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Review

A potential species of next-generation probiotics? The dark and light sides of *Bacteroides fragilis* in health

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ABSTRACT

Bacteroides fragilis (*B. fragilis*) is a commensal Gram-negative obligate anaerobe that resides in the mammalian lower gut and can profoundly affect the susceptibility of the host to inflammatory diseases. Previous studies have identified *B. fragilis* as a common opportunistic pathogen in clinical infections and suggested that it may be responsible for a range of diseases involving a permeable intestinal barrier. However, recent studies of the relationship between nontoxigenic *B. fragilis* and the immune system have indicated that several *B. fragilis* strains may be potential probiotic. In the present review, we summarize the factors influencing the intestinal abundance of *B. fragilis* and discuss the biological interactions between this microbe and the host. Immune system development, age, individual dietary habits, physical condition, drug intake and personal lifestyle habits can all affect the abundance of *B. fragilis* in the human intestine. Polysaccharide A or outer membrane vesicles from nontoxigenic *B. fragilis* may mediate beneficial interactions with the host, whereas enterotoxigenic *B. fragilis* toxin or lipopolysaccharide may stimulate colitis or even systemic inflammation.

Generally, this review summarizes the biological characteristics of *B. fragilis* and describes future application of probiotics.

1. Introduction

Bacteroides fragilis (B. fragilis) is one of the most prevalent members of the genus *Bacteroides* (Phylum Bacteroidetes, Class Bacteroidia, Order Bacteroidales, Family Bacteroidaceae). This Gram-negative obligate anaerobe is commonly found in the human gut flora but has also been detected in the mouth, upper respiratory tract and female genital tract. Microscopically, *B. fragilis* is a rod-shaped cell with rounded ends. On blood agar, *B. fragilis* colonies reach 1–3 mm in diameter, with a smooth, circular and translucent to semi-opaque appearance with little convexity and slight or no hemolysis. On *Bacteroides* bile esculin (BBE) medium, *B. fragilis* hydrolyzes esculin and thus blackens the surrounding environment. *B. fragilis* was initially included in the *Bacteroides fragilis* group, an informal taxonomic group proposed in 1989, and was later designated as a unique species comprising multiple strains, including the immunotypical strains NCTC 9343 (ATCC 25285),

Abbreviations: B. fragilis, Bacteroides fragilis; NTBF, Nontoxigenic *Bacteroides fragilis*; ETBF, Enterotoxigenic *Bacteroides fragilis*; *bft*, *Bacteroides fragilis* toxin genes; BFT, *Bacteroides fragilis* toxin; PSA, Polysaccharide A; PSB, Polysaccharide B; OMVs, Outer Membrane Vesicles; CCF, Commensal Colonization Factors; IgA, Immunoglobulin A; TLR2, Toll-like Receptor 2; T6SSs, Type VI Secretion System; BSAP-1, Bacteroidales Secreted Antimicrobial Protein-1; BfUbb, *Bacteroides fragilis* Eukaryotic-like Ubiquitin Protein; GA, Genetic Architectures; TH1, T Helper 1; TH2, T Helper 2; SCFAs, Short-chain Fatty Acids; APCs, Antigen-presenting Cells; MHC, Major Histocompatibility Complex; IL, Interleukin; Tregs, T Regulatory Cells; DNBS, 2,4-Dinitrobenzene Sulfonic Acid; IBD, Intestinal Bowel Diseases; AAD, Antibiotic-associated Diarrhea; EAE, Experimental Autoimmune Encephalomyelitis; MIA, Maternal Immune Activation; ASD, Autism Spectrum Disorder; MTX, Methotrexate; FAP, Familial Adenomatous Polyposis; BF-LPS, *Bacteroides fragilis* Lipopolysaccharide; AD, Alzheimer's Disease; GI, Gastrointestinal; HGT, Horizontal Gene Transfer; RND, Resistance-Nodulation-Division; MATE, Multidrug and Toxic Efflux

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multidrug-resistant strain HMW615 and sequenced clinical strain YCH46 or 638R (Song, Liu, & Finegold, 2015; Husain et al., 2017; Lee & Mazmanian,2010). The first discovered *B. fragilis* strain was isolated as a pathogen from infected patients and initially named *Bacillus fragilis* (Wexler & Goodman, 2017). Subsequent studies revealed that this species is frequently detected in patients with various inflammatory conditions, such as infections of the abdomen, skin and soft tissue, bone and joint, female reproductive tract, central nervous system and lower respiratory tract infection, as well as inflammatory bowel disease, endocarditis, bacteremia and septicemia (Alexiou et al., 2017; Brook & Itzhak, 2004; Chen et al., 2018; Goldstein, Citron, Tyrrell, Leoncio, & Merriam, 2017; Huang, Ma, & Gong, 1995; Kierzkowska et al., 2017; Ou, Lan, Lin, Tsai, & ChangChien, 2010; Singh, Goyal, Padhi, & Aoun, 2013; Zhao, Jaber, & Lukiw, 2017). However, *B. fragilis* also commonly colonizes mucosal surfaces in the lower gastrointestinal tracts of various mammals, including humans, neonatal lambs, beef calves, infant rabbits and piglets (Bjerke et al., 2011; Border, Firehammer, Shoop, & Myers, 1985; Collins, Bergeland, Myers, & Shoop, 1989; Myers, Shoop, Collins, & Bradbury, 1989). This bacterial species has been reported to metabolize both diet- and host-derived polysaccharides as sources of carbon and energy and to be tolerant of oxygen exposure (Spence, Wells, & Smith, 2006).

Previous publications suggest that *B. fragilis* strains can be classified into two subtypes: nontoxigenic *B. fragilis* (NTBF) strains that do not harbor or secrete *B. fragilis* toxin (BFT), and enterotoxigenic *B. fragilis* (ETBF) strains that harbor *bft* genes encoding *B. fragilis* toxin in their pathogenicity islands (BfPAI) (Table 1) (Franco et al., 1999; Nikitina et al., 2015). ETBF strains are pathogenic and may induce energy metabolism dysfunction and intestinal and extra-intestinal disorders. In contrast, NTBF strains are commonly considered to be beneficial commensal residents that may antagonize ETBF via interspecific competition. These beneficial strains promote gut health by releasing certain advantageous molecules, one of which have been clearly identified as polysaccharide A (PSA) (Wagner et al., 2016). However, it remains unclear how *B. fragilis* delivers these effector molecules to host cells. One possible means of delivery is secretion via outer membrane vesicles (OMVs), which are enriched with outer membrane components such as active proteins, phospholipids and polysaccharides (Mashburn-Warren, McLean, & Whiteley, 2008). Notably, the OMVs secreted by ETBF and NTBF strains were found to exhibit distinctively different levels of metabolic activity (Zakharzhevskaya et al., 2017; Zakharzhevskaya et al., 2017).

