1 **TITLE**

2 ***Faecalibacterium prausnitzii*: from microbiology to diagnostics and**

3 **prognostics**

4 **RUNNING TITLE**

5 *F. prausnitzii:* from microbiology to diagnostics

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23 **CONFLICTS OF INTEREST**

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25

ABSTRACT

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 play 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 give 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.

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 plays 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 and inflammatory bowel diseases (Archer *et al.,* 1998, Christl *et al.,* 1996). Butyrate can reduce intestinal mucosa inflammation through inhibiting NF-κB transcription factor activation (Inan *et al.,* 2000), upregulating PPARγ (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) (Qiu *et al.,* 2013, Sokol *et al.,* 2008b). 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-κB activation, IL-8 production (Sokol *et al.,* 2008b) and upregulation of regulatory T cells 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-κB 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 mucus O-glycans, and may help to maintain suitable proportions of different cell types of secretory linage in the intestinal epithelium (Wrzosek *et al.,* 2013). Finally, a restoration of serotonin (a key neurotransmitter in the gastrointestinal tract that affects motility (Ohman and Simren 2007)) level to normal 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 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 *Faecalibacterium prausnitzii* depletion in gut diseases (Balamurugan *et al.,* 2008, de Goffau *et al.,* 2013, Frank *et al.,* 2007, Furet *et al.,* 2010, Hansen *et al.,* 2012, Jia *et al.,* 2010, Kabeerdoss *et al.,* 2013, Karlsson *et al.,* 2013, Machiels *et al.,* 2013, Martinez-Medina *et al.,* 2006, McLaughlin *et al.,* 2010, Miquel *et al.,* 2013, Qin *et al.,* 2010, Rajilic-Stojanovic *et al.,* 2011, Sobhani *et al.,* 2011, Sokol *et al.,* 2008a, Sokol *et al.,* 2009, Swidsinski *et al.,* 2005, Swidsinski *et al.,* 2008, Vermeiren *et al.,* 2012, Willing *et al.,* 2009), which has prompted interest in considering this bacterium as a new generation probiotic.

Taken all together these findings indicate that *F. prausnitzii* plays a crucial role maintaining gut physiology and host well-being. 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.

(i) 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 OD_{650} that cultures can reach (Duncan *et al.,* 2002, Lopez-Siles *et al.,* 2012)**.**

Despite 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 OD_{650}~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 play 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*

120 *al.,* 2000). Interestingly, it has been reported that treatment with this compound may
121 improve Crohn's disease (CD) as it will serve as a healing factor in inflamed, damaged
122 soft tissues of the gut (Salvatore *et al.,* 2000). Therefore, given the capability to ferment
123 this carbohydrate by *F. prausnitzii*, it would be of interest to explore the effect of
124 restoring this beneficial gut bacterium in CD patients undergoing this treatment.

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

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

142 **(ii) Effect of gut physicochemical conditions**

Tolerance to changes in gut physiological factors can play 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 (Foditsch *et al.,* 2014, Lopez-Siles *et al.,* 2012), the range of pH found in the colon. While 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 duodenum (pH range 5.7-6.4) (Nadal *et al.,* 2007) and in the terminal ileum (Lopez-Siles *et al.,* 2014, Lopez-Siles *et al.,* 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 (Barkas *et al.,* 2013, Nugent *et al.,* 2001), 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 inflammatory bowel disease (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) (Foditsch *et al.,* 2014, Lopez-Siles *et al.,* 2012), suggesting that alterations in bile salts concentrations may determine a variation in *F. prausnitzii* subtype composition. As CD patients also feature an altered bile salt

168 composition (Lapidus and Einarsson 1998, Pereira *et al.,* 2003), further studies need to
169 be conducted to determine if *F. prausnitzii* features higher sensitivity to certain types of
170 bile salt components, and to establish whether or not different bile salt profiles alter *F.*
171 *prausnitzii* subtype composition.

