

MINI REVIEW

Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics

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There is an increasing interest in *Faecalibacterium prausnitzii*, one of the most abundant bacterial species found in the gut, given its potentially important role in promoting gut health. Although some studies have phenotypically characterized strains of this species, it remains a challenge to determine which factors have a key role in maintaining the abundance of this bacterium in the gut. Besides, phylogenetic analysis has shown that at least two different *F. prausnitzii* phylogroups can be found within this species and their distribution is different between healthy subjects and patients with gut disorders. It also remains unknown whether or not there are other phylogroups within this species, and also if other *Faecalibacterium* species exist. Finally, many studies have shown that *F. prausnitzii* abundance is reduced in different intestinal disorders. It has been proposed that *F. prausnitzii* monitoring may therefore serve as biomarker to assist in gut diseases diagnostics. In this mini-review, we aim to serve as an overview of *F. prausnitzii* phylogeny, ecophysiology and diversity. In addition, strategies to modulate the abundance of *F. prausnitzii* in the gut as well as its application as a biomarker for diagnostics and prognostics of gut diseases are discussed. This species may be a useful potential biomarker to assist in ulcerative colitis and Crohn's disease discrimination.

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Introduction

Faecalibacterium prausnitzii has been consistently reported as one of the main butyrate producers found in the intestine (Barcenilla *et al.*, 2000; Duncan *et al.*, 2002). Butyrate has a crucial role in gut physiology and host wellbeing. It is the main energy source for the colonocytes and it has protective properties against colorectal cancer (CRC) and inflammatory bowel diseases (IBD; Christl *et al.*, 1996; Archer *et al.*, 1998). Butyrate can reduce intestinal mucosa inflammation through inhibiting NF- κ B transcription factor activation (Inan *et al.*, 2000), upregulating PPAR γ (Schwab *et al.*, 2007) and inhibiting interferon gamma (IFN- γ ; Klampfer *et al.*, 2003).

Additional anti-inflammatory properties have been attributed to this species through its capability to induce a tolerogenic cytokine profile (with very low secretion of pro-inflammatory cytokines like IL-12 and IFN- γ , and an elevated secretion of the anti-inflammatory cytokine IL-10; Sokol *et al.*, 2008b; Qiu *et al.*, 2013). In line with this findings, *F. prausnitzii* cells or their cell-free supernatant

have been reported to reduce the severity of acute (Sokol *et al.*, 2008b), chronic (Martin *et al.*, 2014) and low grade (Martin *et al.*, 2015) chemical-induced inflammation in murine models. These anti-inflammatory effects were partly associated with secreted metabolites capable of blocking NF- κ B activation, IL-8 production (Sokol *et al.*, 2008b) and upregulation of regulatory T cell production (Qiu *et al.*, 2013). Recently seven peptides that derive from a single microbial anti-inflammatory molecule, a 15 kDa protein, have been identified in *F. prausnitzii* cultures supernatant, and their capability to block NF- κ B pathway has been demonstrated (Quevrain *et al.*, 2015).

F. prausnitzii supernatant has also been shown to attenuate the severity of inflammation through the release of metabolites that enhance the intestinal barrier function and that affect paracellular permeability (Carlsson *et al.*, 2013; Martin *et al.*, 2015). The mechanism by which *F. prausnitzii* ameliorates permeability seems to be related with expression of certain tight junction proteins, but not with an enhancement of claudin expression (Carlsson *et al.*, 2013). Besides, a recent study performed using a gnotobiotic model has shown that *F. prausnitzii* could also influence gut physiology through mucus pathway and the production of the mucus O-glycans, and may help to maintain suitable proportions of different cell types of secretory lineage in the intestinal epithelium (Wrzosek *et al.*, 2013). Finally,

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a restoration of serotonin (a key neurotransmitter in the gastrointestinal tract that affects motility (Ohman and Simren, 2007)) to normal level has been evidenced in murine models treated with either *F. prausnitzii* or its supernatant (Martin et al., 2015), and this species anti-nociceptive effect in non-inflammatory irritable bowel syndrome (IBS)-like murine models has been recently evidenced (Miquel et al., 2016).

Besides, over the last few years an increasing number of studies have reported on *F. prausnitzii* depletion in gut diseases (Swidsinski et al., 2005; Martinez-Medina et al., 2006; Frank et al., 2007; Balamurugan et al., 2008; Sokol et al., 2008a, 2009; Swidsinski et al., 2008; Willing et al., 2009; Furet et al., 2010; Jia et al., 2010; McLaughlin et al., 2010; Qin et al., 2010; Rajilic-Stojanovic et al., 2011; Sobhani et al., 2011; Hansen et al., 2012; Vermeiren et al., 2012; de Goffau et al., 2013; Kabeerdoss et al., 2013; Karlsson et al., 2013; Machiels et al., 2013; Miquel et al., 2013), which has prompted interest in considering this bacterium as a new generation probiotic.

Taken all together these findings indicate that *F. prausnitzii* has a crucial role in maintaining gut physiology and host wellbeing. It still remains elusive however which gut factors modulate *F. prausnitzii* presence in the gut, and the extent of their influence.

Factors supporting *F. prausnitzii* presence in the gut

Carbon sources used by *F. prausnitzii* for growth

F. prausnitzii isolates can grow well using simple carbohydrates (Table 1), but some differences exist between strains in their capability to ferment more complex carbohydrates such as those that are either host or diet derived, as observed by the maximum optical density at 650 nm wavelength (OD₆₅₀) that cultures can reach (Duncan et al., 2002; Lopez-Siles et al., 2012).

