The role of *Faecalibacterium prausnitzii* in health and disease

Zain M. Cheema[1]
[1]Department of Biochemistry & Biomedical Sciences, Faculty of Health Sciences, McMaster University, Canada
*Corresponding author: cheemaz@mcmaster.ca

Abstract
The human body is host to numerous complex microbial communities that comprise the human microbiome. These microbes and their dynamic interactions with each other and with the host, play critical roles in human development and health. Although mostly considered beneficial, bacteria within the microbiome may contribute to disease as infectious agents, through mediation of antibiotic resistance, and by participation in immune phenomenon as drivers of chronic inflammatory diseases. This review highlights the current research on the gut microbiota, with a particular focus on *Faecalibacterium prausnitzii* and its role in maintaining intestinal health. *F. prausnitzii* is a species of obligate anaerobic bacteria found in the human gastrointestinal tract. This species has been widely associated with human health and is found at lower numbers in a wide variety of human diseases including inflammatory bowel disease (IBD). Based on the current research landscape, however, it is evident that majority of research on *F. prausnitzii* is associative in nature and for this reason, culture-dependent studies are needed to further elucidate the role of this gut bacteria in diseases such as IBD.

Introduction
The human gastrointestinal (GI) tract is home to a diverse group of bacteria, archaea, viruses, and fungi.[1-4] Together this collection of microorganisms is referred to as the 'gut microbiota'.[1-4] Interestingly, majority of these microorganisms are bacteria, approximately equal in number to the amount of human eukaryotic cells in the body.[2,5] In contrast, the term 'gut microbiome' refers to the collective genomes, encoding more than three million genes, of the microorganisms that inhabit the gut. [1-4] In the last 15 years, the role of the gut microbiota in maintaining intestinal health has become increasingly evident.[3] Not only do these microorganisms play a key role in harvesting energy, they also prevent the colonization of pathogens and maintain host immunity.[1,3-4] However, a change in the microbial composition of the gut can lead to the development of disease pathology.[4,6] Dysbiosis or the imbalance between protective commensal bacteria and harmful opportunistic bacteria is proposed to be the underlying cause of several diseases including inflammatory bowel disease (IBD). [1,4,6] IBD refers to a group of disorders characterized by chronic inflammation in the GI tract.[7] With regard to the role of dysbiosis in IBD, research suggests that the depletion of *Faecalibacterium prausnitzii*, a major commensal bacterium, is associated with the disease pathology of IBD.[7-8] The purpose of this review is to summarize the current research on the gut microbiota, discuss the role of *F. prausnitzii* in intestinal health, examine the factors promoting *F. prausnitzii* presence in the gut, and lastly consider the role of *F. prausnitzii* in IBD. Additionally, this review seeks to highlight areas in this field of research that need to be further clarified or addressed.

Composition of the gut microbiota
The human gut microbiota is colonized by at least 1000 different species of bacteria.[1,4] This process of colonization begins right after birth as the infant becomes exposed to the outside environment.[9] Additionally, factors such as the maternal microbiota composition and mode of delivery have been shown to influence early colonization of the gut.[9] Research suggests that two main phyla of bacteria dominate the early infant gut: Actinobacteria and Proteobacteria.[1,9] However, as the infant grows older, the gut microbiota continues to evolve and increase in microbial diversity. [1,9] As the individual reaches adulthood, the composition of the gut microbiota becomes relatively stable, but can still be altered by factors such as diet, lifestyle, antibiotic treatment, illness,

aging, and the environment.[1,9] Although twin studies have suggested a potential role of genetics as a determinant of microbiota composition, the extent and nature of its role remains under debate and further research is needed.[9] Furthermore, due to differences in experience, every individual develops a unique gut bacterial composition.[1,3,9] However, in general, the adult microbiota is composed primarily of two main phyla: Firmicutes and Bacteroidetes.[1,9] Other phyla including Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria, although present, are at a much lower proportion.[4]