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

182 Finally, the availability of essential nutrients to support *F. prausnitzii* may
183 influence the distribution of this species in the gut. A recent study based on a functional
184 metabolic map of *F. prausnitzii* strain A2-165 has predicted its inability to synthesize
185 the amino acids alanine, cysteine, methionine, serine, and tryptophan (Heinken *et al.,*
186 2014). Auxotrophy for vitamins and cofactors as biotin, folate, niacin, panthothenate,
187 pyridoxine and thiamine has been observed by further analysis of other *F. prausnitzii*
188 strain genomes, and some discrepancy between strains seems to exist in relation to
189 riboflavin production, which could be due to inter-strain differences (Heinken *et al.,*
190 2014, Magnusdottir *et al.,* 2015). In contrast, this species has been predicted as a
191 cobalamin producer (Magnusdottir *et al.,* 2015). Evidence that some IBD patients are
192 predisposed to feature cobalamin deficiency has been reported (Battat *et al.,* 2014), but

193 the cause of this condition has not been established yet. As there is a lack of consistent
194 clinical data that indicates predisposition of IBD patients to this deficiency (Battat *et al.,*
195 2014), it would be interesting to establish if it is associated with depletion of cobalamin-
196 producers in the gut.

197 Collectively, these findings provide a plausible explanation why *F. prausnitzii* is
198 reduced in abundance in patients with gut disease. Besides, it points out crucial
199 requirements in physicochemical conditions for survival of this species, which can be
200 applied in the future to use this bacterium to treat intestinal disorders related to its
201 depletion.

202 **(iii) *F. prausnitzii* in relation to other members of gut microbiota**

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

212 Recent studies in rat models have revealed that *F. prausnitzii* needs the prior
213 presence of *B. thetaiotaomicron* to colonize the gut (Wrzosek *et al.,* 2013). The inability
214 to maintain *F. prausnitzii* mono-associated animal models has been repeatedly observed
215 (Hoffmann *et al.,* 2015, Wrzosek *et al.,* 2013) and a mouse model has also been
216 described in which *F. prausnitzii* implantation in the gastrointestinal tract requires prior
217 preparation with *E. 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 the two 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 and co-workers (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.

(i) *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 (i.e. species defined by genome comparisons, but without phenotypic properties defined yet (Rossello-Mora and Amann 2015, Schloter *et al.,* 2000)). 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* strains genomes is required to provide conclusive information to establish whether or not the two phylogroups belong to different genomospecies or genomovars (i.e. strains which are phylogenetically different but phenotypically indistinguishable (Rossello-Mora and Amann 2015, Schloter *et al.,* 2000)).

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

(ii) 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, Lopez-Siles *et al.,* 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

276 corroborate the presence of these rare phylotypes, and would provide an opportunity to
277 elucidate the taxonomy within the genus *Faecalibacterium*.
278

F. PRAUSNITZII POPULATIONS IN HEALTHY AND DISEASED GUT

(i) *F. prausnitzii* population composition and richness

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

IBD, colorectal cancer (CRC), irritable bowel syndrome (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.

(ii) *F. prausnitzii* load

Several studies have reported *F. prausnitzii* depletion in adult CD (Frank *et al.,* 2007, Fujimoto *et al.,* 2013, Martinez-Medina *et al.,* 2006, Miquel *et al.,* 2013, Sokol *et al.,* 2008b, Sokol *et al.,* 2009, Swidsinski *et al.,* 2008, Willing *et al.,* 2009), UC (Kabeerdoss *et al.,* 2013, Lopez-Siles *et al.,* 2014, Lopez-Siles *et al.,* 2016, Machiels *et al.,* 2013, McLaughlin *et al.,* 2010, Sokol *et al.,* 2009, Swidsinski *et al.,* 2005, Vermeiren *et al.,* 2012) and CRC (Balamurugan *et al.,* 2008, Lopez-Siles *et al.,* 2016)