Although most *F. prausnitzii* strains are able to ferment inulin (Table 1), the findings show that only two of them can grow well on this substrate (final OD₆₅₀ ~ 0.8). This supports the observed stimulation of this species in nutritional interventions with this prebiotic (Ramirez-Farias et al., 2009), and suggests that only some members of *F. prausnitzii* population are selectively stimulated by inulin (Chung et al., 2016). Strains of this species have a limited ability to utilize other polysaccharides found in the gut lumen such as arabinogalactan, xylan and soluble starch (Louis et al., 2007). Most of the isolates can grow on apple pectin and are able to use some pectin derivatives (Lopez-Siles et al., 2012). *In vitro* studies suggested that, under physiological conditions, *F. prausnitzii* can have a key role in fermentation of some types of pectin and that it can compete successfully with other gut bacteria for this substrate

(Lopez-Siles et al., 2012). These results are supported by the fact that pectinolytic enzymes have been found encoded in the *F. prausnitzii* reference genome (Heinken et al., 2014). Besides, an *in vivo* study has shown that Firmicutes are promoted in apple pectin-fed rats (Licht et al., 2010). Taken together this suggests that pectin or pectin derivatives could be used as a novel prebiotic approach to stimulate *F. prausnitzii* (Chung et al., 2016).

In addition, *F. prausnitzii* strains can also utilize N-acetylglucosamine (Lopez-Siles et al., 2012), a constituent of the glycoproteins found in gut mucosa (Salvatore et al., 2000). Interestingly, it has been reported that treatment with this compound may improve Crohn's disease (CD) as it will serve as a healing factor in inflamed, damaged soft tissues of the gut (Salvatore et al., 2000). Therefore, given the capability to ferment this carbohydrate by *F. prausnitzii*, it would be of interest to explore the effect of restoring this beneficial gut bacterium in CD patients undergoing this treatment.

Table 1 Substrates of different origin metabolized by *Faecalibacterium prausnitzii* isolates *in vitro* (batch pure cultures) as reported by (Duncan, et al. 2002; Lopez-Siles, et al. 2012)

Substrate	No. of utilizers	No. of strains tested
<i>Simple carbohydrates^a</i>		
Glucose	11	11
Fructose	4	4
Cellobiose	10	11
Maltose	10	11
Galactose	9	10
Galacturonic acid	7	9
Sucrose	2	4
Melezitose	1	4
Trehalose	1	4
Rhamnose	1	11
<i>Amino acids^b</i>		
Arginine	4	4
Histidine arylamide	4	4
Glycine arylamide	2	4
<i>Diet-derived^c</i>		
Fructo-oligosaccharides	4	4
Pectin (apple)	10	10
Inulin (chicory)	9	11
<i>Host-derived^d</i>		
Glucosamine HCl	10	10
N-acetylglucosamine	9	10
Glucuronic acid	6	10

^aOther simple carbohydrates tested but non-metabolized are mannitol (0/3), melibiose (0/4), raffinose (0/4), ribose (0/4), fucose (0/10), arabinose (0/11) and xylose(0/11).

^bOther amino acids tested but non-metabolized are alanine (0/4), glutamic acid (0/4), glutamyl (0/4), leucine (0/4), leucine-glycine (0/4), phenylalanine (0/4), proline (0/4), pyroglutamic acid (0/4), serine (0/4), tyrosine (0/4).

^cOther diet-derived carbohydrates not metabolized are arabinogalactan (0/10), citrus pectin (0/10), polygalacturonic acid (0/10), xylan (0/10) and potato starch (8/11) which depends on the solubility of the starch as *F. prausnitzii* does not metabolize starch.

^dOther host-derived carbohydrates not metabolized are chondroitin sulfate (0/10), heparin (0/10), hyaluronic acid (0/10), pig gastric mucin (0/10).

Finally, *F. prausnitzii* isolates are unable to utilize mucin or mucopolysaccharides (Lopez-Siles *et al.*, 2012), although some controversy exists because it has been shown that mucin may stimulate growth of this species (Sadaghian Sadabad *et al.*, 2015). The mechanism by which *F. prausnitzii* would benefit from mucin metabolism remains unknown, and further studies to reveal its interaction with mucin-degraders would be of interest.

F. prausnitzii has the ability to switch between substrates derived from the diet or the host. This capability should be explored further to define novel strategies to restore *F. prausnitzii* populations in the diseased gut by using some of these carbohydrates alone or in combination as prebiotics. *In vivo* studies on healthy human volunteers revealed a clear stimulation of *F. prausnitzii* after various prebiotic treatments (Ramirez-Farias *et al.*, 2009; Benus *et al.*, 2010; Hooda *et al.*, 2012). It remains to be established which particular subtypes of *F. prausnitzii* populations change under prebiotic intakes. In addition, it would be interesting to conduct metatranscriptomic studies to determine if *F. prausnitzii* genes participate in breakdown of these substrates. Besides, this will also provide some clues on cross-feeding relationships between *F. prausnitzii* and other members of the gut microbiota.

Effect of gut physicochemical conditions

Tolerance to changes in gut physiological factors can have a role in determining the ability of an organism to survive in this environment, and they contribute to the temporal/spatial organization of different gut microbes (Parfrey and Knight 2012).