Role of the gut microbiota in intestinal health

The gut microbiota plays a critical role in the maintenance of intestinal health, as well as nutrition, immune development, and host defense.[7] One of the primary role of gut bacteria is the production of compounds essential for GI health.[7,10] Not only do commensal bacteria synthesize vitamins, but they also play a critical role in the fermentation of dietary fibers.[7] The phyla Firmicutes and Bacteroidetes have found to be involved in the production of short chain fatty acids (SCFAs) via fermentation.[7,10] These SCFAs are absorbed by the surrounding colonic epithelial cells (CECs) and are able to regulate cellular processes such as altering cell growth and gene expression.[7] The three major SCFAs produced include: butyrate, acetate, and propionate.[7] Among these, butyrate has been shown to be a primary contributor of intestinal health.[1,4,7] Majority of this SCFA is produced by *Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii and Ruminococcus bromii.*[10] By acting as an energy source for CECs, butyrate promotes cell proliferation.[7] Additionally, butyrate has also been shown to be an important regulator of tight-junction proteins (TJPs) in the GI tract.[7,10] Increased levels of butyrate lead to an increased expression of TJPs, thereby promoting intestinal barrier integrity and preventing bacterial translocation across the epithelium.[7,10] Recent evidence suggests that butyrate might also promote *B*-oxidation in CECs, which increases uptake of oxygen, and therefore creates an anaerobic gut environment unfavourable for the colonization of pathogenic facultative anaerobes.[11] Finally, emerging research in this field also suggests anti-inflammatory activity of butyrate in the gut. In contrast to butyrate, both acetate and propionate have shown to play a systemic role in the body.[7,11] Propionate is taken up by the liver, while acetate moves into peripheral organs such as the muscles.[7,10]

The interaction between the gut microbiota and immune system is critical for the maintenance of intestinal health and barrier integrity.[7] In particular, the presence of the gut microbiota influences development of the mucosal immune system.[1,7] Experiments have indicated that germ-free mice (deficient in a gut microbiota) display an underdeveloped and impaired immune system.[7] When the microbiota is reintroduced in these mice, majority of the immune system function is restored.[7] Bacteria in the gut, primarily of the class Clostridia, have been shown to play a role in the differentiation and proliferation of regulatory T cells (Tregs) via butyrate production.[7] Tregs are immune cells responsible for regulating and suppressing other immune cells of the body.[7] These cells play a critical role in maintaining immunological tolerance and preventing autoimmune reactions.[7] Research suggests that individuals with inflammatory gut diseases such as IBD are found to have much lower levels of butyrate-producing bacteria.[7-8] Additionally, species of gut bacteria such as *Escherichia coli* and *Citrobacter rodentium* have been described to contribute to the induction and development of helper T cells such as Th17.[7]

Finally, the gut microbiota is also responsible for protecting the GI tract from pathogenic bacteria. This concept is referred to as 'colonization resistance'.[12] Commensal bacteria are able to protect the intestine from pathogens in two ways: directly and indirectly.[7,12] By consuming essential nutrients or through the production of anti-microbial molecules such as bacteriocins, commensal bacteria are able to directly prevent the colonization of pathogens.[7,12] For example, species of the genus *Bifidobacterium* have been shown to inhibit colonization of intestinal pathogens, such as *Clostridium difficile*, via production of antimicrobial organic acids and peptides.[13] Recent evidence has also demonstrated the role of contact-dependent interbacterial antagonism, notably in the phylum