304 subjects, and concur with the view that down-shifts in *F. prausnitzii* numbers occur
305 under several pathological disorders. In contrast, other studies have reported no
306 depletion in *F. prausnitzii* levels in CRC (Balamurugan *et al.,* 2008, Sobhani *et al.,*
307 2011, Wang *et al.,* 2012), and even increased *F. prausnitzii* abundance in *de-novo*
308 pediatric CD patients (Hansen *et al.,* 2012). Besides, a consensus on whether or not IBS
309 patients feature a depletion of *F. prausnitzii* has not been reached since both studies
310 reported normal counts (Duboc *et al.,* 2012, Jia *et al.,* 2010, Kassinen *et al.,* 2007,
311 Lopez-Siles *et al.,* 2014, Lopez-Siles *et al.,* 2016, Malinen *et al.,* 2005, Rigsbee *et al.,*
312 2012, Swidsinski *et al.,* 2005, Swidsinski *et al.,* 2008) and studies reporting lower
313 numbers in IBS patients of alternating type (Rajilic-Stojanovic *et al.,* 2011) have also
314 been published. The variety of symptoms featured by IBS patients makes IBS
315 diagnostics complex, which in turn is likely to make it difficult to establish whether or
316 not *F. prausnitzii* is affected in this intestinal condition. Altogether, the exact role that
317 *F. prausnitzii* plays in the pathogenesis of these diseases cannot be established at this
318 stage. On the one hand an external factor can cause a downshift in *F. prausnitzii*, but
319 also this species depletion can be a contributing factor to disease aggravation. In this
320 case, restoration of normal counts of this species should be explored as a way to achieve
321 healing and/or attenuate disease progression.

322 Although the depletion of *F. prausnitzii* is not a specific phenomenon that occurs
323 in a particular disease, the level of depletion as well as which components of the *F.*
324 *prausnitzii* population are affected can be different between diseases. Depletion in
325 phylogroup I abundance is a general feature in abnormal gut conditions, while
326 phylogroup II reduction seems to be specific to CD patients, usually with ileal disease
327 location (Lopez-Siles *et al.,* 2016). This could be the consequence of several factors
328 (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 (e.g. ATCC27768) are required in order 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.

(i) *F. prausnitzii* load as diagnostic supporting tool

The usefulness of gut microbiota assessment to support intestinal diseases 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 (Lopez-Siles *et al.,* 2014, Lopez-Siles *et al.,* 2016, Swidsinski *et al.,* 2008). 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), while 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.

(ii) *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

366 distinguish within IBD subtypes, particularly those with shared location of
367 inflammation, but the number of studies that have explored this issue is limited (Lopez-
368 Siles *et al.,* 2014, Lopez-Siles *et al.,* 2016).

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

377 As the discrimination power of F-E index is limited for some disease subtypes, it
378 could be worth to include additional biomarker characteristics of UC dysbiosis such as
379 *Roseburia hominis* (Machiels *et al.,* 2013), CD dysbiosis such as *Ruminococcus gnavus*,
380 *R. torques*, *Dialister invisus* or *Bifidobacterium adolescentis* (Joossens *et al.,* 2011,
381 Martinez-Medina *et al.,* 2006, Png *et al.,* 2010), as well as other bacterial indicators of
382 gut health such as *Akkermansia muciniphila* (Png *et al.,* 2010). A combination of
383 microbiological indicators with host serological data is also an approach to be further
384 explored to improve diagnostics accuracy, since it has been reported that active CD and
385 UC can be differentiated through monitoring fecal *F. prausnitzii* abundance in
386 conjunction with leukocyte counts (Swidsinski *et al.,* 2008)

387 **(iii) *F. prausnitzii* load as a biomarker of disease progression and treatment**
388 **success.**

389 Given the chronic behavior of IBD, it would be interesting to have a prognostic
390 biomarker for flare-ups. High *F. prausnitzii* counts in feces have been associated with