The optimal pH for *F. prausnitzii* growth ranges between 5.7 and 6.7 (Lopez-Siles *et al.*, 2012; Foditsch *et al.*, 2014), the range of pH found in the colon. Although there are differences in tolerance between strains in the pH range of 5–5.7 (Lopez-Siles *et al.*, 2012), no growth was observed at pH values between 3.5 and 4.5 (Foditsch *et al.*, 2014). This suggests that pH influences *F. prausnitzii* distribution along the gut. This species has been detected also in the duodenum (pH range 5.7–6.4; Nadal *et al.*, 2007) and in the terminal ileum (Lopez-Siles *et al.*, 2014, 2016) in healthy subjects and patients with gut disorders. As it has been reported that ulcerative colitis (UC) and CD patients often have acidic stools (Nugent *et al.*, 2001; Barkas *et al.*, 2013), it remains to be demonstrated whether or not local pH in the gut is modulating *F. prausnitzii* abundance and composition in patients with gut disorders such as IBD.

F. prausnitzii is also highly sensitive to a slight increase in physiological concentrations of bile salts because its growth is compromised by concentrations of 0.5% (wt/vol). This provides a plausible explanation for the reduced abundance of *F. prausnitzii* exhibited by CD patients, as increased bilirubin

concentrations have been reported in these patients, especially in those with ileal disease involvement, and who have undergone intestinal resection (Lapidus and Einarsson, 1998; Pereira *et al.*, 2003). Besides, differences in tolerance among isolates have been reported, especially at a bile salt concentration of 0.1% (wt/vol) (Lopez-Siles *et al.*, 2012; Foditsch *et al.*, 2014), suggesting that alterations in bile salt concentrations may determine a variation in *F. prausnitzii* subtype composition. As CD patients also feature altered bile salt composition (Lapidus and Einarsson, 1998; Pereira *et al.*, 2003), further studies need to be conducted to determine if *F. prausnitzii* features higher sensitivity to certain types of bile salt components, and to establish whether or not different bile salt profiles alter *F. prausnitzii* subtype composition.

F. prausnitzii is extremely oxygen-sensitive (Duncan *et al.*, 2002), but it is capable of withstanding low levels of oxygen found in the intestinal mucosa by using extracellular electron transfer in the presence of flavine and cysteine or glutathione (Khan *et al.*, 2012). Recently, it has been demonstrated that strain A2-165 can retain viability in ambient air for 24 h when formulated with these antioxidants and inulin as a cryoprotectant (Khan *et al.*, 2014). Because oxygen gradient has an important role in defining the spatial organization of microbes in the colon (Swidsinski *et al.*, 2005; Parfrey and Knight, 2012), it would be interesting to determine if there are differences in oxygen tolerance among *F. prausnitzii* subtypes, and if it correlates with inflamed state of the mucosa.

Finally, the availability of essential nutrients to support *F. prausnitzii* may influence the distribution of this species in the gut. A recent study based on a functional metabolic map of *F. prausnitzii* strain A2-165 has predicted its inability to synthesize the amino acids alanine, cysteine, methionine, serine and tryptophan (Heinken *et al.*, 2014). Auxotrophy for vitamins and cofactors such as biotin, folate, niacin, panthothenate, pyridoxine and thiamine has been observed by further analysis of other *F. prausnitzii* strain genomes, and some discrepancy between strains seems to exist in relation to riboflavin production, which could be due to inter-strain differences (Heinken *et al.*, 2014; Magnúsdóttir *et al.*, 2015). In contrast, this species has been predicted as a cobalamin producer (Magnúsdóttir *et al.*, 2015). Evidence that some IBD patients are predisposed to feature cobalamin deficiency has been reported (Battat *et al.*, 2014), but the cause of this condition has not been established yet. As there is a lack of consistent clinical data that indicates predisposition of IBD patients to this deficiency (Battat *et al.*, 2014), it would be interesting to establish if it is associated with depletion of cobalamin-producers in the gut.

Collectively, these findings provide a plausible explanation why *F. prausnitzii* is reduced in abundance in patients with gut disease. Besides, it points out crucial requirements in physicochemical

conditions for survival of this species, which can be applied in the future to use this bacterium to treat intestinal disorders related to its depletion.

F. prausnitzii in relation to other members of gut microbiota

F. prausnitzii co-occurs with several members of the *C. coccoides* group and Bacteroidetes in the gut (Qin et al., 2010). It has been suggested that *F. prausnitzii* may rely on other species like *Bacteroides* for cross-feeding. In co-culture experiments it has been observed that *F. prausnitzii* fermentative activity continues whereas *B. thetaiotaomicron* is fermenting pectin (Lopez-Siles et al., 2012; Chung et al., 2016). This could partially be explained by the acetate produced by the latter, which enhances *F. prausnitzii* growth (Heinken et al., 2014). Besides, initial fermentation of pectin by *B. thetaiotaomicron* can release pectin derivatives which can then be used by *F. prausnitzii*.

Recent studies in rat models have revealed that *F. prausnitzii* needs the prior presence of *B. thetaiotaomicron* to colonize the gut (Wrzosek et al., 2013). The inability to maintain *F. prausnitzii* mono-associated animal models has been repeatedly observed (Wrzosek et al., 2013; Hoffmann et al., 2015) and a mouse model has also been described in which *F. prausnitzii* implantation in the gastrointestinal tract requires prior preparation with *Escherichia coli* (Miquel et al., 2015). Correlation between these two species has been found in IBD patients (Lopez-Siles et al., 2014). Positive or negative correlation was observed depending on the disease location. This suggests the effect of one population on the other although the influence of host factors cannot be ruled out. Depending on patients' condition, these correlations involved specifically one or both phylogroups of *F. prausnitzii* (Lopez-Siles et al., 2016), so future studies of co-culture experiments could further elucidate the interactions between *E. coli* and *F. prausnitzii*.

Taxonomy and phylogeny of *F. prausnitzii*

Duncan et al. (2002) established that the genus *Faecalibacterium* is related to members of *Clostridium* cluster IV (*Clostridium leptum* group), within the Firmicutes phylum, Clostridia class and Ruminococcaceae family. Currently, *F. prausnitzii* is the only *Faecalibacterium* species which has been successfully isolated.