2. Intestinal colonization and inter-bacterial competition of *B. fragilis* strains

B. fragilis comprises approximately 1–2% of cultured fecal bacteria (Sears, Geis, & Housseau, 2014). The abundance of this species in the host is enriched during birth, and weak mucosal selectivity allows multiple *B. fragilis* strains to coexist within an infant host (Bjerke et al., 2011). Interestingly, the persistence of *B. fragilis* increases between 4 months and 1–2 years of age, which corresponds with the development of the adaptive immune system (Rudi, Storrø, Oien, & Johnsen, 2012). Following maturation of the human gut microbiota, specific strains of *B. fragilis* are more likely to colonize the intestine. Studies have shown that members of Phylum Bacteroidetes comprise the most stable bacterial component within the host intestine over time (i.e., decades) and that a single strain typically dominates the species (Faith et al., 2013; Nagpal et al., 2017; Truong, Tett, Pasolli, Huttenhower, & Segata, 2017).

Various factors, including diet, physical condition, drug intake and lifestyle habits, have been reported to affect the abundance of *B. fragilis* in the gut. Of these, diet has the greatest influence on the gut abundance of this organism. Studies of dietary interventions in a Mongolian population revealed that a switch from the traditional diet, which contains high levels of protein and fat, to a carbohydrate-rich diet led to significant changes in the abundance of *B. fragilis* (Li et al., 2016). In Thailand, an analysis of the intestinal microbial communities of 60 healthy children from two regions with different dietary preferences found that the abundance of *B. fragilis* was higher in children from the region where meat and carbohydrates comprised greater proportions of the diet (La-Ongkham, Nakphaichit, Leelavatcharamas, Keawsompong, & Nitisinprasert, 2015). Moreover, a strict vegetarian diet was shown to increase the abundance of commensal microbes, such as *B. fragilis*, in six obese subjects with type 2 diabetes and/or hypertension (Kim, Hwang, Park, & Bae, 2013). Another study of 913 young infants (age: 1 month) fed with maternal vitamin D supplementation and direct supplementation of the infant both revealed a positive correlation of vitamin D with the abundance of *B. fragilis* (Talsness et al., 2017). Previous studies showed that the daily intake of *Lactobacillus casei* Shirota-containing beverage for 6 months could significantly decrease the level of *B. fragilis* in obese children (Nagata, Chiba, Wang, & Yamashiro, 2017). The intake of heat-killed *L. kunkeei* YB38 at doses of $\geq$10 mg/day for two weeks also significantly reduced the level of *B. fragilis* in 29 female subjects (Asama et al., 2016). Additionally, compared with healthy people, the abundance of *B. fragilis* in the gut bacterial microbiome usually increases in patients with diseases such as fungal keratitis, acute appendicitis, inflammatory bowel disease, familial adenomatous polyposis and colorectal cancer (Child et al., 2018; Dejea et al., 2018; Kalyana Chakravarthy et al., 2018; Walters, 2014). Besides, the changes of *B. fragilis* are different for patients with different age groups in the same disease, the abundance of *B. fragilis* increases in pediatric patients with spondyloarthritis (SpA), the reverse phenomenon is observed in adult patients (Stoll et al., 2018). A study based on the populations of healthy Indonesians demonstrated that *B. fragilis* were significantly higher in younger compared to elderly individuals (Rahayu et al., 2018). Analyses of stool samples collected from patients with type 2 diabetes after short-term metformin treatment revealed reductions in the abundance of *B. fragilis* (Sun et al., 2018). However, anti-tuberculosis therapy caused a significant increase in the abundance of *B. fragilis* (Hu et al., 2018). The lifestyle and physiological status of the host also affects the intestinal abundance of *B. fragilis*. For example, a lack of exercise may lead to the significant enrichment of *B. fragilis* and other

Table 1
Bacteroides fragilis strains reported in the literature.

Strain [reference no.]	Type	Isolation	Strain type	Reference
B. fragilis YCH46	NTBF	Bacteremia, Japan (Kuwahara et al., 2002)	No	Nikitina et al., 2015
B. fragilis NCTC 9343/ATCC 25285	NTBF	Appendiceal abscess	Yes	Nikitina et al., 2015
B. fragilis 638R	NTBF	Clinical isolate	No	Nikitina et al., 2015
B. fragilis ZY-312	NTBF	Feces from a healthy infant	No	Wang et al., 2017
B. fragilis BOB25	ETBF	Stool sample from a patient with dysbiosis	No	Nikitina et al., 2015
B. fragilis 86-5443-2-2	ETBF	Pig intestine	No	Thiele Orberg et al., 2017
B. fragilis ATCC 43858	ETBF	Infant with diarrhea, Arizona, USA	No	Hecht et al., 2017
B. fragilis ATCC 43859	ETBF	Infant with diarrhea, Montana, USA	No	Hecht et al., 2017
B. fragilis VPI13784	ETBF	Lamb intestine	No	Wu, Lim, Huang, Saidi, & Sears, 1998
B. fragilis Korea 570	ETBF	Clinical isolate, Seoul, Korea	No	Chung et al., 1999

Data were obtained from the literature and the American Type Culture Collection website.

Bacteroides species (Sket et al., 2017). Previous studies showed that *B. fragilis* had a positive association with obesity, the abundance of *B. fragilis* was higher in obese children when compared with the lean ones (Ignacio et al., 2015).

In the intestine, *Bacteroides* species possess a class of polysaccharide utilization loci that are critical for rapid adaptation to the intestinal environment, bacterial-host interactions and, to a certain degree, cellular protection. In the obligate anaerobe *B. fragilis*, the four-gene operon *osuABCD* is associated with oxygen-induced starch utilization and survival under aerobic conditions (Spence et al., 2006). Commensal colonization factors (ccfABCDE) also comprise a distinctive form of the polysaccharide utilization locus (Wexler & Goodman, 2017). In *B. fragilis*, this CCF system has been reported to mediate gut colonization by regulating capsule expression, as mutant *B. fragilis* lacking *ccf* genes are unable to colonize (Donaldson et al., 2018; Lee et al., 2013). *B. fragilis* can produce at least eight distinct capsular polysaccharides (Krinos et al., 2001). The extensive and variable expression of combinations of surface polysaccharides may provide an essential foundation for the colonization and function of *B. fragilis* in the human colon. Studies have shown that *B. fragilis* mutants lacking surface polysaccharide expression cannot easily colonize the intestine. However, these mutant strains use an alternative pathway to reestablish the expression of multiple capsular polysaccharides and achieve stable commensalism (Liu, Lee, Vanlare, Kasper, & Mazmanian, 2008). In contrast to classical views, which suggest that the immune system has evolved to prevent microbial colonization, recent studies have revealed that immunoglobulin A (IgA), which is secreted by the host in the gut, can bind mucus and enhance the mucosal colonization of *B. fragilis*. To occupy this defined mucosal niche, the bacterial CCF system regulates the expression of capsular polysaccharides on *B. fragilis* to attract IgA binding and thus mediate stable colonization (Donaldson et al., 2018). Additionally, *B. fragilis* requires both PSA and the Toll-like receptor 2 (TLR2) pathway to colonize a specific niche in mice (Round et al., 2011).

