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Review

Investigations of *Bacteroides* spp. towards next-generation probiotics

Huizi Tan[a,b], Qixiao Zhai[a,b,c,*], Wei Chen[a,b,d,e]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China
[b] School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China
[c] International Joint Research Laboratory for Probiotics at Jiangnan University, Wuxi, Jiangsu 214122, China
[d] National Engineering Research Center for Functional Food, Jiangnan University, Wuxi, Jiangsu 214122, PR China
[e] Beijing Innovation Center of Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing, 100048, PR China

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ABSTRACT

Probiotics play important roles on sustaining or reconstructing the homeostasis of intestinal microbiota, which is one of the key factors in alleviating diseases and maintaining the healthy condition of the host. Preclinical trials indicate that *Bacteroides* genus is widely considered as source of novel beneficial candidates for attenuating inflammation by regulating lymphocytes and cytokine expression, controlling metabolism and preventing cancer. Furthermore, the first case of authorization of *Bacteroides xylanisolvens* in food by the European Commission opens the gate for further investigation and application of this promising community. With this paper, we summarized current investigations of discovering beneficial *Bacteroides* strains, exploring their interaction mechanisms with the host, and evaluating the potential safety risks during commercialization.

1. Introduction

The intestinal microbiota, including microorganisms of bacteria, archaea and eukarya, colonize in hosts since birth and change according to the alterations of dietary habitats, health conditions, and ages. The population of these significant symbionts also varies depending on the location of colonization (Donaldson, Lee, & Mazmanian, 2016). Disruption to the intestinal microbiota, including low bacterial diversity, reduction of beneficial microbial products and accumulation of virulent agents, can give rise to inflammation, frailty, and reduced cognitive function (Claesson et al., 2012), for which the most relevant treatments are regarded as probiotics, prebiotics, and antibiotics.

In 2001, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) released the official definition of probiotics: “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2001). Traditional probiotics, corresponding to strains or species generally within *Lactobacillus* and *Bifidobacterium* genera, and a few strains from *Bacillus*, *Weissella*, *Enterococcus*, *Escherichia coli*, and *Saccharomyces* (Ranadheera, Naumovski, & Ajlouni, 2018), play important roles on the host by regulating immune responses and improving health conditions. For instance, *Bifidobacterium lactis* HN019 restores the impaired immunity in the elderly by stimulating $CD4^+$ and $CD25^+$ T lymphocytes and enhancing the tumoricidal activities of natural killer cells and the phagocytic capacity of mononuclear and polymorphonuclear phagocytes (Gill, Rutherfurd, Cross, & Gopal, 2001); *Lactobacillus casei* (Shirota) improves deficits in mood and memory functions associated with frequent constipation (Benton, Williams, & Brown, 2007); *L. paracasei* and *B. longum* are effective for management of blood cholesterol and pressure (de Almada, Almada, Martinez, & Sant'Ana, 2016). Probiotics have been widely adopted in daily diet as food ingredients or supplements, such as cheese, yogurts and cream (Balthazar et al., 2018; Champagne, Cruz, & Daga, 2018; Sperry et al., 2018), and are predicted to reach a global turnover value of 46.55 billion US dollars by 2020 (O'Toole, Marchesi, & Hill, 2017).

Along with the improvements in the bacterial culture methodologies and sequencing techniques, strains or species with beneficial features but outside the range of traditional probiotics have been gradually identified, and are considered to be the next-generation probiotics or the live biotherapeutics products. *Akkermansia muciniphila*, a relatively new species with negative correlation to obesity and diabetes, is capable of ameliorating glucose tolerance and hepatic insulin sensitivity, and ultimately preventing metabolic endotoxemia in the host (Cani & de Vos, 2017; Everard et al., 2013). *Faecalibacterium prausnitzii*, one of the major butyrate-producers in the mammalian intestine, has been observed to suppress the inflammation by blocking the NF-κB pathway and inducing regulatory T cells, and thereby stimulate the apoptosis of the carcinoma cells in colon (Breyner et al., 2017; Cani & de Vos, 2017). However, these two species are difficult to culture and have yet to be approved as food supplements. *Bacteroides* is another group of

* Corresponding author at: No 1800 Lihu Avenue, Binhu District, Wuxi, Jiangsu 214122, PR China.
E-mail address: zhaiqixiao@jiangnan.edu.cn (Q. Zhai).