F. prausnitzii intraspecies diversity

More recent phylogenetic characterization of isolates determined that this species includes two phylogroups, which share 97% 16S rRNA gene sequence similarity (Lopez-Siles et al., 2012). Although genomic coherence remains to be explored, *in silico* analyses of sequenced genomes (Table 2) reveals that the average nucleotide identity (ANI) between isolates S3L/3 (phylogroup I) and L2/6 (phylogroup II) is below 94%, thus supporting the hypothesis that these would belong to two different genomospecies (that is, species defined by genome comparisons, but without phenotypic properties defined yet (Schloter et al., 2000; Rossello-Mora and Amann, 2015)). Besides, isolates S3L/3 and M21/2 (both from phylogroup I) share ANI values over 97% confirming that they belong to the same genomospecies. The accurate sequencing and annotation of several *F. prausnitzii* strain genomes is required to provide conclusive information to establish whether or not the two phylogroups belong to different genomospecies or genomovars (that is, strains which are phylogenetically different but phenotypically indistinguishable (Schloter et al., 2000; Rossello-Mora and Amann, 2015)).

With regard to phenotypic coherence, no statistically significant differences have been found concerning carbohydrate fermentation or tolerance to changes in gut environmental conditions, although there are indicators that differences do exist between the members of the two phylogroups (Table 3). For instance, *F. prausnitzii* S3L/3 has been shown to

Table 2 ANI values for paired comparisons between *F. prausnitzii* strains whose genome has been fully sequenced

<i>F. prausnitzii</i> isolate	ANI ^b values				<i>F. prausnitzii</i> isolate	ANI ^m values			
	KLE1255 (ND)	A2-165 (II)	L2/6(II)	SL3/3(I)		KLE1255 (ND)	A2-165 (II)	L2/6(II)	SL3/3(I)
M21/2 (I)	85.26	83.29	82.11	96.70^c	M21/2(I)	89.02	88.52	88.07	97.34^c
KLE1255 (ND)	–	82.79	82.46	84.70	KLE1255 (ND)	–	88.31	88.65	88.82
A2-165 (II)	82.77	–	82.60	82.74	A2-165(II)	88.31	–	88.23	88.28
L2/6(II)	82.33	82.87	–	81.61	L2/6(II)	88.65	88.23	–	87.99

Abbreviations: ANI, Average nucleotide identity; DDH, DNA-DNA hybridization; ND, not determined. Phylogroup for each strain is indicated in brackets.

^aANI^b, ANI based on BLAST searches of 1 kb genome fragments against a target genome.

^bANI^m, ANI based on the MUMmer algorithm that does not require the artificial generation of 1 kb fragments.

^cIt has been shown that ANI values higher than 94% embraces organisms sharing DDH values higher than 70% which are considered to be genomospecies. ANI^b has better application for distant genomes comparison, whereas both algorithms give nearly identical values in the high identity range (80–100%). Values corresponding to the same genomospecies are indicated in boldface.

Table 3 Summary of *F. prausnitzii* phylogroups I and II characteristics

	Phylogroup I	Phylogroup II
Strains	ATCC27768, M21/2, S3L/3, S4L/4	A2-165, L2-6, L2-15, L2-39, L2-61, HTF-A, HTF-B, HTF-C, HTF-E, HTF-F, HTF-I, HTF-75H, HTF-60C
Gut distribution	Feces and mucosa	Feces and mucosa
Genome size (mean Mb ± s.d.) ^a	3.17 ± 0.06	3.21 ± 0.16
GC content (mean % ± s.d.) ^a	55.85 ± 0.49	56.45 ± 0.21
Genes content (mean ± s.d.) ^a	2881.5 ± 92.6	2892.5 ± 102.5
Proteins content (mean ± s.d.) ^a	2778.5 ± 46.0	2725.5 ± 43.1
<i>Carbohydrate utilization (mean OD₆₅₀ ± s.d.)^b</i>		
Glucose	0.750 ± 0.311	0.428 ± 0.228
Cellobiose	0.665 ± 0.277	0.383 ± 0.312
Maltose	0.685 ± 0.247	0.603 ± 0.273
Galacturonic acid	0.373 ± 0.208	0.165 ± 0.086
Galactose	0.435 ± 0.369	0.630 ± 0.183
Apple pectin	0.408 ± 0.108	0.270 ± 0.224
Inulin	0.115 ± 0.065	0.510 ± 0.440
Glucuronic acid	0.150 ± 0.113	0.360 ± 0.410
N-Acetylglucosamine	0.615 ± 0.224	0.388 ± 0.369
Glucosamine HCl	0.345 ± 0.177	0.267 ± 0.336
<i>Tolerance to pH (mean growth rate ± s.d.)^b</i>		
6.7	0.210 ± 0.070	0.256 ± 0.151
6.2	0.192 ± 0.050	0.245 ± 0.159
5.75	0.081 ± 0.039	0.108 ± 0.042
<i>Tolerance to bile salts (mean maximum OD₆₅₀ ± s.d.)^b</i>		
0%	0.717 ± 0.427	0.613 ± 0.202
0.12%	0.174 ± 0.223	0.071 ± 0.150
0.25%	0.032 ± 0.037	0.014 ± 0.014
0.5%	0.026 ± 0.033	0.002 ± 0.005
<i>SCFA production (mM ± s.d.)^c</i>		
Formate	3.508 ± 2.730	15.190 ± 11.856
Acetate	-8.917 ± 11.288	-3.192 ± 9.256
Butyrate	18.524 ± 11.151	23.882 ± 5.386
D-Lactate	2.014 ± 1.992	2.435 ± 0.865
Association with host metabolites (adapted from (Li, <i>et al.</i> 2008))	Decrease in dihydrothymine and an increase in 4-hydroxyphenylacetyl glycine	Decreased levels of 3-aminoisobutyrate, taurine, 3, 5-hydroxybenzoate, dimethylamine, 2-hydroxyisobutyrate, glycolate and increased lactate and glycine
Abundance in gut disorders (adapted from (Hippe <i>et al.</i> , 2016, Lopez-Siles <i>et al.</i> , 2016))	Depletion in IBS, CRC and IBD patients, particularly in active CD	Depletion in CD patients, especially those with intestinal resection. Associated to type 2 diabetes.