391 lower Crohn's disease activity index (CDAI) and C-reactive protein levels (Fujimoto *et*
392 *al.,* 2013). *F. prausnitzii* level recovery has been reported in feces during remission
393 (Sokol *et al.,* 2009, Swidsinski *et al.,* 2008), while it has been observed that in mucosa,
394 depletion of this species occurs regardless of patients disease activity status (Kabeerdoss
395 *et al.,* 2013, Lopez-Siles *et al.,* 2014, Lopez-Siles *et al.,* 2016, Willing *et al.,* 2009), and
396 particularly compromises phylogroup I (Lopez-Siles *et al.,* 2016). Differences in the
397 methodology or the cohort engaged as well as the type of sample analyzed may be a
398 confounding factor that is preventing an unanimous outcome about the usefulness of *F.*
399 *prausnitzii* to predict flare-ups. Subsequent follow-up studies are needed to conclusively
400 establish which clinical data of the patients correlate with the quantity of *F. prausnitzii*
401 colonizing the gut.

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

410 As far as therapies are concerned, treatments with infliximab and high-dose
411 cortisol have been associated with an increase of *F. prausnitzii* levels (Swidsinski *et al.,*
412 2008). Chemotherapy and interferon α-2b reverse the depletion of *F. prausnitzii* in
413 patients with neuroendocrine tumour of the midgut, whereas somatostatin analogues
414 have no influence on this species (Dorffel *et al.,* 2012). These results suggest that
415 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 (Busquets *et al.,* 2015, Lopez-Siles *et al.,* 2014, Lopez-Siles *et al.,* 2016). 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.

(iv) 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 interest 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 speceis 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 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, in order to monitor disease progression since chronic colonic inflammation can lead to tumour 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.

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FIGURE LEGENDS

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; UC, ulcerative colitis; CD, Crohn's disease; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; CRC, colorectal cancer; 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.

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 ulcerative colitis; E3, pancolitis or universal colitis; I-CD, ileal Crohn's disease; IC-CD, ileocolonic Crohn's disease; C-CD, colonic Crohn's disease; F, total *F. prausnitzii* load; PHG I, *F. prausnitzii* phylogroup I load; PHG II, *F. prausnitzii* phylogroup II load; F-E index, *F. prausnitzii-*

819 *E. coli* index; AUC, area under the ROC curve; ROC, receiver operating characteristic
820 curve.
821 **Figure 3.** *F. prausnitzii* populations in healthy gut and in patients with inflammatory
822 bowel disease (IBD). In IBD patients, alteration of gut environment may affect *F.*
823 *prausnitzii* population composition and load. These differences can be monitored to
824 discriminate within IBD subtypes.
825