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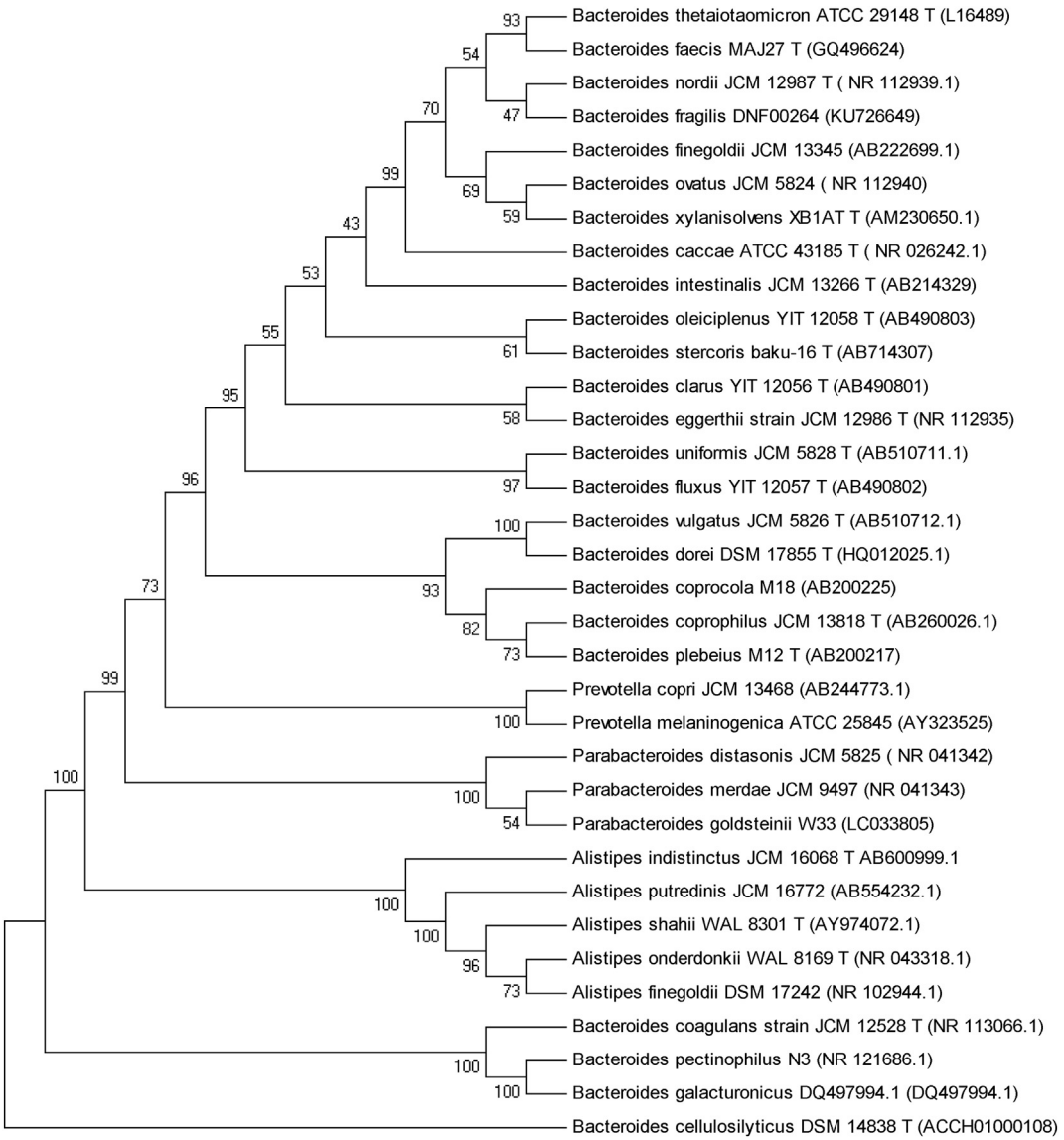

Fig. 1. Phylogenetic tree of the species among *Bacteroidales* (order) that have been identified in the human intestine. The 16 s rRNA sequences of the representatives from each species were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/). The figure was generated by the Mega v5.0 software with Bootstrap values based on 1000 replicates for the neighbor-joining and maximum-parsimony (Tamura et al., 2011).

promising candidates that has attracted major attentions from scientists as model organisms or community for the investigations of the intestinal microbiota field, due to their powerful adaption characteristics in the host and especially the underlying benefits.