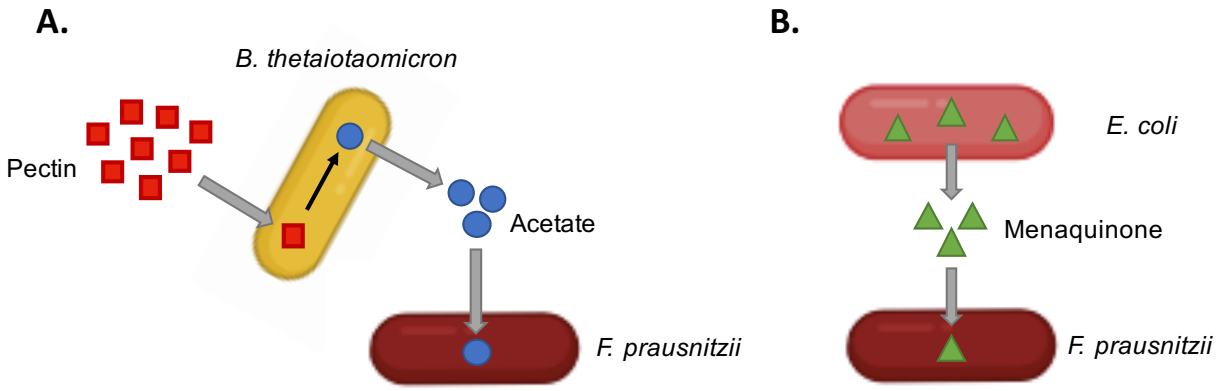

Figure 1. *Examples of syntrophy with* F. prausnitzii. (A) Acetate, which is produced by B. thetaiotaomicron due to pectin fermentation, is rapidly taken up by F. prausnitzii to promote growth. (B) Menaquinone, directly produced by E. coli, is taken up by F. prausnitzii and used in the electron transport chain to facilitate anaerobic respiration.

Bacteroidetes. By injecting toxic effectors into nearby cells, a mechanism known as the type VI secretion system occurs, in which gut commensals are able to prevent the colonization of harmful bacteria and, thereby, maintain a stable gut environment.[14] On the other hand, indirect methods of colonization resistance involve the role of commensal bacteria in the production of pro-inflammatory molecules that prime or enhance the immune response against pathogenic bacteria.[7,12] Together, these mechanisms are critical for preventing GI infections.

It is important to note that a slight disruption in any of the functions mentioned above may lead to the development of disease pathology such as obesity, malnutrition, IBD, neurological disorders, and cancer.[4,6] Dysbiosis or a change in composition of the gut microbiota has been suggested to be one of the primary underlying cause of this disruption.[4,6] It is evident in patients with IBD that there is a decrease in gut bacteria with anti-inflammatory properties and an increase in those that contribute to inflammation.[4,8] Although the levels of many different bacterial species are altered in IBD, the most consistent finding suggests a lower abundance of *F. prausnitzii* in patients with IBD.[15] The role of *F. prausnitzii* in gut health and disease will be further addressed in the following portion of this review.

Role of *F. prausnitzii* in intestinal health

F. prausnitzii is one of the three most abundant bacterial species found in the GI tract, accounting for approximately 6-8% of the gut microbial community in healthy individuals.[15] *F. prausnitzii* is a member of the Firmicutes phylum and the only known species in the genus *Faecalibacterium*.[8,15] It is a Gram-positive, strict anaerobic bacterium.[8,15] Currently, majority of the research on *F. prausnitzii* is based on metagenomic studies of the gut microbiota.[16] These studies have shown altered levels of *F. prausnitzii* in patients with a range of metabolic diseases such as colorectal cancer, obesity, celiac disease, and IBD.[16] However, this data is associative in nature and cannot be used to suggest a causal role of *F. prausnitzii* in disease pathology.[16] Instead, culture dependent studies are required to address the role of *F. prausnitzii* in health and disease.[16] Unfortunately, due to the difficulty in growing this extremely oxygen sensitive (EOS) bacterium, a limited number of studies have assessed the function and underlying biology of *F. prausnitzii*.[16]

Factors promoting *F. prausnitzii* in the gut

When considering isolation strategies for *F. prausnitzii*, it is important to consider the bacteria's growth requirements. Current literature provides insight regarding the role of specific carbon sources, gut physiological conditions, and various bacteria in the growth of *F. prausnitzii*. However, the effects of each of these factors has been evaluated

using a limited number of *F. prausnitzii* strains. One study, in particular, showed that simple carbohydrates including fructose, glucose, cellobiose, and maltose were fermented by 90-100% of *F. prausnitzii* strains.[17] Additionally, diet-derived apple pectin has been shown to promote *F. prausnitzii* growth in culture.[17] Both *in vitro* and *in vivo* studies have demonstrated an increase in *Firmicutes* abundance after introduction of pectin as an energy source.[17-18] Lastly, host-derived carbon sources including glucosamine HCl and N-acetylglucosamine have also been shown to be fermented by *F. prausnitzii* and thereby promote growth.[17] This data suggests a use of any of the above mentioned carbon sources as prebiotics for individuals suffering from IBD.[19] In other words, these carbons sources can be administered to restore *F. prausnitzii* levels in the diseased gut.[19] Furthermore, the aforementioned carbon sources may also be added as supplements to media in order to better improve culture-dependent isolation strategies for *F. prausnitzii*. The success of such strategy should be addressed and evaluated in the future. With regard to mucin, glycoproteins that make up the mucus covering on epithelial cells, little is known about its effect on *F. prausnitzii*.[20-21] Conflicting research about the ability of *F. prausnitzii* to utilize mucin exists in current literature and future studies should focus on addressing this issue.[20]