The intestinal microbial competition of *B. fragilis* is supported by two pervasive ecological drivers: non-contact-dependent secretory antimicrobial proteins and the contact-dependent Type VI secretion system (T6SSs). The first known *Bacteroidales* secreted antimicrobial protein-1 (BSAP-1) is released in *B. fragilis* OMVs, which contain membrane attack/perforin (MACPF) domains that are used to lyse other bacterial cells or infect host cells via pore formation (Chatzidaki-Livanis et al., 2014). BSAP-1 is an important competitive factor that affects the composition of the human intestinal microflora at the strain level. Competitive-colonization experiments in mice confirmed that a BSAP-1-producing *B. fragilis* strain could antagonize and outcompete a BSAP-1-sensitive *B. fragilis* strain. Furthermore, metagenomic analyses revealed that BSAP-1-sensitive and -producing strains did not coexist in the human gut (Roelofs, Coyne, Gentyala, Chatzidaki-Livanis, & Comstock, 2016). Eukaryotic-like ubiquitin protein (BfUbb), which is also produced by *B. fragilis*, was recently confirmed as a novel secreted antimicrobial protein that can increase strain competitiveness during intraspecies antagonism (Chatzidaki-Livanis et al., 2017). Contact-dependent T6SSs are another class of interference competition factor detected widely in *Bacteroides* strains found in humans. T6SSs comprise multiprotein complexes and are used by *Bacteroides* species as a delivery system for toxin effectors that mediate interbacterial antagonism pathways. This system is homologous to the contractile tails of T4 bacteriophages, which can inject toxic effector proteins directly into adjacent microbes (Sana, Lugo, & Monack, 2017). Numerous *Bacteroides* strains in the human intestinal system use different T6SS loci, including a large percentage of intestinal *B. fragilis* strains that harbor T6SS loci (Coyne, Roelofs, & Comstock, 2016). The T6SS locus can segregate into three different genetic architectures (GA): GA1, GA2 and GA3. In *B. fragilis*, GA3 plays a significant role in inter-strain competition and may facilitate competition for dominance during the early life of the host. Notably, previous studies revealed GA3 enrichment among strains in infant microbiomes, whereas *B. fragilis* strains lacking GA3 T6SS were more commonly observed in adults (Verster et al., 2017). Another study of GA3 T6SS revealed that *B. fragilis* strains equipped with this locus could antagonize most other *Bacteroides* strains in the human gut (Chatzidaki-Livanis, Geva-Zatorsky, & Comstock, 2016).

In summary, *B. fragilis* can achieve preliminary colonization of the intestinal mucus via polysaccharide utilization loci and capsular polysaccharide expression. Furthermore, T6SSs and at least two antimicrobial proteins produced by *B. fragilis* are indispensable for the predominant and persistent occupancy of an intestinal niche and the elimination of competitors. However, *B. fragilis* colonization mainly relies on a tolerant host immune system that allows colonization of the gut, and certain host immune molecules (e.g., IgA) have even been shown to stabilize bacterial colonization. The age, diet, physical condition, drug intake and lifestyle of the host all affect the pattern of intestinal colonization. An understanding of the mechanisms of colonization and identification of competitive interactions involving *B. fragilis* may be an important prerequisite to the suitable modification of strains that would enable successful colonization and healthy function in a host (Chatzidaki-Livanis et al., 2017).

3. Immunomodulatory effects of nontoxigenic *B. fragilis* strains on host diseases

To date, commensal NTBF strains have been shown to inhibit inflammation in different organs, including the peritoneum, intestinal tract, brain and lung. These strains can also inhibit infection by pathogenic bacteria and support cancer therapy (Table 2). PSA has been identified as the main functional molecule produced by *B. fragilis* NCTC 9343 (Tan, Zhai, & Chen, 2019). This polysaccharide features a zwitterionic structure composed of repeating oligosaccharide units comprising constituent sugars with free amino and carboxyl groups and is crucial for the favorable biological activity of a strain (Tzianabos et al., 2000). Furthermore, the zwitterionic PSA produced by *B. fragilis* has been identified as an immunomodulatory molecule that plays a role in immune system maturation. T helper 1 (T_H1) and T_H2 cells are subtypes of the effector CD4+ T cell population. In a previous study, either *B. fragilis* NCTC 9343 or PSA could correct a T_H1/T_H2 cell imbalance in germ-free mice and enhance regulatory T cells (Tregs) function (Mazmanian, Liu, Tzianabos, & Kasper, 2005). Similar to BSAP-1, PSA is delivered via OMVs secreted from the surfaces of *B. fragilis* cells. Recent studies have revealed the immunomodulatory mechanisms used by *B. fragilis* NCTC 9343 and the related PSA and OMVs in the contexts of different diseases. A newly discovered strain, *B. fragilis* ZY-312, has also been confirmed to exert beneficial immunomodulatory effects in the host.

In addition, short-chain fatty acids (SCFAs) derived from gut microbiota involved carbohydrate fermentation (including *B. fragilis*), such as primarily acetate, propionate, and butyrate, have beneficial functions include providing energy for the colonic mucosa and maintain colonic homeostasis. (D'Argenio & Mazzacca, 1999). For instance, oral administration of *B. fragilis* could significantly increase the SCFAs concentration in the intestinal contents of *salmonella*-infected rats, which may further reduce inflammation and restore the integrity of gut barrier (Bukina et al., 2018).

3.1. Abscess

The immunomodulatory properties of the *B. fragilis* capsular polysaccharide have been elucidated during two decades of research (Tzianabos, Onderdonk, Rosner, Cisneros, & Kasper, 1993). Studies have demonstrated that subcutaneous administration of the *B. fragilis* NCTC 9343 (ATCC 25285) and ATCC 23745 capsular complex, which comprises PSA and polysaccharide B (PSB), can protect against the formation of intra-abdominal abscesses after an intraperitoneal injection of the *B. fragilis* capsular complex. Although this protective activity might not meet traditional criteria for antigen specificity, it may

Table 2
Reported beneficial immunomodulatory effects of *Bacteroides fragilis*.