Figure 1

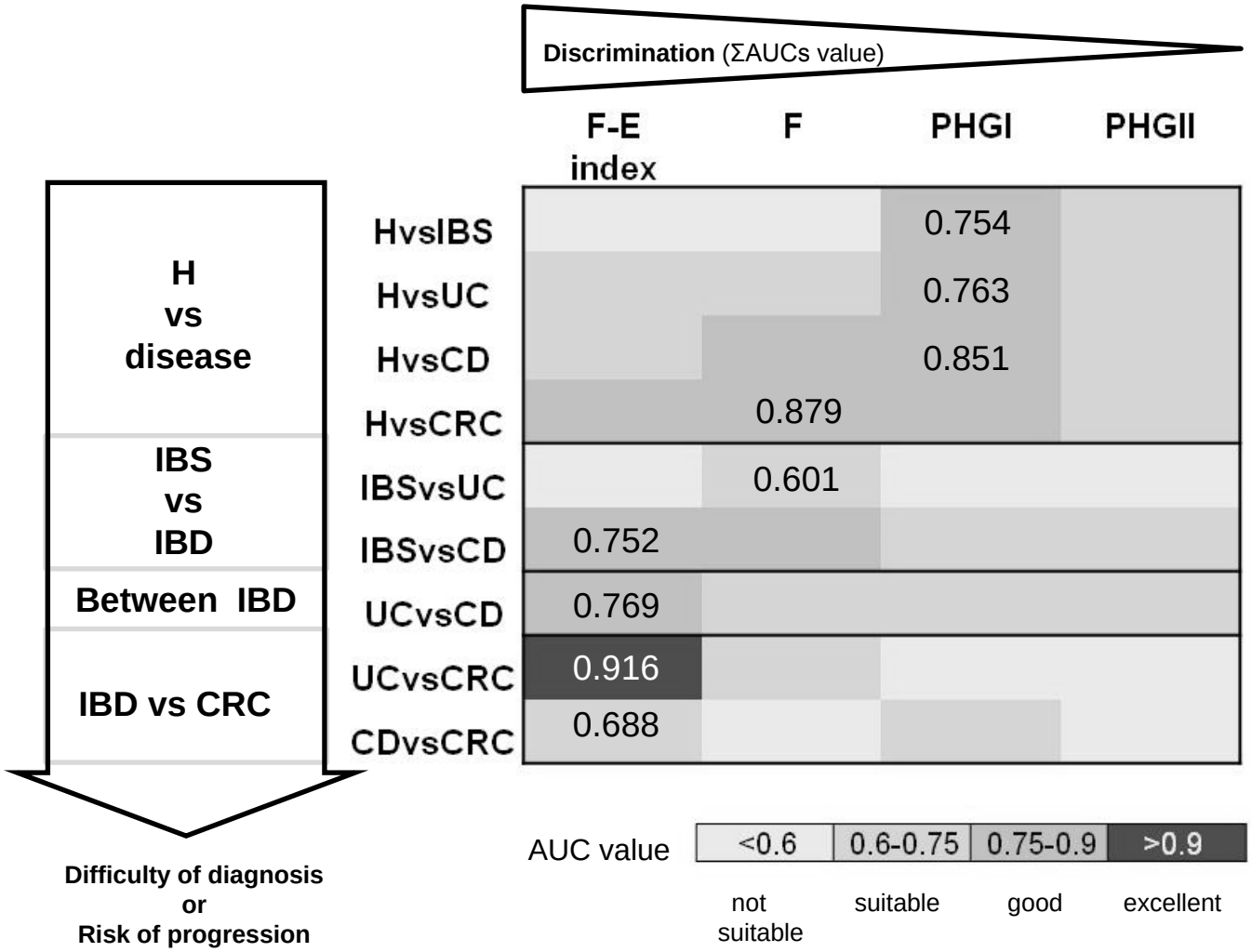

Discrimination (ΣAUCs value)
F-E index
F
PHGI
PHGII
H vs disease
IBS vs IBD
Between IBD
IBD vs CRC
HvsIBS
HvsUC
HvsCD
HvsCRC
IBSvsUC
IBSvsCD
UCvsCD
UCvsCRC
CDvsCRC
0.754
0.763
0.851
0.879
0.601
0.752
0.769
0.916
0.688
Difficulty of diagnosis or Risk of progression
AUC value
<0.6
0.6-0.75
0.75-0.9
>0.9
not suitable
suitable
good
excellent