Abbreviations: CD, Crohn's disease; CRC, colorectal cancer; GC, guanine and cytosine; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; OD, optical density; SCFA, short chain fatty acids. No statistically significant differences have been found between the members of the two phylogroups for any of the characteristics analyzed.

^aFor these calculations phylogroup I included isolates M21/2 and S3L/3 and phylogroup II consisted of L2/6 and A2-165 isolates.

^bFor these calculations ATCC27768, M21/2, S3L/3 and S4L/4 (phylogroup I) and A2-165, L2-15, L2-39, L2/6, HTF-F and HTF-75H (phylogroup II) were used (Lopez-Siles, *et al.* 2012).

^cShort chain fatty acids produced by strains ATCC27768, M21/2, S3L/3 and S4L/4 (phylogroup I) and A2-165 and L2-6 (phylogroup II) on yeast casitone fatty-acids medium supplemented with 0.5% (wt/vol) glucose (Lopez-Siles *et al.*, 2012).

produce significantly higher amounts of metabolites derived from phenylalanine, tyrosine and tryptophan metabolism than strain M21/2, despite both belonging to phylogroup I (Russell *et al.*, 2013). The link of *F. prausnitzii* with tyrosine metabolism has been corroborated in fecal samples of healthy subjects (Jansson *et al.*, 2009). Because the release of different metabolites by gut bacteria can have direct effect on different host signaling pathways, it is possible that within *F. prausnitzii* populations there are members that interact in a different manner with the host. Supporting this hypothesis, it has been demonstrated that *F. prausnitzii* ATCC27768 (phylogroup I) and *F. prausnitzii* A2-165 (phylogroup II)

are associated with the modulation of host metabolites related to different pathways (Li *et al.*, 2008; Jansson *et al.*, 2009; Table 3). Prevalence and/or abundance of both phylogroups varies among patients suffering gut disorders such as CD, UC and type 2 diabetes (Lopez-Siles *et al.*, 2015, 2016; Hippe *et al.*, 2016), and further metabolomic studies are needed to establish the effects of that in host wellbeing.

Approaching the real diversity of the genus Faecalibacterium

Recent studies on species diversity and abundance in healthy and diseased gut samples however suggest

that other *F. prausnitzii* phylotypes exist (Lopez-Siles et al., 2015, 2016) and the presence of other species within the *Faecalibacterium* genus cannot be ruled out. These have been estimated by molecular methods analyzing the overall bacterial community in fecal samples to represent around 2% of *Faecalibacterium* sequences (Tap et al., 2009; Walker et al., 2011), and corroborated using species-specific primers (Lopez-Siles et al., 2015). Interestingly, rare phylotypes have been mainly recovered from subjects with gut disease (Lopez-Siles et al., 2016). Further studies based on next generation sequencing may help to corroborate the presence of these rare phylotypes, and would provide an opportunity to elucidate the taxonomy within the genus *Faecalibacterium*.

***F. prausnitzii* populations in healthy and diseased gut**

F. prausnitzii population composition and richness

Overall a decrease in gut microbiota diversity has been reported in the mucosa of IBD patients (Tamboli et al., 2004; Seksik et al., 2006; Barnich and Darfeuille-Michaud, 2007; Ott et al., 2008; Sokol et al., 2008a; Chassaing and Darfeuille-Michaud, 2011). In particular, fewer types of Firmicutes, mostly from Ruminococaceae, were observed in feces of CD patients (Scanlan et al., 2006). Regarding *F. prausnitzii* population, subtype richness is also lower in IBD patients, which frequently tends to only possess one of the two main phylogroups (Lopez-Siles et al., 2015).

IBD, CRC, IBS and healthy subjects feature a different composition of *F. prausnitzii* subtypes (Lopez-Siles et al., 2015). Although some phylotypes have been specifically associated to each condition, the main members of the *F. prausnitzii* population (four phylotypes, two phylogroups) have been detected in all the subject groups but with a different distribution between conditions (Lopez-Siles et al., 2015). As factors explaining these differences remain unknown, further studies of isolation and characterization of strains from patients suffering intestinal disorders are needed to test the effect of either host or gut physicochemical factors on different *F. prausnitzii* subtypes.