Tolerance to different physiological conditions in the gut is a key determinant of the ability for bacteria to colonize the GI tract.[17] Experiments testing the pH tolerance of various strains of *F. prausnitzii* show that the optimal pH for growth ranges between 5.7 and 6.7.[18] This data is reinforced by the colonization patterns of *F. prausnitzii* in the GI tract. *F. prausnitzii* is found at higher levels in the duodenum, which has a pH range of 5.7-6.4 in healthy subjects.[22] Additionally, evidence shows that *F. prausnitzii* is highly sensitive to bile salts.[17] Although strain-strain variability exists, *F. prausnitzii* growth is compromised when bile salts concentrations reach 0.5% (wt/vol) or above.[17] This data might explain why Crohn's disease (CD) patients, who exhibit higher bile salt concentration in their gut, have decreased levels of *F. prausnitzii*.[15] Furthermore, based on functional metabolic maps of *F. prausnitzii*, it has become evident that certain strains of this bacteria are unable to synthesize molecules such as cysteine, biotin, and riboflavin.[23] These results signify the importance of having such molecules present in the gut environment for use by *F. prausnitzii*.[23] Nonetheless, this data varies among strains and further research using a larger collection of isolates is required.

Syntrophy, otherwise referred to as 'cross-feeding', is the phenomenon where one bacterial species is dependent on the products of another species.[24] Examples of this phenomenon are widely evident when looking at the diverse microbial communities present in the GI tract.[24] Interestingly, *F. prausnitzii* has been shown to rely on other bacterial species in the gut for cross-feeding.[17,25] Past studies have observed this relationship between *F. prausnitzii* and *Bacteroides thetaiotaomicron* – a Gram-negative, strict anaerobe, found to be abundant in the human gut microbiota.[17] Co-culture experiments containing these two bacteria in media supplemented with pectin showed enhanced growth of *F. prausnitzii*.[17] Specifically, acetate, a SCFA produced by *B. thetaiotaomicron* via pectin fermentation, was observed to be rapidly taken up by *F. prausnitzii* (Figure 1A).[17] These results are supported by previous studies showing the importance of acetate in *F. prausnitzii* growth.[23] Furthermore, experiments in germ-free mice have demonstrated that *F. prausnitzii* is unable to colonize the gut individually.[25] However, when co-colonized with another bacterium such as *B. thetaiotaomicron*, successful colonization is observed.[25] Interestingly, a similar cross-feeding relationship has recently become evident between *F. prausnitzii* and *E. coli*.[26] A group of scientists have evaluated the role of *E. coli* as a 'helper' strain for multiple species of bacteria including *F. prausnitzii*.[26] Using co-culture experiments, the researchers demonstrated that *E. coli* induced growth of *F. prausnitzii* (Figure 1B).[26] Additionally, they tested a library of mutant *E. coli* strains to evaluate the underlying mechanism of induction.[26]

The researchers concluded from the experiments that the *E. coli* genes involved in menaquinone biosynthesis were responsible for inducing *F. prausnitzii* growth.[26] Follow-up genome sequencing of *F. prausnitzii* further supported this notion when a lack of genes responsible for the menaquinone biosynthesis pathway were identified.[26] In majority of Gram-positive bacteria including *F. prausnitzii*, menaquinone participates in the electron transport chain in order to facilitate anaerobic respiration.[26] However, in the case of *F. prausnitzii*, menaquinone is not synthesized on its own but is instead acquired from the external environment.[26] For this reason, *E. coli*, a major producer of menaquinone, is able to induce *F. prausnitzii* growth in culture.[26] With regard to improving current *F. prausnitzii* isolation techniques, future efforts should focus on elucidating the role of menaquinone in the cross-feeding relationship between *F. prausnitzii* and *E. coli*.