2. Characteristics of *Bacteroides*

In healthy adults, 20–80% of the intestinal microbiota corresponds to Bacteroidetes (phylum), including genera of *Bacteroides*, *Parabacteroides*, *Prevotella* and *Alistipes* (Huttenhower et al., 2012) *Bacteroidales* (order) is the most abundant group of gram-negative bacteria which flourish in the human intestine at high densities of 10^9–10^{11} CFU per gram of feces (Zitomersky, Coyne, & Comstock, 2011), and constitutes over 30 species which are more closely related than members in other bacteria orders, as shown in Fig. 1. And, *Bacteroides* is considered as one of the major genera of the core microbiota module, of which species with relative abundance of over 1% refers to *B. uniformis*, *B. vulgatus*, *B. caccae*, and *B. thetaiotaomicron* (Jeffery, Lynch, & O'Toole, 2016; Qin et al., 2010; Tap et al., 2009). However, the *Bacteroides* genus displays significant differentiation in diversities among individuals (Kurokawa et al., 2007).

Bacteroides are obligate anaerobes and are non-spore-forming, non-motile, rod-shaped with round ends (Krieg, Ludwig, Euzeby, & Whitman, 2001). Most of the cells are resistant to 20% bile salt and are highly enriched in colonic mucus (Donaldson et al., 2016). Brucella laked blood, kanamycin, vancomycin plates are generally used for isolating *Bacteroides* strains from colon biopsies and fecal samples (Zitomersky et al., 2011). Modified medium with specific carbon source has been discovered for purifying low-abundant species (Tan, Zhao, Zhang, Zhai, & Chen, 2018), which promote the further investigations of these novel commensal candidates. One of the well-acknowledged characteristics of *Bacteroides* species is the strong polysaccharides degradation systems. The microorganisms produce short chain fatty acids (SCFA) by consuming non-digestible plant- or animal-based glycan derived from daily food intake or intestinal mucus, which provide both nutrients and energy to the host (Koropatkin, Cameron, & Martens, 2012). The polysaccharide utilization loci (PULs) of *Bacteroides* are composed of homologs of starch-utilization system (Sus), such as SusC and SusD, which aimed at binding the target glycans, regulators for sensing the target glycans, and over 200 genes encoding glycoside

Table 1
Promising *Bacteroides* candidates for next-generation probiotics.

Bacteroides strains	source	Target disorders	Beneficial modulatory mechanisms	Study model	Safety evaluation
B. fragilis NCTC9343	Human gut samples	*H. hepaticus* infection (Mazmanian,et al., 2008)	Normalizes of the impaired Treg/Th17 and Th1/Th2 profiles by PSA secretion, eliminate inflammation and facilitate colonization (Mazmanian et al., 2005; Round et al., 2011)	*In vivo* Animal model	Not available
B. fragilis NCTC9343	Human gut samples	Oxazolone-induced experimental colitis (An et al., 2014)	Inhibits the invariant natural killer T cells by expressing sphingolipids to prevent over-activation during infection (An et al., 2014)	*In vivo* Animal model	Not available
B. fragilis ZY312	Human feces	*V. parahaemolyticus* infection (Li et al., 2017)	Protects the morphology of LoVo and RAW 264.7 cells from wrinkling and lysis, and decreases the colonization of *V. parahaemolyticus* in mouse intestine (Li et al., 2017)	*In vitro* & *In vivo* Animal model	No adverse effects on normal and nude mice (Wang et al., 2017)
B. fragilis ZY312	Human feces	Antibiotic-associated diarrhea (Zhang et al., 2018)	Improves the disturbed microbiota and impaired gut barrier caused by antibiotics *via* upregulating mucus-filled goblet cells and tight junction protein (Zhang et al., 2018)	*In vivo* Animal model	No adverse effects on normal and nude mice (Wang et al., 2017)
B ovatus V975	Originally from human gut samples, constructed with KGF-2	DSS-induced colitis (Hamady et al., 2010)	Represses the secretion of pro-inflammatory cytokines under the stimulation of prebiotics, promote mucin production (Hamady et al., 2010)	*In vivo* Animal model	Not available
B ovatus V975	Originally from human gut samples, constructed with TGF-β1	DSS-induced colitis (Hamady et al., 2011)	Downregulates the secretion of TNF-αand IL-1β under the co-treatment with xylan (Hamady et al., 2011)	*In vivo* Animal model	Not available
B. uniformis CECT7771	Human feces	Overweight-associated disorders (Cano, Santacruz, Moya, & Sanz, 2012)	Downregulates cholesterol and triglyceride concentrations; repress the serum glucose, insulin, and leptin (Cano, Santacruz, Moya, & Sanz, 2012)	*In vivo* Animal model	No obvious damage in normal and immune-deficient mice (Fernandez-Murga & Sanz, 2016)
B. dorei D8	Human feces	High-fat diet-induced cardiovascular diseases (Gerard et al., 2007)	Efficiently converts cholesterol into coprostanol (Gerard et al., 2007)	*In vitro*	Not available
B. fragilis NCTC9343	Human gut samples	Autism spectrum disorders (Hsiao et al., 2013)	Corrects the intestinal integrity, microbiota composition, and serum metabolites, to improve the impaired communicative, sensorimotor, stereotypic and anxiety-like behaviors (Hsiao et al., 2013)	*In vivo* Animal model	Not available
B. ovatus D-6	Human feces	Cancer (Ulsemer et al., 2013)	Capsular polysaccharides possess sufficient immunogenicity as cancer vaccines for developing the anti-TFα IgM and IgG antibodies in host (Ulsemer et al., 2013)	*In vivo* Animal model	Not available
B. xylanisolvens DSM23964	Human feces	Cancer (Ulsemer et al., 2016)	Boosts the level of natural anti-TFα IgM antibodies in healthy adults (Ulsemer et al., 2016)	*In vivo* Human trial	Non-pathogenicity confirmed in mouse and human (Ulsemer, Toutounian, Kressel, et al., 2012; Ulsemer, Toutounian, Schmidt, Karsten, & Goletz, 2012; Ulsemer, Toutounian, Schmidt, Leuschner, et al., 2012), has been authorized as supplements in milk products by the European Commission (Brodmann et al., 2017)