F. prausnitzii load

Several studies have reported *F. prausnitzii* depletion in adult CD (Martinez-Medina et al., 2006; Frank et al., 2007; Sokol et al., 2008b, 2009; Swidsinski et al., 2008; Willing et al., 2009; Fujimoto et al., 2013; Miquel et al., 2013), UC (Swidsinski et al., 2005; Sokol et al., 2009; McLaughlin et al., 2010; Vermeiren et al., 2012; Kabeerdoss et al., 2013; Machiels et al., 2013; Lopez-Siles et al., 2014, 2016) and CRC (Balamurugan et al., 2008; Lopez-Siles et al., 2016) subjects, and concur with the view that down-shifts in *F. prausnitzii* numbers occur under several pathological disorders. In contrast, other

studies have reported no depletion in *F. prausnitzii* levels in CRC (Balamurugan et al., 2008; Sobhani et al., 2011; Wang et al., 2012), and even increased *F. prausnitzii* abundance in *de novo* pediatric CD patients (Hansen et al., 2012). Besides, a consensus on whether or not IBS patients feature a depletion of *F. prausnitzii* has not been reached since both studies reported normal counts (Malinen et al., 2005; Swidsinski et al., 2005, 2008; Kassinen et al., 2007; Jia et al., 2010; Duboc et al., 2012; Rigsbee et al., 2012; Lopez-Siles et al., 2014, 2016) and studies reporting lower numbers in IBS patients of alternating type (Rajilic-Stojanovic et al., 2011) have also been published. The variety of symptoms featured by IBS patients makes IBS diagnostics complex, which in turn is likely to make it difficult to establish whether or not *F. prausnitzii* is affected in this intestinal condition. Altogether, the exact role that *F. prausnitzii* has in the pathogenesis of these diseases cannot be established at this stage. On the one hand an external factor can cause a downshift in *F. prausnitzii*, but also this species depletion can be a contributing factor to disease aggravation. In this case, restoration of normal counts of this species should be explored as a way to achieve healing and/or attenuate disease progression.

Although the depletion of *F. prausnitzii* is not a specific phenomenon that occurs in a particular disease, the level of depletion as well as which components of the *F. prausnitzii* population are affected can be different between diseases. Depletion in phylogroup I abundance is a general feature in abnormal gut conditions, whereas phylogroup II reduction seems to be specific to CD patients, usually with ileal disease location (Lopez-Siles et al., 2016). This could be the consequence of several factors (physicochemical, host-related or microbiome-related) that may vary between disorders and can affect either some or all *F. prausnitzii* members. In turn, these different populations can have a direct effect in host wellbeing. For instance, a recent study has shown different *F. prausnitzii* profiles in obese subjects with and without developed type two diabetes (Hippe et al., 2016), suggesting that differences in phylotypes may lead to differences in inflammatory status in the host, thus having an influence on disease development. Currently, studies on anti-inflammatory properties of *F. prausnitzii* have been performed with strain A2-165, from phylogroup II. Similar studies conducted with strains representative of phylogroup I (for example, ATCC27768) are required to determine whether or not there are differences between phylogroups regarding anti-inflammatory activity.

Future perspectives: potential use of *F. prausnitzii* as a healthy gut microbiota biomarker.

F. prausnitzii load as diagnostic supporting tool

The usefulness of gut microbiota assessment to support intestinal disease diagnostics and/or

prognostics has gained interest during the last few years. Some studies have pointed out that the abundance of fecal or mucosa-associated *F. prausnitzii* is a potential biomarker to discriminate between gut disorders (Swidsinski *et al.*, 2008; Lopez-Siles *et al.*, 2014, 2016). In particular, *F. prausnitzii* is a good biomarker to discriminate CD and CRC from healthy subjects as well as CD from IBS (Figure 1). Of interest, *F. prausnitzii* phylogroup I is particularly good in discriminating healthy subjects from gut disease cohorts including IBS, IBD and CRC (Lopez-Siles *et al.*, 2016), whereas phylogroup II has a limited use as biomarker. This could be partially explained by the fact that phylogroup II load is less reduced in intestinal disease.

It is difficult however to establish the use of a single bacterial species as a general biomarker for all disease types. *F. prausnitzii* in conjunction with *E. coli* abundance as a complementary indicator (F-E index) has been proven to be a better biomarker than *F. prausnitzii* alone (Lopez-Siles *et al.*, 2014). This index allows good discrimination of CRC patients from other gut disorders, especially UC. The F-E index is also a good biomarker to differentiate UC and IBS patients from those with CD. However, the heterogeneity of disease subtypes is preventing discrimination between conditions.

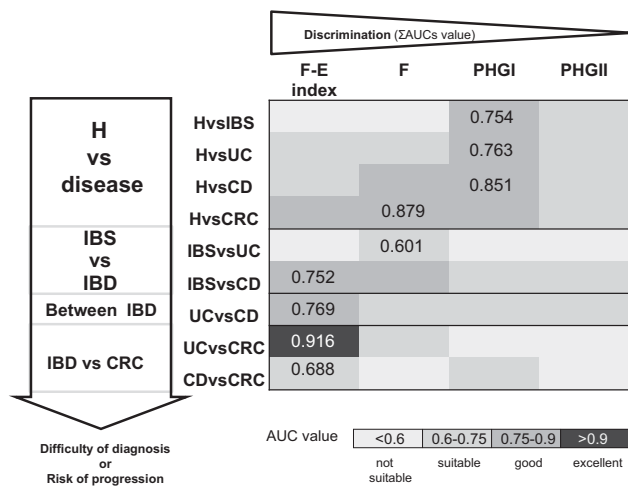


Figure 1 Biomarker of choice to discriminate between conditions. Selected pair wise comparisons of conditions are represented taking into account the difficulty of diagnosis or the risk of progression. The four options of biomarkers (*F. prausnitzii*, the two phylogroups or the *F. prausnitzii-E. coli* index calculated as (Lopez-Siles *et al.*, 2014)), have been ranked according to their discriminative power estimated as the sum of all the AUC values for all the pair wise comparisons taking into account all the conditions. For each comparison, the highest AUC value achieved is depicted. H, healthy control group; F, total *F. prausnitzii* load; PHG I, *F. prausnitzii* phylogroup I load; PHG II, *F. prausnitzii* phylogroup II load; F-E index, *F. prausnitzii-E. coli* index; AUC, area under the ROC curve; ROC, receiver operating characteristic curve.