Reference (author, year [reference no.])	Disease target	Strain/functional substance	In-vivo model	Significant findings
Tzianabos, Kasper, Cisneros, Smith, & Onderdonk, 1995	Intra-abdominal abscess	*B. fragilis* NCTC 9343 and ATCC 23745/PSA and PSB	Rat model of intra-abdominal sepsis induced by intraperitoneal injection of *B. fragilis* capsular polysaccharide complex	Subcutaneous administration of *B. fragilis* PSA and PSB protected against intra-abdominal abscesses
Tzianabos et al., 1999.	Intra-abdominal abscess	*B. fragilis* NCTC 9343/PSA	Rat model of experimental sepsis induced by *B. fragilis*	*B. fragilis* PSA induced an immunomodulatory and protective response against abscess formation that depended on CD4+ T cells and IL-2
Mazmanian et al., 2008	IBD/*Helicobacter hepaticus* infection	*B. fragilis* NCTC 9343/PSA	Mouse model of experimental colitis induced by *Helicobacter hepaticus*	PSA protected mice from colitis in a manner dependent a functional IL-10-producing CD4+ T cells
Round & Mazmanian, 2010	IBD	*B. fragilis* NCTC 9343/PSA	Mouse model of experimental colitis induced by 2,4,6-trinitrobenzene sulfonic acid	*B. fragilis* PSA mediated the conversion of CD4+ T cells to T regulatory cells and induced the production of IL-10 via Toll-like receptor 2 signaling to prevent and cure experimental colitis
Ochoa-Reparaz et al., 2010	MS	*B. fragilis* NCTC 9343/PSA	Mouse model of EAE induced by oral antibiotic treatment	*B. fragilis* PSA protected mice from central nervous system demyelination and may thus protect against MS in humans
Shen et al., 2012	IBD	*B. fragilis* NCTC 9343/PSA	Mouse model of experimental colitis induced by 2,4,6- trinitrobenzene sulfonic acid	Outer membrane vesicles delivered PSA to dendritic cells for TLR2 recognition, resulting in regulatory T cell activity and IL-10 production
Sommese et al., 2012	*Bartonella henselae* infection	*B. fragilis* NCTC 9343/PSA	Infection of mice by *Bartonella henselae*	*B. fragilis* reduced damage from *B. henselae* infection
Hsiao et al., 2013	ASD	*B. fragilis* NCTC 9343	Offspring of a mouse model of maternal immune activation	*B. fragilis* corrected gut defects and ASD-related behavioral abnormalities in offspring
Wang, Begum-Haque, et al., 2014; Wang, Telesford, et al., 2014	MS	*B. fragilis* NCTC 9343/PSA	Mouse model of active optimal EAE induced by injection of peptides and *Bordetella pertussis* toxin	*B. fragilis* PSA may prevent EAE via TLR2- mediated CD39 signaling; CD39 may be associated with the Th17/Tregs balance
Johnson et al., 2015	Asthma (Airway inflammation)	*B. fragilis* PSA	C57BL/6 mice treated with intranasal ovalbumin	*B. fragilis* PSA can protect against airway inflammation by enhancing CD4+T cell expansion and IL-10 production
Pagliuca et al., 2016	Cat-scratch disease (*Bartonella henselae* infection)	*B. fragilis* NCTC 9343	Experimental mice administered *Bartonella henselae* on days 1–7	Colonization with *B. fragilis* reduced inflammatory liver damage induced by *B. henselae*
Chang et al., 2017	IBD	*B. fragilis* NCTC 9343	Mouse model of experimental colitis induced by dextran sulfate sodium	*B. fragilis* prevented colitis, possibly via the TLR2/IL-10 signaling pathway
Li et al., 2017	*Vibrio parahaemolyticus* Infection	*B. fragilis* ZY-312	Experimental mice treated with *lux*-expressing *V. parahaemolyticus*	*B. fragilis* secreted substances that prevented *V. parahaemolyticus* infection
Zhang et al., 2018	AAD	*B. fragilis* ZY-312	Rat model of AAD induced by an antibiotic cocktail	*B. fragilis* strain ZY-312 ameliorated AAD-related diarrhea and restored intestinal barrier function and enterocyte regeneration
Johnson et al., 2018	Pulmonary inflammation	*B. fragilis* PSA	C57BL/6 mice treated with intranasal ovalbumin	PSA protected against pulmonary inflammation in a mechanism dependent on T cell-T cell cooperative and IL-10
Zhou et al., 2018	Adverse drug reaction to methotrexate	*B. fragilis* ATCC 25285	BALB/c mice injected with methotrexate	*B. fragilis* alleviated methotrexate-induced intestinal mucositis
Sittipo et al., 2018	CRC	*B. fragilis* NCTC 9343/PSA	Human colon carcinoma cell lines SW620 and HT29	PSA protected against CRC by inhibiting cell proliferation and impairing cell migration and invasion

PSA, polysaccharide A; PSB, polysaccharide B; IBD, inflammatory bowel disease; IL, interleukin; MS, multiple sclerosis; EAE, experimental autoimmune encephalitis; ASD, autism spectrum disorder; AAD, antibiotic-associated diarrhea; CRC, colorectal cancer

specifically target a motif of oppositely charged groups on polysaccharides. Thereby, the observed protection would be T cell-dependent (Onderdonk, Iii, Tzianabos, & Kapser, 1999). Subsequent studies have also shown that the zwitterionic polysaccharides produced by *B. fragilis* mediate $CD4^+$ T cell responses. The observed responses required the internalization of PSA by antigen-presenting cells (APCs), which then presented PSA antigens on major histocompatibility complex (MHC) class II molecules (Tzianabos et al., 2000). $CD4^+$ T cells further confer protection against abscesses induced by pathogenic bacteria by producing cytokines such as interleukin (IL)-2, interferon-γ and IL-10. Particularly, IL-2 was identified as an essential inhibitor of abscess formation (Tzianabos et al., 1999). As mentioned below, other studies have reported associations of *B. fragilis* PSA-associated diseases with concrete immune mechanisms, including the archetypical example of the immunoregulatory mechanism of *B. fragilis* NCTC 9343 PSA.

3.2. Colitis

Studies of mice indicate that PSA released by *B. fragilis* NCTC 9343 has significant effects on the treatment of experimental colitis and may protect against weight loss and inflammation. As a useful anti-inflammatory molecule, PSA was shown to mediate a healthy immune response and prevent *Helicobacter hepaticus*-induced colitis in mice by expanding the population of IL-10-producing $CD4^+CD45Rb^{low}$ T cells and suppressing the production of pro-inflammatory IL-17 (Mazmanian, Round, & Kasper, 2008). The inflammation inhibition depending on IL-10 production of this immune response requires both TLR2 expression on $CD4^+$T cells and TLR2 signaling (Round et al., 2011). Similarly, in germ-free mice, *B. fragilis* mono-colonization requires TLR2 signaling to induce the development of $CD4^+Foxp3^+$ T regulatory cells and enhance the inflammation suppressive capacity by IL-10 production (Chang et al., 2017). Remarkably, PSA not only prevents but also cures colitis in animal models (Round & Mazmanian, 2010).

PSA packaged in OMVs also ameliorates animal colitis, similar to the effects of orally administered purified PSA (Shen et al., 2012). Moreover, a recent study demonstrated an interaction mechanism wherein *B. fragilis* OMVs could activate a non-canonical host autophagy pathway and thus protect against 2,4-dinitrobenzene sulfonic acid (DNBS)-induced colitis. This mechanism simultaneously required the expression of the host IBD-associated genes *ATG16L1* and *NOD2* (Chu et al., 2016).