Figure 2

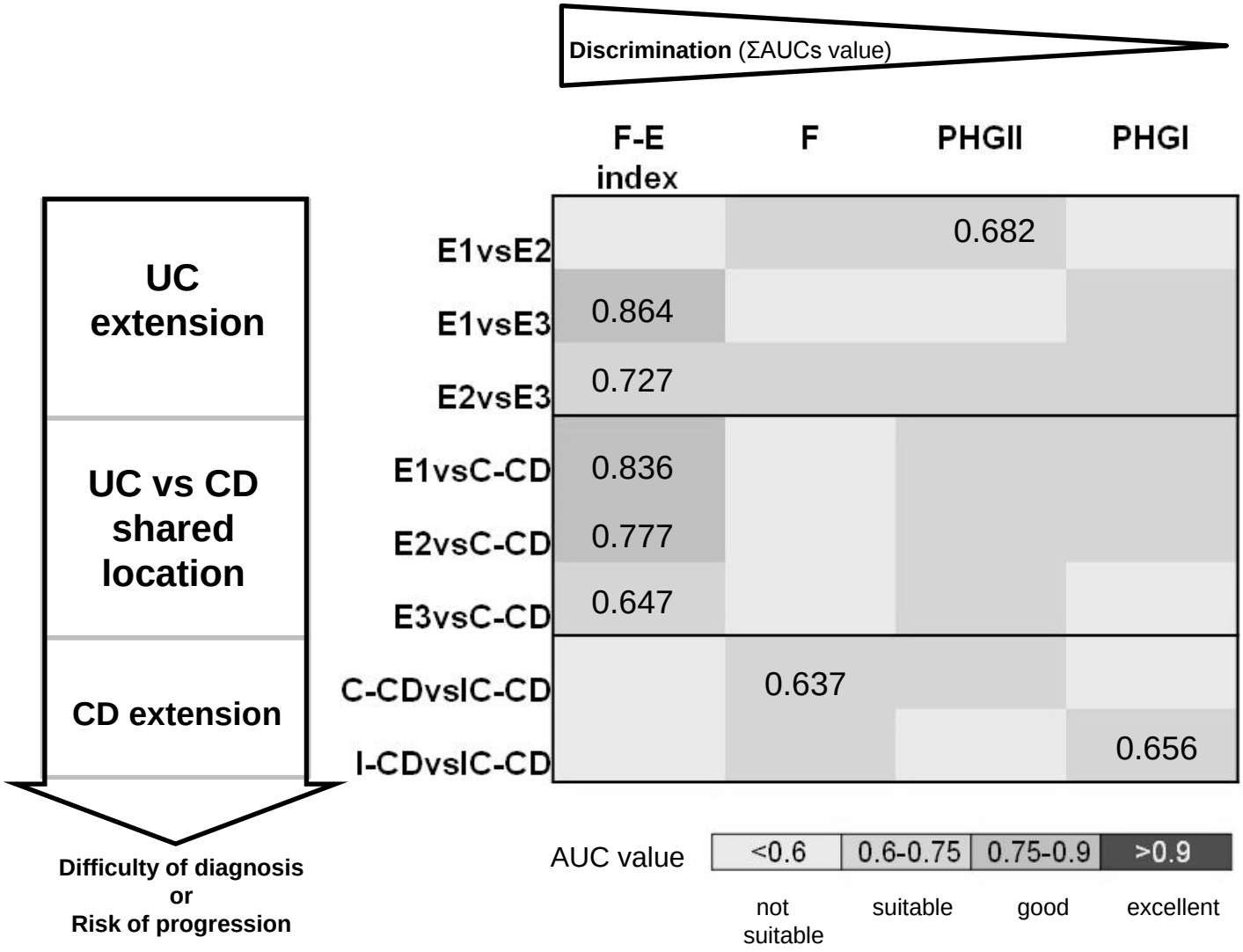
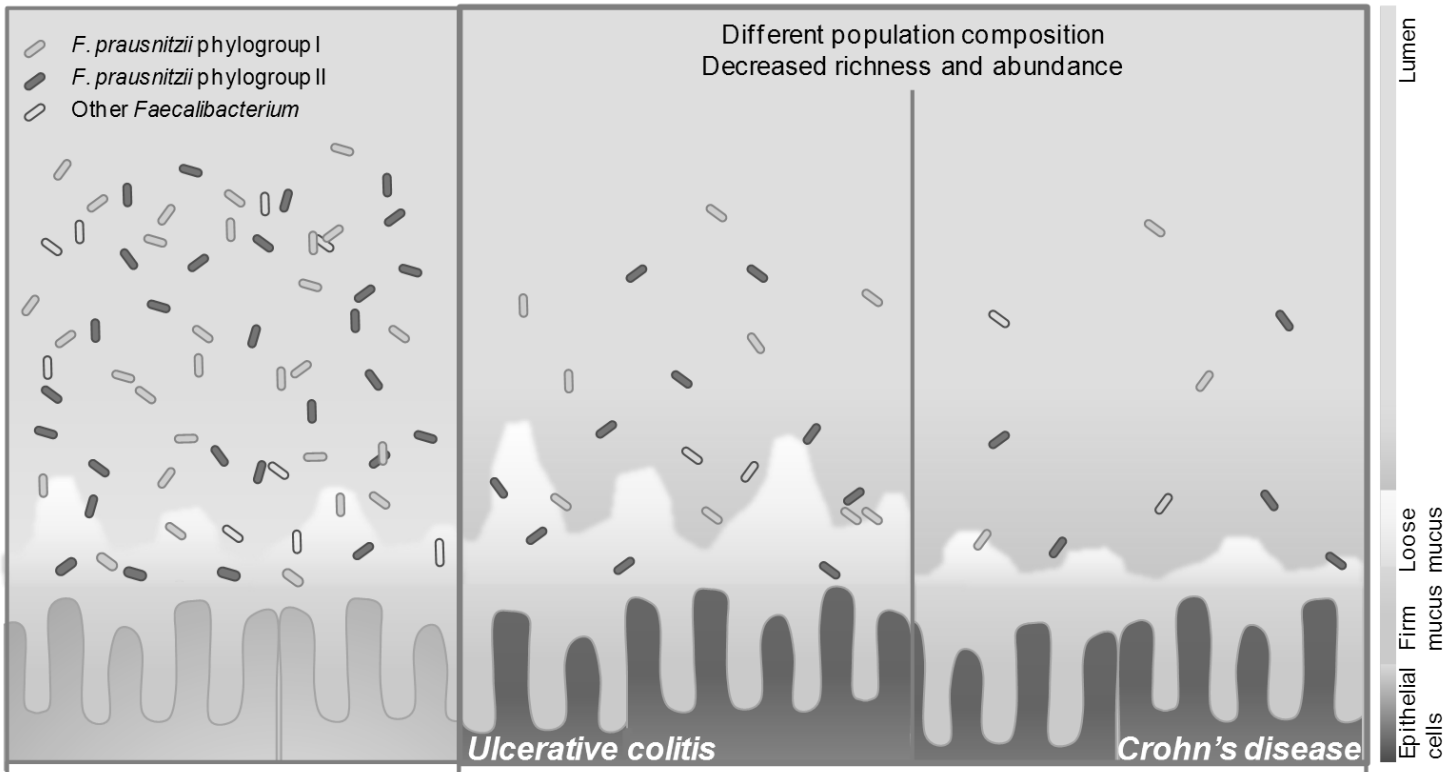