F. prausnitzii load as IBD subtype biomarker
An accurate discrimination between UC and CD is of relevance due to differences in treatment and management between these two entities (Mowat *et al.*, 2011). An unmet need in IBD diagnostics is to have a fast and reliable biomarker to distinguish within IBD subtypes, particularly those with shared location of inflammation, but the number of studies that have explored this issue is limited (Lopez-Siles *et al.*, 2014, 2016).

We observed that F-E index is a suitable biomarker to discriminate ulcerative proctitis and left-sided UC from pancolitis (Lopez-Siles *et al.*, 2014), which is of interest for clinicians to monitor risk of extension of the inflamed area in UC (Figure 2). This index was shown also to distinguish between all UC patients regardless of their disease subtypes and those with C-CD with suitable accuracy (Figure 2). In contrast, *F. prausnitzii* alone or phylogroup quantification showed limited ability to discriminate between IBD subtypes. Whether or not *F. prausnitzii* phylogroup quantification in conjunction with *E. coli* counts are more accurate biomarkers remains to be explored.

As the discrimination power of F-E index is limited for some disease subtypes, it could be worth to include additional biomarker characteristics of UC dysbiosis such as *Roseburia hominis* (Machiels *et al.*, 2013), CD dysbiosis such as *Ruminococcus gnavus*, *R. torques*, *Dialister invisus* or *Bifidobacterium*

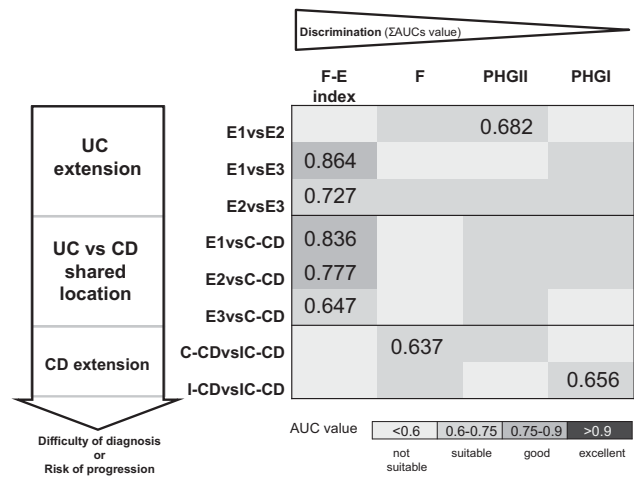


Figure 2 Biomarker of choice to discriminate between IBD locations. Selected pair wise comparisons of conditions are represented taking into account the difficulty of diagnosis or the risk of progression. The four options of biomarkers (*F. prausnitzii*, the two phylogroups or *F. prausnitzii-E. coli* index calculated as (Lopez-Siles *et al.*, 2014)), have been ranked according to their discriminative power estimated as the sum of all the AUC values for all the pair wise comparisons taking into account all the conditions. For each comparison, the highest AUC value achieved is depicted. E1, Ulcerative proctitis, E2, Distal or left-sided UC; E3, pancolitis or universal colitis; I-CD, ileal CD; IC-CD, ileocolonic CD; C-CD, colonic CD; F, total *F. prausnitzii* load; PHG I, *F. prausnitzii* phylogroup I load; PHG II, *F. prausnitzii* phylogroup II load; F-E index, *F. prausnitzii-E. coli* index; AUC, area under the ROC curve; ROC, receiver operating characteristic curve.

adolescentis (Martinez-Medina *et al.*, 2006; Png *et al.*, 2010; Joossens *et al.*, 2011), as well as other bacterial indicators of gut health such as *Akkermansia muciniphila* (Png *et al.*, 2010). A combination of microbiological indicators with host serological data is also an approach to be further explored to improve diagnostics accuracy, since it has been reported that active CD and UC can be differentiated through monitoring fecal *F. prausnitzii* abundance in conjunction with leukocyte counts (Swidsinski *et al.*, 2008).

F. prausnitzii load as a biomarker of disease progression and treatment success

Given the chronic behavior of IBD, it would be interesting to have a prognostic biomarker for flare-ups. High *F. prausnitzii* counts in feces have been associated with lower CD activity index and C-reactive protein levels (Fujimoto *et al.*, 2013). *F. prausnitzii* level recovery has been reported in feces during remission (Swidsinski *et al.*, 2008; Sokol *et al.*, 2009), whereas it has been observed that in mucosa, depletion of this species occurs regardless of patients disease activity status (Willing *et al.*, 2009; Kabeerdoss *et al.*, 2013; Lopez-Siles *et al.*, 2014, 2016), and particularly compromises phylogroup I (Lopez-Siles *et al.*, 2016). Differences in the methodology or the cohort engaged as well as the type of sample analyzed may be a confounding factor that is preventing an unanimous outcome about the usefulness of *F. prausnitzii* to predict flare-ups. Subsequent follow-up studies are needed to conclusively establish which clinical data of the patients correlate with the quantity of *F. prausnitzii* colonizing the gut.

Several studies have shown that *F. prausnitzii* numbers are reduced in resected CD patients in comparison with those without resection (Sokol *et al.*, 2008b; Lopez-Siles *et al.*, 2014). We observed that this phenomenon is replicated with phylogroup counts (Lopez-Siles *et al.*, 2016), with more evident depletion of phylogroup II. However, whether this shift is a consequence of these patients featuring a more acute disease, or if it is the outcome of the surgery is still unclear. It would be interesting to conduct follow-up studies to assess the usefulness of this biomarker to precisely predict when such interventions might be needed.