ZY-312, a novel non-enterotoxigenic *B. fragilis* strain isolated from the feces of a healthy infant, was recently confirmed as a potential next-generation probiotic candidate from phylum Bacteroidetes. In addition to its effects on adaptive immunity, studies reported that *B. fragilis* is also associated with the innate immune system. *B. fragilis* ZY-312 was shown to enhance phagocytic activity in macrophage and induce polarization to the M1 phenotype (Deng et al., 2016; Wang et al., 2017). Further studies revealed that *B. fragilis* ZY-312 could alleviate diarrhea and increase the microbial abundance in a rat model of antibiotic-associated diarrhea (AAD). All of these effects promoted intestinal barrier restoration and enterocyte regeneration, and these mechanisms may explain the strategy underlying AAD therapy (Zhang et al., 2018).

The SCFAs produced by *B. fragilis* can also increase the number of colonic Tregs and protect against colitis (Smith et al., 2013). An in vitro study also showed that the SCFAs derived from *B. fragilis* can inhibit the sporulation of *Clostridium perfringens* which might cause Diarrheas in patients (Wrigley, 2004).

3.3. Central nervous system disease

B. fragilis has been reported to have indirect effects on the host, which are attributed to its unique immunoregulatory functions. PSA secreted by *B. fragilis* is a potent regulator and inhibitor of central nervous system demyelinating disease. In one study of a mouse model of experimental autoimmune encephalomyelitis (EAE), purified *B. fragilis* PSA was shown to serve as a prophylaxis and treatment against changes in gut bacteria and development of disease in response to oral antibiotic treatment (Ochoa-Reparaz et al., 2010). This protective mechanism was found to depend on IL-10 production (Ochoa-Reparaz et al., 2010). Further investigation revealed that *B. fragilis* PSA may prevent EAE via TLR2-mediated CD39 signaling and that the expression of CD39 on $CD4^+$ cells may be associated with a balance between IL-17-secreting Th17 and IL-10-secreting Tregs (Wang, Begum-Haque, et al., 2014; Wang, Telesford, et al., 2014).

The above-mentioned finding indicates complex interactions among the gut mucosal tissue, brain and spinal cord. Meanwhile, a gut-microbiome-brain connection has also been discovered in the offspring of mice with maternal immune activation (MIA) induced by the intraperitoneal injection of 20 mg/kg poly(I:C) during pregnancy. These offspring exhibit characteristics of autism spectrum disorder (ASD) with social impairments and gastrointestinal barrier defects (Smith, Li, Garbett, Mirnics, & Patterson, 2007). However, the oral administration of *B. fragilis* ameliorated both neurodevelopmental disorders and gastrointestinal abnormalities in these deficient offspring (Hsiao et al., 2013).

3.4. Airway inflammation

Recent studies have shown that *B. fragilis* PSA can protect against airway inflammation and experimental asthma by inducing the expansion of $CD4^+$T cells and production of IL-10. Accordingly, PSA may be useful for the treatment of a wide range of manifestations of human asthma (Johnson, Jones, & Cobb, 2015). Consistent with the systemic nature of immunity, studies have confirmed that PSA-activated effector/memory T cells can cooperate with $FoxP3^+$ Tregs in lung tissue to prevent pulmonary inflammation. First, the PSA antigen is internalized and presented on MHC class II molecules expressed on the APC surface, where it promotes the activation of $CD4^+$T cells in the intestinal tract. Next, communication between effector T cells and resident $FoxP3^+$ Tregs in the lung amplifies the suppressive capacity of Tr1 cells, eventually leading to the release of the anti-inflammatory cytokine IL-10 and prevention of pulmonary inflammation (Johnson, Jones, & Cobb, 2018). As mentioned above, capsular polysaccharide from *B. fragilis* inhibits airway inflammation via a mechanism dependent on $CD4^+$ T cell activation and subsequent T cell-driven IL-10 production (Fig. 1).

3.5. Bacterial infection

B. fragilis NCTC 9343 has been reported to protect against various pathogens, including *Helicobacter hepaticus* and *Bartonella henselae* (Pagliuca et al., 2016; Sommese et al., 2012). A recent study found that certain substances secreted by the novel *B. fragilis* strain ZY-312 could prevent *Vibrio parahaemolyticus* infection (Li et al., 2017). Another study demonstrated that depending on the T6SS competition mechanism, symbiotic NTBFs could restrict the acquisition of pathogenic ETBFs and prevent colitis (Hecht et al., 2016). As mentioned above, BSAP-1 is an important competitive factor that affects the strain-level composition of microbiota. Therefore, we speculate that *B. fragilis* uses both known competition mechanisms during interspecies competition and may also compete with pathogens.

3.6. Cancer

B. fragilis NCTC 9343 also effectively promotes anti-cancer immunosurveillance (Routy et al., 2018). Oral treatment with *B. fragilis* or *B. fragilis* polysaccharides may reverse non-responsiveness to CTLA-4 blockade anticancer therapy (Vetizou et al., 2015). However, the multiple adverse effects, particularly the pro-inflammatory effects, are not well-tolerated by many patients. Fortunately, a recent mouse study found that *B. fragilis* alleviated the adverse effects induced by