hydrolases, transferases, or polysaccharide lyases for depolymerizing the target glycans (Bolam & Koropatkin, 2012; Martens et al., 2011). *B. thetaiotaomicron* carries hybrid two-component system proteins which facilitate the recruitment of the enzymes that involved in the degradation of polysaccharides (Sonnenburg et al., 2006), and the switch of gene subsets during utilization of different substrates depends on their availability and preference (Sonnenburg et al., 2005).

Thus, the polysaccharides utilization systems of the *Bacteroides* species improve their ecological fitness by the guarantee of a wide spectrum of target polysaccharides, especially non-digested dietary fibers. A recent study declared that the simple glycan released from xylan could help the growth of probiotics like *Bifidobacterium* (Rogowski et al., 2015). Mucin will not be digested by most *Bacteroides* species unless it is the only nutrition source available for growth, which eliminates the pathogenic possibilities of *Bacteroides* to directly attack the protective barrier of the intestine in the host (Zitomersky et al., 2013).

3. A stable community in gut microbiome: ecology of *Bacteroides*

Bacteroidetes are much more stable compared to Firmicutes in the human gut across lifetime and generations (Faith et al., 2013).In addition to their powerful nutrients utilization capabilities, which give rise to advantages in niches occupation over other intestinal commensals, syntrophic interactions are well established among *Bacteroides* species as one could ferment certain polysaccharides and liberate breakdown products for others which are unable to grow on the polysaccharides alone (Rakoff-Nahoum, Coyne, & Comstock, 2014). Furthermore, a cross-feeding strategy among the *Bacteroides* members even with a cost to one side was revealed by Rakoff-Nahoum and colleagues (Rakoff-Nahoum, Foster, & Comstock, 2016). *B. ovatus* synthesizes outer surface glycoside hydrolases which are unnecessary for its growth, only to facilitate the colonization of *B. vulgatus* and in return creates better living conditions for its own due to the detoxification of inhibitory substances.

Despite such symbiosis, different patterns of competition also exist in the *Bacteroides* community. One strategy involves secretion of antimicrobial proteins encoded with a membrane attack complex/perforin domain, such as *B. fragilis 638R* inhibits *B. fragilis* NCTC9343 (Chatzidaki-Livanis, Coyne, & Comstock, 2014), and *B. uniformis* ATCC 8492 antagonized by *B. uniformis* CL03T00C23 (Roelofs, Coyne, Gentyala, Chatzidaki-Livanis, & Comstock, 2016). Unlike traditional bacteriocin, no immunity, modification, or transportation genes were defined in the antimicrobial clusters. The producers synthesize antimicrobial proteins with minor differences from the target proteins of the sensitive strains encoded by orthologous genes to protect themselves, such as the porin-like outer membrane protein for *B. fragilis* and the O-antigen glycan of LPS for *B. uniformis* (Roelofs, Coyne, Gentyala, Chatzidaki-Livanis, & Comstock, 2016). And a Eukaryotic-like ubiquitin protein is crucial during the secretion of the antimicrobial toxins (Chatzidaki-Livanis et al., 2017). Another strategy is to express contact-dependent Type VI secretion systems (T6SSs), which are widely existed in gram-negative bacteria aimed at antagonism (Chatzidaki-Livanis, Geva-Zatorsky, & Comstock, 2016; Russell et al., 2014). The mechanisms of these two competition strategies differ in the targeting range and the effects achieved, as the T6SSs perform a wider inhibition spectrum than the secreted antimicrobial proteins, with niche restriction rather than exclusive effects on the sensitive targets (Coyne, Roelofs, & Comstock, 2016).