Discrimination (ΣAUCs value)
F-E index
F
PHGII
PHGI
UC extension
UC vs CD shared location
CD extension
E1vsE2
E1vsE3
E2vsE3
E1vsC-CD
E2vsC-CD
E3vsC-CD
C-CDvsIC-CD
I-CDvsIC-CD
0.682
0.864
0.727
0.836
0.777
0.647
0.637
0.656
Difficulty of diagnosis or Risk of progression
AUC value
<0.6
0.6-0.75
0.75-0.9
>0.9
not suitable
suitable
good
excellent

Figure 3



Healthy	Inflammatory Bowel Disease
Population regulated by •Gut environmental factors •Nutrients availability •Competition with gut symbionts •Immunologic homeostasis etc	Factors regulating population are altered •↓pH, ↑bile salts, … •↓vitamins, ↓N-acetylglucosamine … • Different microbial community composition • Inflammatory process

Table 1. Substrates of different origin metabolised 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
Simple carbohydrates[a]		
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
Amino acids[b]		
Arginine	4	4
Histidine arylamide	4	4
Glycine arylamide	2	4
Diet-derived[c]		
Fructo-oligosacharides	4	4
Pectin (apple)	10	10
Inulin (chicory)	9	11
Host-derived[d]		
Glucosamine HCl	10	10
N-acetylglucosamine	9	10
Glucuronic acid	6	10

[a] Other simple carbohydrates tested but non-metabolised are mannitol (0/3), melibiose (0/4), raffinose (0/4), ribose (0/4), fucose (0/10), arabinose (0/11) and xylose(0/11)

[b] Other amino acids tested but non-metabolised 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)

[c] Other diet-derived carbohydrates not metabolised 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 metabolise starch.