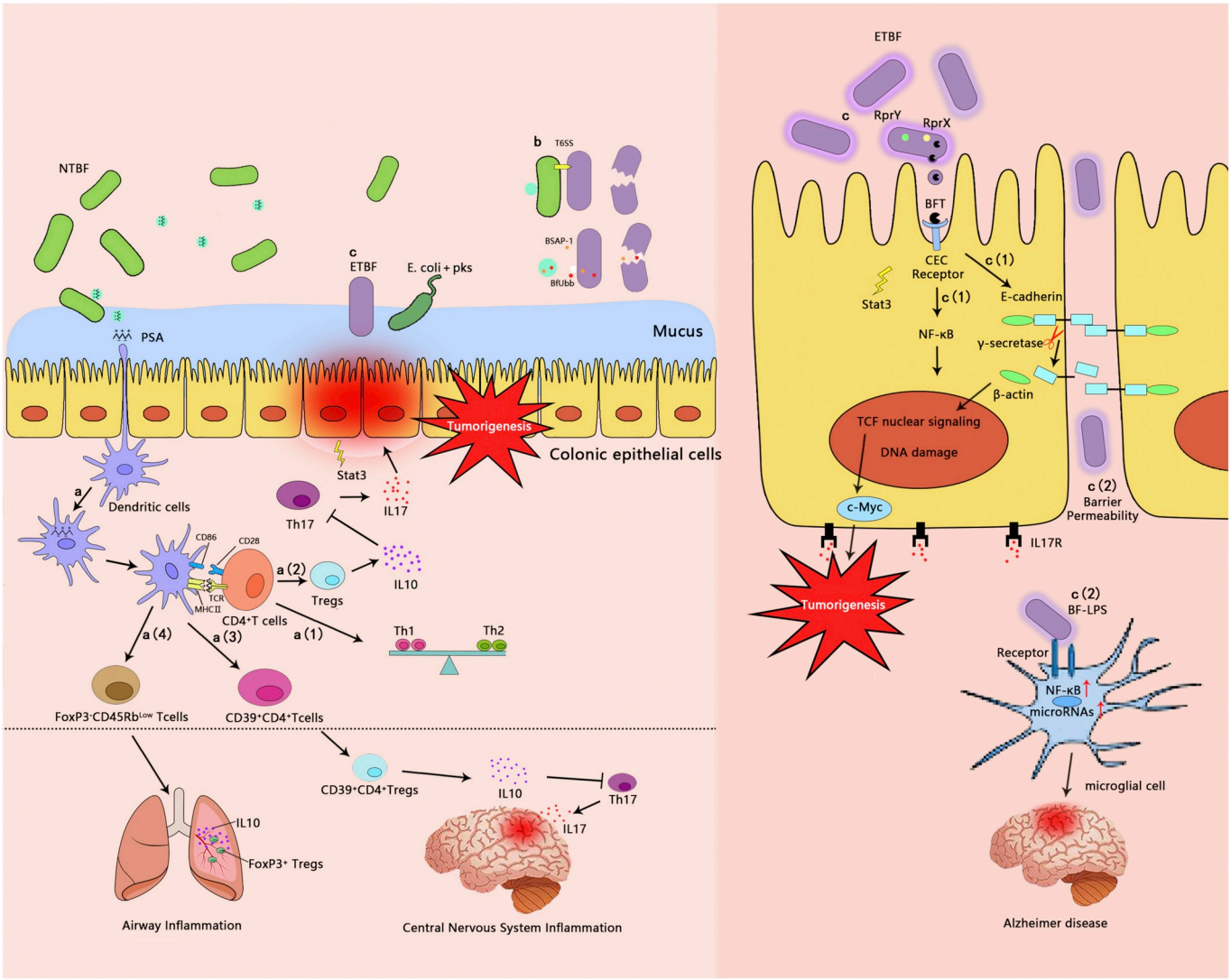

Fig. 1. Potential immune mechanisms underlying the interaction between *Bacteroides fragilis* and the human intestine.

a. Immune regulation: Polysaccharide A (PSA), which is delivered via outer membrane vesicles (OMVs), was confirmed as the major beneficial molecule produced by nontoxigenic B. fragilis (NTBF). PSA is recognized, internalized and presented on the surfaces of antigen-presenting cells (e.g., dendritic cells) in the context of major histocompatibility complex class II molecules to induce CD4 + T cell activation. CD86 promotes the generation of IL10-producing T cells. (1) In germ-free mice, PSA corrects the T helper 1 (TH1)/TH2 cell imbalance and thus improves the development of the immune system. (2) In inflammatory bowel disease (IBD), the subsequent production of the anti-inflammatory cytokine IL-10 by Tregs can inhibit an inflammatory response by IL17-producing TH17 cells, thus ameliorating IBD. (3) In central nervous system inflammation, PSA activates CD39 signaling on CD4 + T cells and Tregs to suppress the Th17 inflammatory response. (4) In airway inflammation, PSA activates intestinal effector Foxp3^{-}CD45Rb^{Low} T cells, which then communicate with resident FoxP3^{+}Tregs in the lungs to prevent pulmonary inflammation.

b. Interbacterial competition: *B. fragilis* uses two weapons of interbacterial competition: contact-independent secreted antimicrobial proteins, including BSAP-1 and BfUbb, which can lyse bacteria via pore formation and contact-dependent Type VI secretion systems (T6SSs), which are homologous to the contractile tails of T4 bacteriophages. These T6SSs inject toxic effector proteins directly into adjacent microbes. NTBF has been shown use these competitive mechanisms to inhibit enterotoxigenic *B. fragilis* (ETBF) and other bacterial pathogens.

c. Pathogenic mechanism: ETBF may asymptomatically colonize the normal colon. However, these organisms can cause disease following disruption of the ETBF toxin regulator system, RprXY, or an intestinal leak. The interaction between ETBF and an *Escherichia coli* strain carrying the genotoxic pks gene island can allow the former to facilitate recolonization of the latter in the mucosal tissue. This interaction promotes IL-17 induction, which eventually leads to tumorigenesis. (1) *B. fragilis* enterotoxin (BFT), which is produced by ETBF, can bind a putative colonic epithelial (CEC) receptor to stimulate γ-secretase-regulated β-actin-T-cell-factor nuclear signaling and E-cadherin cleavage. These processes promote the expression of the proto-oncoprotein c-Myc. Upon recognition by the CEC receptor, BFT also activates the IL-17 receptor and NF-κB signaling pathways to triggers sustained Stat3 activation in epithelial and immune cells and mucosal Th17 immune responses. Both pathways may lead to tumorigenesis. (2) Barrier permeability may allow ETBF to enter the bloodstream and pass through the blood-brain barrier. *B. fragilis* lipopolysaccharide (BF-LPS) may be recognized by TLR2, TLR4 and/or CD14 on microglial cells, which elicits expression of the inflammatory transcription factor NF-kB complex and pro-inflammatory microRNAs. These factors may contribute to the development of Alzheimer's disease (AD).

Table 3
Studies on the pathogenicity of ETBF

Reference (author, Year [reference no.])	Disease	Strain/virulence factors	Mechanism of action
Wu et al., 1998		ETBF VPI13784/(BFT-1) or 86-5443-2-2/(BFT-2)	BFT cleaved E-cadherin in an ATP-independent manner
Wu, Morin, Maouyo, & Sears, 2003		ETBF strain 86-5443-2-2/(BFT-2)	BFT triggered β-actin nuclear signaling in intestinal epithelial cells
Wu et al., 2006	Colitis	High-BFT-expressing strain I-1345/BFT mutant I-1345	BFT binds to an unidentified receptor distinct from E-cadherin on intestinal epithelial cells and triggers colitis
Wu et al., 2007		*B. fragilis* recombinant strain I1345/BFT	BFT stimulated β-actin-T-cell-factor nuclear signaling regulated by γ-secretase and induced E-cadherin cleavage
Rhee et al., 2009	Colitis	*B. fragilis* VPI 13784 (BFT1)/*B. fragilis* 86-5443-2-2(BFT2)/*B. fragilis* Korea 570(BFT3)	ETBF induced colitis in C57BL/6 mice; BFT was sufficient to induce colitis in both germ-free and conventional mice
Wu et al., 2009	Colon cancer	ETBF strain 86-54432-2/(BFT-2)	ETBF but not NTBF colonization triggered colon tumorigenesis via the Stat3 and Th17 signaling pathways
Wick et al., 2014	Colitis	ETBF strain 86-5443-2-2	ETBF colonization induced severe colitis in mice with notable Stat3 activation and a Th17 immune response
Lukiw, 2016	Alzheimer's disease	BF-LPS	BF-LPS is recognized by TLR2, TLR4 and/or CD14 microglial cell receptors, leading to pro-inflammatory neurodegeneration associated with Alzheimer's Disease
Thiele Orberg et al., 2017	Colon cancer	ETBF/BFT	ETBF colonization and the IL-17 response might be characteristics of colon tumorigenesis
Chung et al., 2018	Human colorectal cancer	ETBF strain 086-5443-2-2	ETBF requires IL-17 receptor, NF-κB and Stat3 signaling to trigger tumorigenesis in the distal colon
Zhao & Lukiw, 2018a, 2018b	Alzheimer's disease	BF-LPS	*B. fragilis* contributes to progressive proinflammatory neurodegeneration via NF-κB activation and microRNA-146a induction
Kwong et al., 2018	Colon cancer/ bacteremia	*B. fragilis*	Patients with *B. fragilis* bacteremia have an increased risk of colorectal cancer
Zhao & Lukiw, 2018a, 2018b	Alzheimer's disease	BF-LPS	Gastrointestinal tract microbiome-derived neurotoxins (e.g., BF-LPS) alter the expression of NF-kB-miRNA-directed genes and thus drive the development of Alzheimer's disease
Dejea et al., 2018	Familial adenomatous polyposis	ETBF strain 086-54443-2-2 and pks+ *E.coli* murine NC101	The mucosa of patients with familial adenomatous polyposis patients was highly enriched with genes encoding colibactin (*clbB*) and BFT (*bft*), which induced IL-17 production and DNA damage in murine colon epithelial cells