All these studies show that the stability of *Bacteroides* in the host is determined by the balance of the cooperation in nutrition utilization, exploitative and interference competition. Moreover, the physical exchange of DNA of over 100 kb among *Bacteroides* encoding attachment and utilization of substrates and antagonism functions among *Bacteroides* species also contribute to the ecological and evolutionary dynamics (Coyne, Zitomersky, McGuire, Earl, & Comstock, 2014).

4. Regulatory effects on gut and beyond: intervention with *Bacteroides* spp.

In addition to the unique physiological characteristics and the special ecological dynamics, *Bacteroides* species are under investigations for their underlying beneficial dialogues with the host (Table 1), of which *B. fragilis* NCTC9343 is one of the earliest and most significant achievements. The capsular polysaccharide of *B. fragilis* NCTC9343, termed PSA, can help colonization in the host and alleviate colitis induced by the pathogen *Helicobacter hepaticus* at the same time (Mazmanian, Round, & Kasper, 2008); Moreover, the sphingolipids of *B. fragilis* NCTC9343 can attenuate the oxazolone-triggered experimental colitis (An et al., 2014). *B. fragilis* ZY312, which was isolated from the feces of a healthy infant, is capable of protecting both *in vitro* intestinal and immune cells and *in vivo* models from infection by the food-borne pathogen *Vibro parahaemolyticus* (Li et al., 2017); and alleviating antibiotic-associated syndrome by restoring intestinal microbiota diversity and reconstructing the intestinal barrier (Zhang et al., 2018). The authors of this review have also isolated two *B. fragilis* and *B. ovatus* strains from fecal samples provided by healthy Chinese donors which are capable of downregulating inflammatory responses, such as the induction of TNF-α and repression of IL-10 caused by lipopolysaccharides infection (unpublished data).

Moreover, it has also been proved that *Bacteroides* can be cloned with beneficial agents for better exhibition of modulatory effects. For example, genes encoding the keratinocyte growth factor-2 (KGF-2) or the cytokine of transforming growth factor-β (TGF-β), which are both responsible for the proliferation of intestinal epithelial cells and the maintenance of gut homeostasis, can be inserted into the xylan-utilization operon of *B. ovatus*. Therefore, the reconstructed *B. ovatus* can produce KGF-2 or TGF-β respectively under the stimulation of xylan and display functions of preventing DSS-induced colitis in the form of improving weight lossand reduced colon length, and downregulating the secretions of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 (Hamady et al., 2010; Hamady et al., 2011). Meanwhile, it has been declared that these improvements by *B. ovatus* are even better than the traditional steroid treatment.

However, the beneficial impacts of *Bacteroides* species on the host are not limited to the gastrointestinal tract. The prediction of the modulatory effects of *Bacteroides* in disorders induced by overweight was inspired by the facts that the population of Bacteroidetes in the gut could be recovered through diet therapy for obesity (Ley, Turnbaugh, Klein, & Gordon, 2006), and that there is higher risk of obesity in babies under formula-feeding and with less *B. uniformis* colonized in the intestine (Owen, Martin, Whincup, Smith, & Cook, 2005; Sanchez et al., 2011), has already been confirmed by Sanz and colleagues who demonstrated that the *B. uniformis* CECT7771 is capable of reducing body weight by decreasing the dietary fat absorption, downregulating cholesterol and triglyceride concentrations in both liver and serum, repressing the levels of glucose, insulin, and leptin in blood, and ameliorating the overweight-associated immune dysfunctions (Cano, Santacruz, Moya, & Sanz, 2012). Besides, *B. dorei* D8 possess intervention potentials of high-fat diet-induced cardiovascular diseases due to its efficient conversion capabilities of cholesterol into coprostanol *in vitro* (Gerard et al., 2007), as the serum cholesterol concentration is negatively correlated with the fecal coprostanol/cholesterol ratio (Sekimoto, Shimada, Makanishi, Nakano, & Katayama, 1983).

To date, an increasing number of preclinical evidences underline the significance of the bidirectional brain-gut microbiota interactions. In addition to the impact on the intestinal permeability and immunity, bacterial candidates in the human intestine and their metabolites could contribute to central nervous system and behavior disorders, such as autism spectrum disorders (ASD) (Hsiao et al., 2013), anxiety (Heijtza et al., 2011), depression (Rao et al., 2009), cognitive function (Davari, Talaei, Alaei, & Salami, 2013), and ingestive behavior (Vijay-Kumar et al., 2010). However, probiotics such as *Bifidobacterium longum*

(Bercik et al., 2011) and *Lactobacillus rhamnosus* (Bravo et al., 2011) are able to correct the anxiety- and depression-like behaviors and the following colitis. Notably, *B. fragilis* NCTC9343 also exhibits manifest normalization effects on the ASD-associated defects in terms of intestinal integrity, microbiota composition and serum metabolites, and on the impaired communicative, sensorimotor, stereotypic and anxiety-like behaviors in the offspring of maternal immune activation mice (Hsiao et al., 2013).