Role of *F. prausnitzii* in IBD

As previously described, IBD is a group of metabolic diseases characterized by chronic inflammation of the GI tract.[4,7] There are two main forms of IBD: Crohns disease (CD) and ulcerative colitis (UC).[4,7] Although the exact cause of IBD remains unknown, evidence suggests that a combination of genetic risk and dysbiosis contribute to disease pathology.[7] A reduction in the diversity of the gut microbiota accompanied by lower levels of Firmicutes bacteria can be seen in IBD patients.[8,15] In particular, lower counts of *F. prausnitzii* in the gut microbiota of patients suffering IBD has been reported in several studies.[8,15] It has been proposed that *F. prausnitzii* displays anti-inflammatory properties that may be responsible for maintaining a healthy gut environment.[8,15] For this reason, scientists are currently evaluating the use of *F. prausnitzii* as a probiotic to counterbalance dysbiosis in the gut.[15] Although the exact mechanism by which *F. prausnitzii* maintains intestinal health remains unclear, researchers have proposed several hypotheses. Recent evidence has shown the role of *F. prausnitzii* in butyrate production, thereby maintaining intestinal health and integrity.[8,10,15] Not only does butyrate act as an energy source for CECs, but it has shown to be involved in preventing inflammation. By inhibiting NF-κB and IFN-ɣ, as well as up regulating PPARɣ, a reduction in intestinal inflammation has been observed.[27] In addition, *F. prausnitzii* also displays novel anti-inflammatory properties that have been shown in a DSS colitis murine model.[28] Administration of the cell-free supernatant of *F. prausnitzii* in colitis induced mice led to a reduction in gut inflammation.[28] Furthermore, researchers were successfully able to identify specific peptides in the supernatant responsible for this inhibitory effect.[29] These peptides derive from a 15 kDa protein produced by *F. prausnitzii*, referred to as microbial anti-inflammatory molecule (MAM).[29] Follow-up experiments have shown that MAM is able to demonstrate immunomodulatory activity by blocking both the NF-κB signaling cascade and production of IL-8, a pro-inflammatory cytokine.[29] Due to a very limited number of studies on MAM, future research should focus on evaluating the potential use of MAM as a therapeutic strategy for IBD.

Conclusion

The human body is host to numerous complex microbial communities that comprise the human microbiota. These microbes and their dynamic interactions with each other and with the host play critical roles in human development and health. Specifically, the gut microbiota contributes to intestinal health through the production of metabolites such as butyrate, the development of the mucosal immune system, and by providing protection from pathogenic bacteria via colonization resistance. Unfortunately, a disruption in any of the mentioned functions can lead to severe disease pathology. This is evident when looking at the association between *F. prausnitzii* and IBD. As described in the literature, *F. prausnitzii* is not extensively characterized and there are a limited number of isolates to date. For this reason, future work should focus on development of a culture dependent methodology for the isolation of a diverse set of *F. prausnitzii* strains. Genomic and phenotypic assays of various isolates will allow researchers to assess strain-strain variability,

providing further insight into the immunomodulatory role of *F. prausnitzii*.

Overall, the study of the human gut microbiota in health and disease is an emerging field of research. However, the majority of current research is associative in nature and for this reason, culture-dependent studies are essential to further elucidate the role of gut microbes in diseases such as IBD.

Acknowledgements

I would like to thank my principal investigator, Dr. Michael G. Surette, for giving me the amazing opportunity to pursue this research. I would also like to acknowledge the support from the Institute of Infectious Disease Research Summer Student Fellowship in conducting my research project. This work was completed as part of the requirements of BIOMEDDC 4A15, Fall 2018, at McMaster University.

