from human fecal samples of healthy volunteers consuming omnivorous diets.

Isolate code	Laboratory of isolation‡	Volunteer	Sex*	Age (years)	Culture collection	Original isolation
ATCC 27768		1	unknown	unknown	ATTCC 27768	(4)
A2-165	RINH	2	F	34	DSMZ 17677	(2, 11)
L2-15	RINH	3	M	2		(2)
L2-39	RINH	3	M	2		(2)
L2-6	RINH	3	M	2		(2, 11)
L2-61	RINH	3	M	2		(2)
M21/2	RINH	4	F	36		(26)
S3L/3	RINH	5	F	46		(26)
S4L/4	RINH	5	F	46		(26)
HTF-A	GU	6	M	31		This study
HTF-B	GU	6	M	31		This study
HTF-C	GU	6	M	31		This study
HTF-E	GU	7	M	44		This study
HTF-F	GU	7	M	44		This study
HTF-I	GU	8	M	28		This study
HTF-60C	GU	8	M	28		This study
HTF-75H	GU	9	M	65		This study

‡RINH: Rowett Institute of Nutrition and Health, Aberdeen (Scotland), United Kingdom; GU: Groningen University, Groningen, The Netherlands. * F: female, M: male.

Table 2. Growth of *F. prausnitzii* strains on a range of carbohydrate substrates. Values refer to mean OD_{650} ± SD after 24 h.

Substrate[a]	Supplier	Phylogroup I strains :-				Phylogroup II strains :-					
		ATCC 27768	M21/2	S3L/3	S4L/4	A2-165	L2-15	L2-39	L2-6	HTF-75H	HTF-F
Glucose	BDH 10117	0.29±0.02	0.96±0.02	0.92±0.18	0.83±0.43	0.53±0.13	0.29±0.01	0.26±0.05	0.32±0.21	0.32±0.02	0.85±0.07
Cellobiose	Sigma C7252	0.26±0.02	0.87±0.33	0.81±0.11	0.72±0.21	0.63±0.10	0.28±0.01	0.18±0.01	0.02±0.07	0.32±0.05	0.87±0.01
Maltose	Sigma M5885	0.32±0.35	0.85±0.15	0.75±0.07	0.82±0.12	0.62±0.07	0.44±0.11	0.78±0.05	0.22±0.21	0.55±0.10	1.01±0.04
Rhamnose	Sigma R3875	-[b]	-	-	0.12±0.03	-	-	-	-	-	-
Galacturonic acid	BDH 571670	0.12±0.00	0.31±0.04	0.45±0.04	0.61±0.06	0.21±0.04	0.12±0.01	0.07±0.02	-	-	0.26±0.02
Galactose	BDH G0750	0.24±0.05	0.95±0.03	0.44±0.02	0.11±0.09	0.80±0.08	0.75±0.28	-	0.61±0.12	0.33±0.28	0.66±0.25
Pectin, apple	BDH 38052	0.31±0.09	0.40±0.04	0.36±0.03	0.56±0.02	0.66±0.01	0.08±0.00	0.07±0.00	0.24±0.02	0.18±0.07	0.39±0.07
Starch, potato	BDH 102713	-	0.06±0.01	0.09±0.02	-	0.07±0.03	-	0.06±0.01	0.07±0.02	0.08±0.06	0.05±0.02
Inulin,chicory	Sigma I2255	0.21±0.27	0.10±0.00	0.08±0.01	0.07±0.01	0.80±0.05	-	-	0.09±0.18	0.18±0.07	0.97±0.26
Glucuronic acid	Fluka 71560	0.09±0.00	-	0.28±0.05	0.08±0.01	0.83±0.02	-	-	-	0.08±0.03	0.17±0.03
N-acetyl glucosamine	Sigma A8625	0.34±0.03	0.88±0.04	0.67±0.00	0.57±0.03	0.98±0.01	0.18±0.06	0.07±0.00	-	0.20±0.02	0.51±0.24
Glucosamine HCl	BDH 962240	0.15±0.01	0.31±0.01	0.58±0.01	0.34±0.11	0.95±0.15	0.13±0.03	0.08±0.02	0.14±0.03	0.14±0.03	0.16±0.07

[a]None of the strains grew on arabinose, fucose, xylose, arabinogalactan, polygalacturonic acid, pectin (citrus), mucin (pig gastric), chondroitin sulphate, hyaluronic acid and heparin. No growth was detected on xylan by final pH change. All substrates were obtained from Sigma.