Furthermore, intestinal commensals participate in cancer immunosurveillance of the host by activating natural antibodies like blood group antigens (Nguyen, Tangvoranuntakul, & Varki, 2005), the alpha-Gal epitope (Macher & Galili, 2008; Yilmaz et al., 2014), and the tumor-specific Thomsen-Friedenreich antigens (TFα) (Ulsemer et al., 2013) through bacterial ligands. For example, the capsular polysaccharides of *B. ovatus* D-6 obtain more immunogenicity than the synthetic TFα which is used as cancer vaccines for developing the anti-TFα IgM and IgG antibodies in host (Ulsemer et al., 2013); meanwhile, the oral administration of *B. xylanisolvens* DSM23964 boosts the level of natural anti-TFα IgM antibodies in healthy adults (Ulsemer et al., 2016).

Collectively, all these discoveries emphasize the possibilities of *Bacteroides* species to be considered as next-generation probiotics, and the application of which in prevention or intervention in dysbiosis of gut microbiota and the associated disorders requires further investigations in the modulatory mechanisms and safety evaluation.

5. Modulation mechanism discoveries: interactions with the immune system

In order to perform the beneficial modulatory effects in the host, the bacterial signatures and metabolites of *Bacteroides* engage with a variety of immunocytes and contribute to the activation of immune responses. Typically, $CD4^+$ T cells are crucial elements that are in charge of autoimmune responses and supervision of carcinogenesis, and can be differentiated into effector T cell subsets. Generally, T helper 1 (Th1) and T helper 2 (Th2) cells possess distinct and opposite immune-regulatory responsibilities, so as T helper 17 (Th17) and regulatory T (Treg) cells; therefore, the Th1/Th2 (Neurath, Finotto, & Glimcher, 2002) or Treg/Th17 (Kimura & Kishimoto, 2010) ratio reveals the homeostasis or dysbiosis of the immune system.

PSA, the well-acknowledged symbiosis factor from *B. fragilis* NCTC9343, is competent to facilitate the proliferation of T cells *via* stimulating the maturation of dendritic cells (Mazmanian, Liu, Tzianabos, & Kasper, 2005). In the meantime, PSA promotes the correction of the disturbed Th1/Th2 balance by upregulating the secretion of the typical Th1 marker, IFN-γ, in the asthma and allergies associated Th2-bias models (Mazmanian et al., 2005). More interestingly, Toll-like receptor 2 is required during the normalization of the impaired Treg/Th17 profiles by PSA through suppressing the expression of IL-17 (Round et al., 2011), which is one of the universal pro-inflammatory pathways shared by pathogens, and ultimately eliminates infections and maintains the colonization niche of the *B. fragilis* at the intestinal epithelial surface.

Additionally, the sphingolipids of *B. fragilis* NCTC9343 is required as supplements of the lipid antigen population to restrict the hyper-expansion of invariant natural killer T cells during neonatal development, and therefore the inflammation and disorders induced by oxazolone can be limited while in adulthood (An et al., 2014). Compared with the traditional probiotics, *B. acidifaciens* boosts the differentiation of B cells in the large intestine of germ-free mice, with significantly larger amount of immunoglobulin A secreted which is capable of promoting the clearance of infectious agents and microorganisms (Yanagibashi et al., 2013).

It is considered that *Bacteroides* actually activate mechanisms to protect the host from fetal damage by initially creating minor inflammation. For instance, the PSA producing *B. fragilis* stimulates the IL-10 producing T cells for suppressing further inflammation after upregulating pro-inflammatory cytokines and abscess formation during leakage into the peritoneum (Cohen-Poradosu, McLoughlin, Lee, & Kasper, 2011). *B. fragilis* ZY312 reinforces the phagocytosis and polarization of bone marrow-derived macrophage cells to classical-M1 phenotype, and dramatically increases the production of IL-12, IL-1β and nitric oxide (Deng et al., 2016). But the IL-10 producing T cells activated by the increased CD80 and CD86 promote the conversion of responses which are associated with alternative-M2 phenotype, and thereby protecting the host from over-inflammatory reactions (Mazmanian & Kasper, 2006).