ETBF, enterotoxigenic *Bacteroides fragilis*; BTF, *B. fragilis* enterotoxin; BF-LPS, *B. fragilis* lipopolysaccharides; NTBF, nontoxigenic *B. fragilis*

methotrexate (MTX), a widely used anticancer immunosuppressant (Zhou et al., 2018). Another recent study showed that PSA induced the production of IL-8 in vitro, which might inhibit the proliferation of colorectal cancer cells and restrict the epithelial-mesenchymal transition (Sittipo et al., 2018). In vivo, *B. fragilis* could prevent the development of colon cancer. A mouse model of AOM/DSS-induced colitis-associated colon cancer exhibited decreased tumorigenesis after *B. fragilis* administration (Lee et al., 2018).

4. Pathogenic mechanism of enterotoxigenic *B. fragilis*

ETBF strains have been implicated in various conditions involving intestinal and extra-intestinal infections, including inflammatory bowel disease (IBD), bacteremia, systemic inflammation and neurological disorders (Table 3). However, asymptomatic colonization with ETBF is also common in adults. In fact, both ETBF and NTBF can chronically colonize mice, although only the former can trigger a pro-carcinogenic multi-step inflammatory cascade (Chung et al., 2018; Rhee et al., 2009). In children, ETBF-associated diarrhea often occurs between the ages of 1 and 5 years. In adults, the incidence of ETBF-associated diarrhea may increase gradually with age. A study of 513 patients with gastroenteritis in Taipei revealed that ETBF infections often occurred in the elderly and during cold, dry winters (Ji et al., 2014). Moreover, ETBF-associated diarrhea differs by geographical location (Lopez et al., 2017; Sears, 2009).

4.1. Colitis and colorectal cancer

ETBFs can release complex toxins. One notable ETBF virulence factor is *B. fragilis* toxin (BFT), a zinc-dependent metalloprotease with three distinct molecular isoforms (BFT-1, -2 and -3) (Chung et al., 1999). A recent study demonstrated that BFT might be activated by fragipain (Sarvari et al., 2017). Additionally, BFT, which interacts hydrophobically and electrostatically with outer membrane components, may be secreted during vesicle formation (Zakharzhevskaya, Tsvetkov, et al., 2017; Zakharzhevskaya, Vanyushkina, et al., 2017). A temporary expansion of ETBF can disrupt the intestinal mucosa and the toxin regulator system RprXY, thus inducing inflammatory bowel disease and colorectal cancer (Hecht, Casterline, Choi, & Bubeck Wardenburg, 2017). ETBF is considered a risk factor for colorectal cancer because it increases the permeability of intestinal epithelial cells and promotes cell proliferation, which is associated with the occurrence of colitis and colorectal neoplasia (Boleij, Hechenbleikner, Goodwin, Badani, & Sears, 2014). Furthermore, a significant association has been detected between the proportion of ETBF and colorectal carcinogenesis (Kwong et al., 2018).

Studies have shown that oral inoculation with ETBF could initiate serious cleavage of the intercellular adhesion protein E-cadherin in the intestinal epithelium, leading to persistent and subclinical colitis in specific-pathogen free mice but rapidly lethal colitis in germ-free mice (Rhee et al., 2009). A further report revealed that BFT produced by ETBF might bind to a putative colonic epithelial receptor to stimulate β-actin-T-cell-factor nuclear signaling in a process regulated by γ-secretase. This binding would induce the cleavage of E-cadherin, thus promoting the expression of the proto-oncoprotein c-Myc and, ultimately, cell proliferation (Wu, Rhee, Zhang, Franco, & Sears, 2007). Moreover, after recognition by a colonic epithelial receptor, BFT was shown to activate the Wnt and NF-κB signaling pathways, leading to sustained Stat3 activation in immune cells and mucosal Th17 immune responses in a mouse model of ETBF-induced colitis (Wick et al., 2014; Wu et al., 2009).

B. fragilis toxin genes were found to be highly enriched in the colonic mucosa of patients with familial adenomatous polyposis (FAP). More than half of patients with FAP are co-colonized by *ETBF* and *E. coli* strains that carry the genotoxic *pks* gene island, which enables synergistic carcinogenic effects. ETBF may degrade the mucosa to facilitate the recolonization of mucosal tissue by *pks* +*E. coli*. Consequently,

the production of reactive oxygen species and IL-17 would increase DNA damage in colonic epithelial cells, leading to carcinogenesis (Dejea et al., 2018; Tomkovich & Jobin, 2018).

4.2. Alzheimer's disease

ETBF may enter the bloodstream consequent to intestinal dysbiosis and barrier dysfunction. Recent research has shown that the extremely pro-inflammatory *B. fragilis* lipopolysaccharide (BF-LPS), which is leaked into the blood via gastrointestinal tract breaches, is the major factor predisposing the host to systemic inflammation. BF-LPS may also pass through the blood-brain barrier and gradually promote the development of Alzheimer's disease (AD). Once recognized by TLR2, TLR4 and/or CD14 on microglial cells, BF-LPS can elicit expression of the NF-kB complex, an inflammatory transcription factor, as well as of pro-inflammatory microRNAs. These events suppress the expression of miRNA-bound mRNAs and trigger a receptor in myeloid/microglial cells, consistent with the observations of sporadic AD in the brain (Lukiw, 2016; Zhao & Lukiw, 2018a, 2018b). Hence, we suppose that neurotoxins associated with the human gastrointestinal (GI)-tract microbiome, such as BF-LPS, may be exceptionally potent drivers of pro-inflammatory degenerative neuropathology.