[b] - = Δ OD_{650} < 0.05. All values in the Table were corrected for growth on basal medium without carbohydrate addition

Table 3. Distribution of pectin-utilizing ability among cultured strains of human colonic anaerobes.

Phylum, species	Strains tested[a]	Pectin utilizers
Bacteroidetes		
Bacteroides thetaiotaomicron	22	22
Bacteroides ovatus	24	23
Bacteroides vulgatus	22	7
Bacteroides fragilis	53	17
Other *Bacteroides* spp.	67	19
Actinobacteria		
Bifidobacterium spp.	41	0
Collinsella (formerly *Eubacterium*) *aerofaciens*	15	0
Firmicutes		
Eubacterium rectale+Roseburia spp.	20; 10[b]	0
Eubacterium eligens	5	3
Eubacterium biforme	5	0
Ruminococcus obeum, torques, gnavus	16	0
Coprococcus spp.	7	0
Peptostreptococcus spp.	8	0
Lactobacillus spp.	6	0
Fusobacterium spp.	10	0
Faecalibacterium prausnitzii	10[b]	8[b]
Ruminococcus albus, bromii, callidus	14	0
Eubacterium siraeum	2[b]	0
Other (unclassified)	7	0

[a] Unless indicated otherwise, data are from Salyers *et al.* 1977 (43,44)

[b] This study. One *B. fibrisolvens* strain is included here along with the *E. rectale* + *Roseburia* related strains tested, which are detailed in the Materials and Methods.

Table S1. Short chain fatty acid (SCFA) formed by *F. prausnitzii* strains alone and in mixed culture (YcFA medium supplemented with 0.5% pectin at 3 pH values).

Initial pH 6.12			SCFA (mM)			
Culture(s)	Formate	Acetate	Propion.	Butyrate	Lactate	Succinate
S3L/3 only	2.4±0.2	-9.6±0.5		12.8±0.1	0.6±0.0	
A2-165 only	5.6±0.3	-6.5±1.7		9.8±0.4		
DSM3376 only	10.8±0.6	13.4±3.0			3.7±0.5	
B5482 only	0.8±0.4	3.0±1.1				8.2±0.3
S3L/3+B5482	1.9±0.2	-4.4±1.1	1.7±1.6	11.7±1.6		3.5±0.5
A2-165+B5482	3.7±0.4	3.3±2.9	1.4±0.6	6.4±0.5		5.3±0.7
3376+B5482	9.5±0.3	14.1±1.7			4.3±1.5	1.4±0.1
S3L/3+3376	8.4±0.1	2.8±1.2		10.9±0.9	2.3±0.2	
A2-165+3376	9.6±0.3	6.1±2.0		8.0±0.6	2.5±0.8	
S3L/3+B5482+3376	8.2±0.1	5.0±1.1		11.8±1.0	2.2±0.4	1.4±0.0
A2-165+B5482+3376	9.6±0.4	7.6±1.9		8.0±0.3	2.5±0.2	1.3±0.1
Initial pH 6.45			SCFA (mM)			
Culture(s)	Formate	Acetate	Propion.	Butyrate	Lactate	Succinate
S3L/3 only	4.4±1.0	-6.9±3.7		13.6±0.5		
A2-165 only	9.6±0.2	-0.6±0.7		10.1±0.3		
DSM3376 only	17.4±1.7	16.3±5.5			1.7±0.1	
B5482 only	0.9±0.1	16.4±1.1	3.5±0.1			11.2±0.3
S3L/3+B5482	2.6±0.4	3.2±3.4	2.1±0.4	10.4±0.4		6.1±0.4
A2-165+B5482	3.7±0.2	7.2±0.3	2.0±0.1	6.3±0.1		7.6±0.0
3376+B5482	17.6±0.4	24.1±1.2	0.2±0.1		1.1±0.1	4.3±0.2
S3L/3+3376	16.0±0.3	12.2±0.8		11.8±0.3	1.4±0.0	
A2-165+3376	17.5±1.1	13.2±3.3		7.8±0.4	1.1±0.2	
S3L/3+B5482+3376	12.8±0.1	8.9±0.4		11.9±0.3	1.1±0.0	2.9±0.1
A2-165+B5482+3376	14.8±1.4	11.7±5.2		6.9±1.0	0.9±0.3	3.1±0.1
Initial pH 6.79			SCFA (mM)			
Culture(s)	Formate	Acetate	Propion.	Butyrate	Lactate	Succinate
S3L/3 only	5.0±0.1	-3.7±0.5		13.2±0.5		
A2-165 only	10.3±0.7	1.1±2.8		8.7±0.3	0.4±0.4	
DSM3376 only	21.5±0.3	24.9±0.9			0.8±0.0	
B5482 only	1.0±0.4	17.9±2.6	4.2±1.2		0.7±0.1	9.9±1.1
S3L/3+B5482	3.2±0.3	14.7±1.1	2.7±0.5	9.8±0.6	0.3±0.4	7.0±0.2
A2-165+B5482	5.4±0.2	16.6±2.7	2.8±0.7	6.2±0.2		7.8±0.6
3376+B5482	15.5±0.6	24.4±1.5	2.3±0.2			7.7±0.1
S3L/3+3376	12.8±2.3	2.6±7.0		8.5±1.5	0.6±0.5	
A2-165+3376	20.0±1.5	19.3±4.2		6.9±0.5	0.7±0.1	
S3L/3+B5482+3376	13.1±2.2	9.7±3.4		8.3±2.3		2.5±2.2
A2-165+B5482+3376	11.7±2.7	9.4±2.9		6.2±1.1		3.7±0.7