Furthermore, various metabolites are also recruited to maintain the stability of the immune system. *Bacteroides* is one of the dominant contributors of SCFA among the intestinal commensals, mostly in the form of acetate and propionate. Accumulated acetate blocks the transportation of toxins between gut lumen and blood (Fukuda et al., 2011); while propionate is capable of inducing the apoptosis of human colon carcinoma cells, and thus avoids the formation of tumors (Cruz-Bravo et al., 2014). Moreover, Vitamin K, which is mainly synthesized by gut microbiota candidates such as *Bacteroides* (Boxma et al., 2012), can help prevent or treat osteoporosis by increasing bone mineral density (Fujita et al., 2012).

6. Potential pathogenicity of *Bacteroides* spp.: critical safety evaluation

Unlike traditional probiotics, such as *Lactobacilli* and *Bifidobacteria*, that the entire species of which are accepted as Generally Regarded as Safe (GRAS) in the USA or authorized for consumption by the European Food Safety Authority (O'Toole et al., 2017), the health-promoting characteristics of *Bacteroides* are strictly strain-dependent. Although PSA produced by nonenterotoxigenic *B. fragilis* attenuates infections and facilitates colonization (Mazmanian et al., 2008), this capsular polysaccharide also acts as symbionts for bacterial growth (Liu, Lee, VanLare, Kasper, & Mazmanian, 2008) and possesses abscess-inducing properties (Surana & Kasper, 2012). Moreover, *B. fragilis* carrying virulent fragilysin genes accelerates inflammation and severe colitis (Yim et al., 2013); *B. fragilis* YCH46 producing fibrinogen-degrading protease may damage the defense system and enhances infections by bacterial invasion in wounded tissues (Chen, Kinouchi, Kataoka, Akimoto, & Ohnishi, 1995). Additionally, *B. caccae* p2Lc3 secrets inflammatory bowel disease-associated antigens which are similar with RagA virulence factor of *Porphyromonas gingivalis* (Wei et al., 2001). Beta-lactamase-producing *Bacteroides* spp. correlate with head and neck infection (Brook, 1988). These studies highlight the importance of strain identification and safety evaluation during scientific and industrial application.

Therefore, the FAO/WHO strongly recommend three-step clinical trials before application of probiotics, including safety assessment and functional characterization; double blind, randomized, placebo-controlled human studies; and efficiency comparisons with standard treatments. However, progress in the exploration of microorganisms with underlying benefits suggests the renewal of the guiding principles for industrial applications. Thus, in 2016, the Food and Drug Administration (FDA) of USA announced that the next-generation probiotics should be developed as dietary ingredients first rather than dietary supplements; and the live biotherapeutics products, defined as “a biological product composed of live organisms for prevention, treatment, or cure a disease or condition of human beings but is not a vaccine”, should follow the process of as the investigational new drug (O'Toole et al., 2017; Sanders, Shane, & Merenstein, 2016).

B. xylanisolvens DSM23964 is the most recent case within the *Bacteroides* genus for being authorized as starters in the fermentation of pasteurized milk products under Novel Food Regulation No 258/97 by the European Commission (Brodmann et al., 2017). The non-pathogenicity of the bacteria was assessed by checking extracellular enzymes, pathogenic factors and antibiotics resistance *in vitro*, and hematological