As far as therapies are concerned, treatments with infliximab and high-dose cortisol have been associated with an increase of *F. prausnitzii* levels (Swidsinski *et al.*, 2008). Chemotherapy and interferon α -2b reverse the depletion of *F. prausnitzii* in patients with neuroendocrine tumor of the midgut, whereas somatostatin analogs have no influence on this species (Dorffel *et al.*, 2012). These results suggest that restoration of the gut conditions due to medication can have an effect on counterbalancing *F. prausnitzii* depletion in the diseased intestine. In contrast, other studies have not found a

medication associated with the recovery of normal levels of this species in the mucosa, suggesting that *F. prausnitzii* would be a poor biomarker to monitor treatment efficacy (Lopez-Siles *et al.*, 2014, 2016; Busquets *et al.*, 2015). However, since these studies are retrospective, further prospective studies are required to establish the usefulness of these biomarkers to monitor long-term treatment efficacy, and to relate impact of medication in this species load in the gut.

Sample of choice to implementation in diagnostics

When analyzing data by sample location, it was observed that colonic biopsies were the most suitable to distinguish disease phenotypes (Lopez-Siles *et al.*, 2014). Although statistical significance was not reached for rectal samples, similar results were obtained. To validate these results would be of value since rectal sigmoidoscopy is a non-invasive method to collect tissue samples which will allow implementing mucosa-associated *F. prausnitzii* quantification in routine clinical practice. Alternatively, the validation in samples collected with rectal swabs, which have been reported to have a great similarity to biopsy specimens (Albenberg *et al.*, 2014) would also be of interest. Nevertheless, it would be of interest to determine if fecal total abundance of *F. prausnitzii* and of both phylogroups can be a suitable biomarker for the detection, follow up and/or classification of IBD phenotypes. The implementation of *F. prausnitzii* counts in feces seems a promising strategy as a biomarker, because it has been already proven to discriminate between active UC and CD patients (Swidsinski *et al.*, 2008) and thus would provide a straightforward method to assess IBD. However, further optimization to fine-tune this tool to achieve discrimination within IBD subtypes and also applicable in patients in remission phases is needed.

Concluding remarks

F. prausnitzii is a metabolically versatile microorganism, and this may explain its wide distribution and high load as part of the gut microbiota in humans. Two phylogroups have been described so far within this species, although the real diversity of the genus remains unknown. *F. prausnitzii* is an important bacterium for human health but, members of this species are very sensitive to changes in gut environment which can limit its distribution, particularly in a diseased gut. Changes in this species population richness and quantity have been observed in several intestinal disorders (Figure 3). There is a lot of information still missing on which phylogroup is important under which conditions in the gut. As the depletion of this species is not homogeneous in all gut diseases however, the use of *F. prausnitzii* as a gold standard measure of a healthy

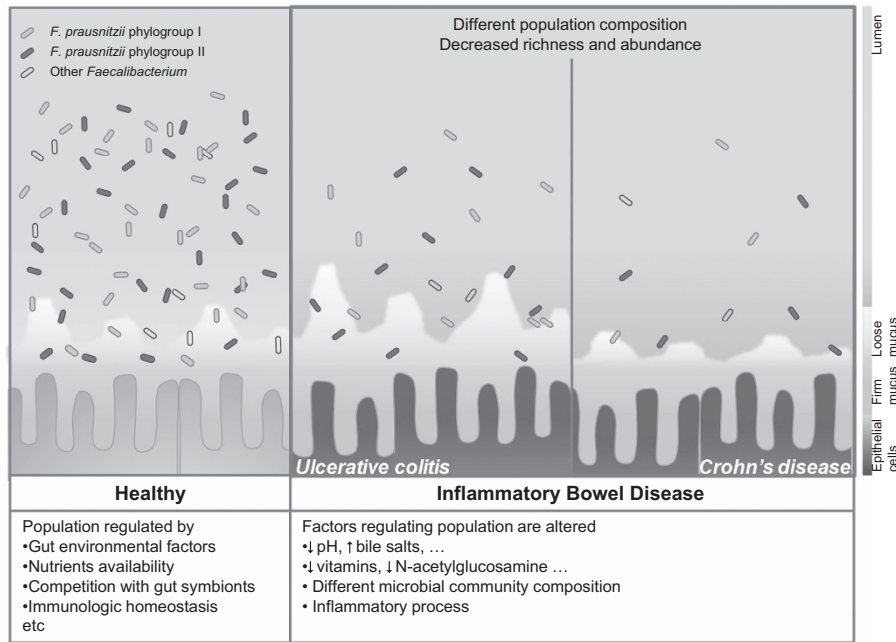


Figure 3 *F. prausnitzii* populations in healthy gut and in patients with IBD. In IBD patients, alteration of gut environment may affect *F. prausnitzii* population composition and load. These differences can be monitored to discriminate within IBD subtypes.

gut microbiota is limited. Nevertheless, it is a good biomarker of certain gut conditions. It has the potential to assist in discriminating between UC and CD subtypes, particularly those with colonic disease location. Besides, discrimination between UC and CRC could be a further application of particular interest for this biomarker, to monitor disease progression since chronic colonic inflammation can lead to tumor formation. As studies in this field are somewhat limited, and a consensus has not yet been established, there is a need to conduct more studies to fully implement *F. prausnitzii* as a biomarker by defining in which medical condition it could be of assistance. Preferably, these studies should be conducted in larger independent cohorts of patients that include individuals from different ethnicities.

Conflict of Interest

The authors declare no conflict of interest.

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