Table S2. Pectin (total sugar) utilization, growth and final pH for the experiments shown in Table S1 and Figure 5 (main paper)

Initial pH 6.12 Strain(s)	Pectin used (%)	Final OD_{650}	Final pH	Counts ($\times 10^7$/ml) *F. prausnitzii*
S3L/3 only	47.0±5.3	0.69±0.17	6.05±0.04	16.2±0.9
A2-165 only	46.8±4.2	0.70±0.02	5.80±0.02	10.6±2.1
DSM3376 only	58.9±5.9	0.76±0.02	5.20±0.02	
B5482 only	47.5±4.8	0.76±0.00	5.44±0.01	
S3L/3+B5482	57.8±4.7	1.01±0.00	5.79±0.02	24.7±0.1
A2-165+B5482	47.9±11.6	0.87±0.01	5.54±0.02	12.0±0.6
3376+B5482	60.0±2.1	0.81±0.02	5.18±0.01	
S3L/3+3376	70.0±2.2	0.98±0.01	5.44±0.02	18.9±1.3
A2-165+3376	64.8±1.2	0.92±0.02	5.37±0.02	16.4±0.3
S3L/3+B5482+3376	66.2±3.8	1.09±0.01	5.46±0.03	17.7±0.3
A2-165+B5482+3376	64.1±4.2	1.05±0.01	5.33±0.01	18.9±1.4
Initial pH 6.45 Strain(s)	Pectin used (%)	Final OD_{650}	Final pH	Counts ($\times 10^7$/ml) *F. prauznitzii*
S3L/3 only	43.5±4.1	0.75±0.01	6.26±0.04	16.4±7.0
A2-165 only	40.4±6.1	0.76±0.01	6.15±0.03	8.8±1.4
DSM3376 only	69.4±7.7	0.93±0.02	5.61±0.02	
B5482 only	80.6±7.4	1.26±0.01	5.73±0.01	
S3L/3+B5482	70.6±3.4	1.23±0.02	5.96±0.01	24.1±1.7
A2-165+B5482	70.0±2.1	1.18±0.02	5.86±0.03	16.6±2.0
3376+B5482	60.7±4.8	1.13±0.04	5.48±0.03	
S3L/3+3376	63.0±1.4	1.14±0.01	5.85±0.03	9.4±0.6
A2-165+3376	74.7±9.5	1.07±0.01	5.73±0.02	3.9±0.0
S3L/3+B5482+3376	77.3±2.8	1.24±0.02	5.79±0.03	18.5±1.6
A2-165+B5482+3376	76.4±7.1	1.17±0.02	5.64±0.03	6.4±1.3
Initial pH 6.79 Strain(s)	Pectin used (%)	Final OD_{650}	Final pH	Counts ($\times 10^7$/ml) *F. prausnitzii*
S3L/3 only	44.3±5.3	0.70±0.04	6.64±0.04	14.5±0.8
A2-165 only	38.3±5.9	0.75±0.03	6.67±0.08	10.1±2.2
DSM3376 only	63.7±1.9	0.89±0.01	6.22±0.03	
B5482 only	84.8±2.3	1.24±0.00	6.36±0.05	
S3L/3+B5482	83.3±6.3	1.25±0.01	6.45±0.04	8.5±1.3
A2-165+B5482	85.4±2.8	1.18±0.00	6.38±0.06	8.8±2.4
3376+B5482	83.9±3.7	1.30±0.02	5.96±0.05	
S3L/3+3376	65.8±2.9	1.09±0.02	6.26±0.02	3.3±0.1
A2-165+3376	65.7±3.4	0.99±0.02	6.18±0.09	7.7±2.5
S3L/3+B5482+3376	76.8±3.1	1.24±0.03	6.16±0.05	5.1±3.3
A2-165+B5482+3376	78.4±4.2	1.21±0.01	6.07±0.04	4.4±1.5