[d] Other host-derived carbohydrates not metabolised are choindrotin sulphate (0/10), heparin (0/10), hyaluronic acid (0/10), pig gastric mucin (0/10)

Table 2. Average nucleotide identity (ANI) values for paired comparisons between *F. prausnitzii* strains whose genome has been fully sequenced. Phylogroup for each strain is indicated in brackets. Values corresponding to the same genomospecies are indicated in boldface.

ANIb* values

F. prausnitzii isolate	KLE1255 (nd)	A2-165 (II)	L2/6(II)	SL3/3(I)
M21/2 (I)	85.26	83.29	82.11	**96.70**[§]
KLE1255 (nd)	●	82.79	82.46	84.70
A2-165 (II)	82.77	●	82.60	82.74
L2/6(II)	82.33	82.87	●	81.61

ANIm values**

F. prausnitzii isolate	KLE1255 (nd)	A2-165 (II)	L2/6(II)	SL3/3(I)
M21/2(I)	89.02	88.52	88.07	**97.34**[§]
KLE1255 (nd)	●	88.31	88.65	88.82
A2-165(II)	88.31	●	88.23	88.28
L2/6(II)	88.65	88.23	●	87.99

nd, not determined

* ANIb, average nucleotide identity based on BLAST searches of 1 kb genome fragments against a target genome.

** ANIm, average nucleotide identity based on the MUMmer algorithm that does not require the artificial generation of 1kb fragments.

ANIb has better application for distant genomes comparison, while both algorithms give nearly identical values in the high identity range (80-100%).

[§] It has been shown that ANI values higher than 94% embraces organisms sharing DNA-DNA hybridization (DDH) values higher than 70% which are considered to be genomospecies.

Table 3. Summary of *F. prausnitzii* phylogroups I and II characteristics. No statistically significant differences have been found between the members of the two phylogroups for any of the characteristics analyzed.

	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±SD)*	3.17±0.06	3.21±0.16
GC content (mean %±SD)*	55.85±0.49	56.45±0.21
Genes content (mean±SD)*	2881.5±92.6	2892.5±102.5
Proteins content (mean±SD)*	2778.5±46.0	2725.5±43.1
Carbohydrate utilisation (mean OD_{650}±SD) **		
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-Acetylgucosamine	0.615±0.224	0.388±0.369
Glucosamine HCl	0.345±0.177	0.267±0.336
Tolerance to pH (mean growth rate±SD)**		
6.7	0.210±0.070	0.256±.0151
6.2	0.192±0.050	0.245±0.159
5.75	0.081±0.039	0.108±0.042
Tolerance to bile salts (mean maximum OD_{650}±SD)**		
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
SCFA production (mM ±SD) [§]		
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, *et al.* 2008))	Decrease in dihydrothymine and an increase in 4-hydroxyphenylacetylglycine	Decreased levels of 3-aminoisobutyrate, taurine, 3,5-hydroxylbenzoate, dimethylamine, 2-hydroxyisobutyrate, glycolate and increased lactate and glycine
Abundance in gut disorders[§§] (adapted from (Hippe *et al.*, 2016, Lopez-Siles *et al.*, 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.

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

** For 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)

§ Short 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 YCFA medium supplemented with 0.5% (wt/vol) glucose (Lopez-Siles *et al*., 2012)

§§ IBS, irritable bowel syndrome; CRC, colorectal cancer; IBD, inflammatory bowel disease; CD, Crohn's disease