References

1. Thursby, E. & Juge, N. Introduction to the human gut microbiota. *Biochem. J.* **474,** 1823–1836 (2017).
2. Gill, S. R. *et al.* Metagenomic analysis of the human distal gut microbiome. *Science.* **312,** 1355–1359 (2006).
3. Cani, P. D. Human gut microbiome: hopes, threats and promises. *Gut* **67,** 1716–1725 (2018).
4. Guinane, C. M. & Cotter, P. D. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. *Therap. Adv. Gastroenterol.* **6,** 295–308 (2013).
5. Sender, R., Fuchs, S. & Milo, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol.* **14,** e1002533 (2016).
6. Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M. & Owen, L. J. Dysbiosis of the gut microbiota in disease. *Microb. Ecol. Health Dis.* **26,** 26191 (2015).
7. Nishida, A. *et al.* Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin. J. Gastroenterol.* **11,** 1–10 (2018).
8. Foditsch, C. *et al.* Isolation and characterization of *Faecalibacterium prausnitzii* from calves and piglets. *PLoS One* **9,** 1–19 (2014).
9. Rodríguez, J. M. *et al.* The composition of the gut microbiota throughout life, with an emphasis on early life. *Microbial Ecology in Health & Disease* **26,** (2015).
10. Morrison, D. J. & Preston, T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **7,** 189–200 (2016).
11. Valdes, A. M., Walter, J., Segal, E. & Spector, T. D. Role of the gut microbiota in nutrition and health. *BMJ* **361,** k2179 (2018).
12. Buffie, C. G. & Pamer, E. G. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.* **13,** 790–801 (2013).
13. Hütt, P., Shchepetova, J., Lõivukene, K., Kullisaar, T. & Mikelsaar, M. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. *J. Appl. Microbiol.* **100,** 1324–1332 (2006).
14. Russell, A. B. *et al.* A type VI secretion-related pathway in bacteroidetes mediates interbacterial antagonism. *Cell Host Microbe* **16,** 227–236 (2014).
15. Lopez-Siles, M. *et al.* Mucosa-associated *Faecalibacterium prausnitzii* phylotype richness is reduced in patients with inflammatory bowel disease. *Appl. Environ. Microbiol.* **81,** 7582–7592 (2015).
16. Martín, R. *et al.* Functional characterization of novel *Faecalibacterium prausnitzii* strains isolated from healthy volunteers: A step forward in the use of *F. prausnitzii* as a next-generation probiotic. *Front. Microbiol.* **8,** 1–13 (2017).
17. Lopez-Siles, M. *et al.* Cultured Representatives of Two Major Phylogroups of Human Colonic Faecalibacterium prausnitzii Can Utilize Pectin, Uronic Acids, and Host-Derived Substrates for Growth. *Applied and Environmental Microbiology* **78,** 420–428 (2011).
18. Licht, T. R. *et al.* Effects of apples and specific apple components on the cecal environment of conventional rats: role of apple pectin. *BMC Microbiol.* **10,** 13 (2010).
19. Chung, W. S. F. *et al.* Modulation of the human gut microbiota by dietary fibres occurs at the species level. *BMC Biol.* **14,** 3 (2016).
20. Sadaghian Sadabad, M. *et al.* A simple coculture system shows mutualism between anaerobic faecalibacteria and epithelial Caco-2 cells. *Sci. Rep.* **5,** 17906 (2016).

21. Perez-Vilar, J. & Hill, R. L. The structure and assembly of secreted mucins. *J. Biol. Chem.* **274,** 31751–4 (1999).
22. Nadal, I. *et al.* Imbalance in the composition of the duodenal microbiota of children with coeliac disease. *J. Med. Microbiol.* **56,** 1669–1674 (2007).
23. Heinken, A. *et al.* Functional Metabolic Map of Faecalibacterium prausnitzii, a Beneficial Human Gut Microbe. *Journal of Bacteriology* **196,** 3289–3302 (2014).
24. Morris, B. E. L., Henneberger, R., Huber, H. & Moissl-Eichinger, C. Microbial syntrophy: interaction for the common good. *FEMS Microbiol. Rev.* **37,** 384–406 (2013).
25. Wrzosek, L. *et al. Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol.* **11,** 61 (2013).
26. Fenn, K. *et al.* Quinones are growth factors for the human gut microbiota. *Microbiome* **5,** 161 (2017).
27. Segain, J. P. *et al.* Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut* **47,** 397–403 (2000).
28. Carlsson, A. H. *et al. Faecalibacterium prausnitzii* supernatant improves intestinal barrier function in mice DSS colitis. *Scand. J. Gastroenterol.* **48,** 1136–1144 (2013).
29. Breyner, N. M. *et al.* Microbial Anti-Inflammatory Molecule (MAM) from *Faecalibacterium prausnitzii* Shows a Protective Effect on DNBS and DSS-Induced Colitis Model in Mice through Inhibition of NF-**κ**B Pathway. *Front. Microbiol.* **8,** 114 (2017).