Legend

Figure S1. Hierarchical cluster of RAPD-PCR fingerprints of *F. prausnitzii* isolates by using Gelcompar II. Four phylogroup I (●) and six phylogroup II (○) isolates have been selected for further phenotypical characterisation. Similarity values are shown at branching points (Similarity coefficient: Different bands; Dendogram type: UPGMA; Tolerance: 1%).

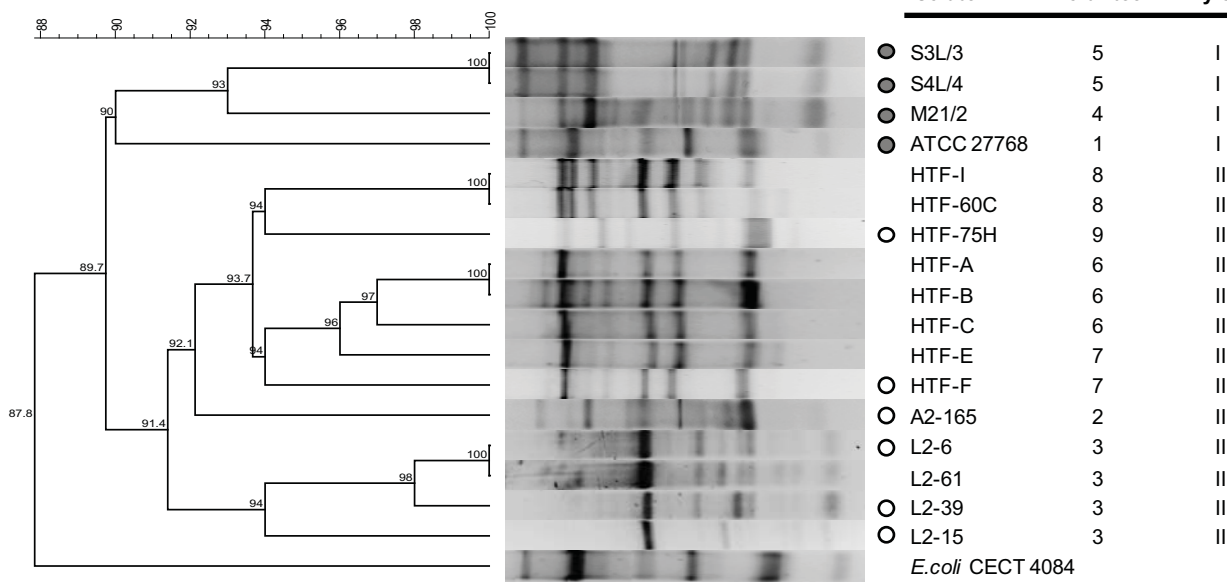

Figure S1. Lopez-Siles et al.