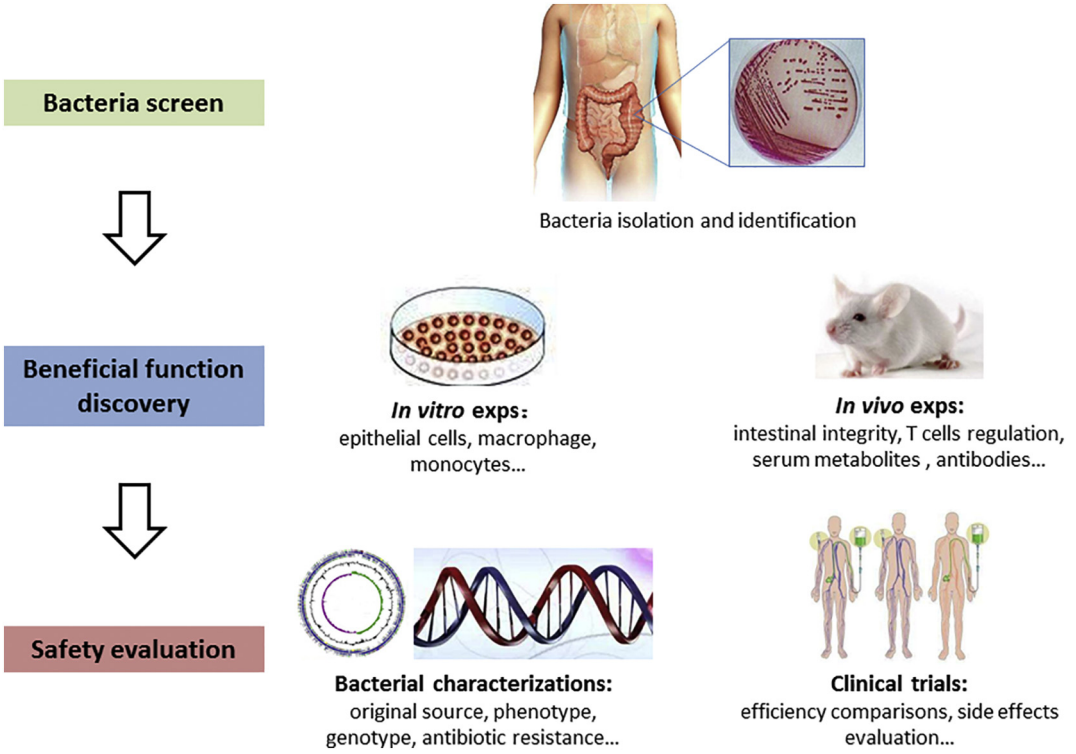

Fig. 2. Graphic summary of the development routine of *Bacteroides* as next-generation probiotics before authorization for industrial application (O'Toole et al., 2017). According to the FDA regulations, the underlying benefits and the corresponding mechanisms of *Bacteroides* strains isolated from human samples were discovered by a serial of *in vitro* and *in vivo* experiments. Complete characterizations of the beneficial strains are crucial, including original source, culture history, phenotype and genotype, antibiotic resistance, and manufacturing method. Clinical trials for safety evaluation is mandatory to make sure no adverse effect would occur in the involved volunteers before final applications.

parameters, serum inflammatory markers, and liver enzyme values both in animal and human trials (Ulsemer, Toutounian, Kressel, et al., 2012; Ulsemer, Toutounian, Schmidt, Karsten, & Goletz, 2012; Ulsemer, Toutounian, Schmidt, Leuschner, et al., 2012), which was also confirmed by the German Federal Institute for Occupational safety in Health. The *B. xylanisolvens* is required to be inactivated as its final form in the real products, and is yet to be included on the qualified presumption of safety (QPS) list.

Besides, the acute ingestion of the anti-inflammatory *B. fragilis* ZY312 had no adverse effects on either normal or nude mice. The bacterium has been tested for the antibiotic resistance, and has been manifested to be non-toxigenic and stable genetically and phenotypically (Wang et al., 2017). No obvious damage was observed in either normal or immune-deficient mice treated with the metabolism-regulatory *B. uniformis* CECT7771, and the treatment even reduced the secretion of inflammatory proteins caused by the intraperitoneal administration of cyclophosphamide (Fernandez-Murga & Sanz, 2016). Therefore, the low risk of these strains offers promising prospects for application.

7. Conclusion

The successful applications of fecal microbiota transplantation in diarrhea associated with recurrent *Clostridium difficile* infection (van Nood et al., 2013) have inspired therapeutic concepts of utilizing single intestinal commensal strain or bacterial mixture to improve intestinal microbiotia dysbiosis-associated diseases (Petrof et al., 2013). Thus, isolation and characterization of new beneficial bacterial candidates is critical. The extended probiotic functions and colonization spots (Donaldson, Lee, & Mazmanian, 2016) in the gastrointestinal track of the next-generation beneficial microorganisms help to better address individual problems and requirements either in its indigenous form or as delivery vehicles.

Bacteroides species are widely considered to be a source of novel beneficial candidates for treating intestinal colitis, immune dysfunctions, and metabolic disorders, and for cancer prevention. Other promising commensal bacteria, such as *Clostridium* and *Faecalibacterium*, have not yet been as deeply investigated as *Bacteorides* spp.

In general, the development of the *Bacteroides* strains with underlying benefits include three major steps (Fig. 2): bacterial isolation from human samples, function discoveries by *in vitro* and *in vivo* experiments, and safety assessments exploring the complete characterizations such as original source, culture history, phenotype and genotype, antibiotic resistance, and manufacturing methods. According to the FDA regulations, clinical trials for safety evaluation is mandatory to make sure no adverse effect would occur in the involved volunteers before final applications.

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Author contributions

H. T. and Q. Z. designed the structure of the paper and drafted the manuscript; W. C. reviewed and revised the manuscript.

Conflicts of interests

The authors declare no conflicts of interests.

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