5. Antibiotic resistance mechanisms of *B. fragilis*

Traditional antibiotics, such as metronidazole, carbapenems and cefoxitin, are commonly and effectively used to treat *B. fragilis* infections (Brook, Wexler, & Goldstein, 2013). However, this bacterial species is remarkably adaptable to its surroundings and can easily develop antibiotic resistance, which is further encouraged by the improper use of antibiotics. Therefore, antibiotic resistance has become the primary hindrance to the treatment of *B. fragilis* infections. This resistance is largely attributed to the genetic plasticity of *B. fragilis*, which refers to inversions, duplications, horizontal gene transfer (HGT) and large-scale chromosomal transfer (Husain et al., 2017). According to the literature, *B. fragilis* carries four CRISPR-Cas systems. Of these, three involve adjacent *cas* genes that closely match Class 1 Type IB, Class 1 Type IIIB and Class 2 Type IIC. In contrast, the CRISPR-Cas system isolated from blood is an atypical Type IIIB system that lacks an adjacent *cas* gene. Furthermore, the CRISPR-Cas systems of *B. fragilis* may regulate endogenous genes associated with the conversion of symbiotes and pathogens (Tajkarimi & Wexler, 2017). Additionally, *B. fragilis* possesses multidrug efflux pump transporter systems that can export toxic antibacterial substrates to the outside environment and may be responsible for conferring resistance to a wide spectrum of antibiotics. Resistance-Nodulation-Division (RND)-type and multidrug and toxic efflux (MATE) are the two main types of efflux pumps expressed in *B. fragilis* Ghotaslou et al., 2018; Ghotaslou, Yekani, & Memar, 2018).

Various mechanisms have been proposed to explain metronidazole resistance in *B. fragilis*, including nitroimidazole reductase (which depends on *nim* genes), RND-type efflux pumps, RecA protein overexpression and ferrous iron transporter (FeoAB) deficiency (Ghotaslou, Bannazadeh Baghi, et al., 2018, Ghotaslou, Yekani, & Memar, 2018). Three different β-lactamases have been described in *B. fragilis*: CepA endogenous cephalosporinase, CfxA β-lactamase and CfiA metallo-β-lactamase (Litterio, Cejas, Gutkind, & Radice, 2017). The presence of an insertion sequence (IS) element upstream to *cfiA* is the main genetic determinant of resistance to carbapenem (Brook et al., 2013).

6. Future perspectives

The Food and Agriculture Organization of the United Nations and World Health Organization define probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2001). Currently, the commercially available probiotics are limited to a narrow range of organisms, including *Lactobacillus* spp., *Bifidobacterium* spp., *Saccharomyces* spp., *Bacillus* spp., *E. coli*, *Enterococci* and *Weissella* spp (Gibson et al., 2017; Hill et al., 2014). In recent years, the field of probiotics has yielded considerable information about the role of the human microbiota in health and disease. A newly proposed concept, next-generation probiotics, overlaps with the emerging concept of live biotherapeutic products in the medical field (O'Toole, Marchesi, & Hill, 2017). NTBF strains have been identified as candidate next-generation probiotics.

Strong proinflammatory T_H17 immune responses are known to induce inflammation in various diseases. We infer that NTBF may protect against inflammatory disease via microbe-host interactions that enhance the suppressive abilities of Tregs throughout the body (Shen et al., 2012). Collectively, *B. fragilis* coordinates the balance between IL-10-secreting Tregs and T_H17 responses, which ultimately protect against inflammatory diseases (Lee & Mazmanian, 2010). A very recent study revealed that the protective effects of NTBF may be independent of PSA, and some other studies suggested that SCFAs fermented from *B. fragilis* might exert beneficial effect (Bukina et al., 2018; Chan et al., 2018). In other words, the beneficial mechanisms associated with the *B. fragilis*–host interaction are highly complex and require further exploration. Other views suggest that *B. fragilis* may control pathogenesis by readjusting the composition of the gut microbiota or by enhancing the intestinal barrier defense system. We believe that the interaction between commensal NTBF and the immune system has profound effects on human health (Ochoa-Reparaz, Mielcarz, Ditrio, et al., 2010; Ochoa-Reparaz, Mielcarz, Wang, et al., 2010). Therefore, a better understanding of the molecular mechanisms underlying microbe-host and microbe-microbe interactions and of the immunomodulatory PSA/OMVs/SCFAs produced by NTBF may provide novel options for disease intervention and therapy.

Currently, antibiotic therapy remains the most efficient method of ETBF infection control. An analysis of diarrheal stool samples from Iran found that metronidazole effectively treated *B. fragilis* infection (Akhi et al., 2016). Furthermore, a novel antimicrobial regimen comprising tazobactam/ceftolozane with metronidazole was found to be useful and safe in Japanese patients with complicated intra-abdominal infections (Mikamo et al., 2019). However, antimicrobial resistance continues to increase among *B. fragilis* strains, and therefore it remains necessary to establish a means of testing the antimicrobial susceptibility of these organisms and to develop new antimicrobial strategies. MBT-ASTRA may be a rapid option for screening the antimicrobial susceptibility of a specific *B. fragilis* infection (Justesen, Acar, Sydenham, Johansson, & Esgai, 2018). NTBF may protect against antibiotic-induced intestinal dysbiosis. The microbial competition mediated by NTBF T6SSs may specifically target pathogens without significantly altering the gut microbiota. Accordingly, this option may be useful as a narrow-spectrum therapy (Raffatellu, 2018). Dietary intervention has also been suggested as an adjuvant therapy for *B. fragilis* infection. A previous study demonstrated that the structural manipulation of a probe scaffold, such as acarbose, could selectively inhibit polysaccharide utilization by gut microbiota, which might regulate the population of *B. fragilis* (Santilli, Dawson, Whitehead, & Whitehead, 2018).

Investigations of *B. fragilis* remain in the animal experiment stage and more testing is needed to determine the safety of this organism. However, the future applications of NTBF are promising and lead to questions about further uses. Current research suggests that *B. fragilis* might be combined with drugs to assist in the treatment of diseases or used to make microecological preparations for disease prevention. Bacterial powders must be microencapsulated to ensure the survival of bacteria during passage through the gastrointestinal tract. However, the safety and validity of such powders should be evaluated in clinical practice, and the factors related to industrial application, including high-density fermentation and high-activity preparation (e.g., freeze-drying process of bacterial powder, long shelf life preservation), should also be considered. Differences in function and safety among the various NTBF strains must also be considered and investigated.

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Contributors

Fengting Sun contributed to literature search and writing the manuscript. Qixiao Zhai and Wei Chen reviewed and revised the manuscript. Qingsong Zhang prepared the pictures and tables. Hao Zhang and Jianxin Zhao guided and advised the topic of this article.

Declarations of Competing Interest

None.

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