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Beneficial effects of *Bifidobacterium longum* subsp. *longum* BB536 on human health: Modulation of gut microbiome as the principal action

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ABSTRACT

Probiotics have shown great promise in promoting human health. Despite the promising evidence, there is little information on the clinical efficacy of many of the available probiotics and their mechanisms of action are often unclear. *Bifidobacterium longum* subsp. *longum* BB536 is a clinically effective, well-established, multifunctional probiotic that has a long history of human use in alleviating gastrointestinal, immunological and infectious diseases. This review summarizes the functional benefits of BB536 from the most relevant clinical and animal studies and offers a theoretical basis for understanding its mechanisms of action. Key clinical findings point out that BB536 could act as a microbiome modulator to orchestrate the physiological activities of gut communities. Specifically, BB536 modulates luminal metabolism, stabilizes gut microbiota, and ultimately drives a fine-tuned homeostatic balance within the host-microbiome interaction. Clinical evidence of effectiveness of BB536 and how such multifunctional activities take place would be valuable for an optimized probiotic selection.

1. Introduction

The human gastrointestinal tract harbours a complex and dynamic microbial ecosystem composed of trillions of microorganisms that are collectively termed the gut microbiota (Thursby & Juge, 2017). These intestinal microbes regulate many aspects of host physiology, including maintenance of immune function, carbohydrate metabolism, and metabolic homeostasis (Nagpal et al., 2018). Important advances have shown that several highly prevalent health disorders, including inflammatory bowel disease (IBD) and acute infectious diarrhoea, and chronic diseases such as type-2 diabetes and obesity, are associated with the altered microbiota composition as well as imbalance host-microbiota interactions (Liang, Leung, Guan, & Au, 2018).

In recent decades, a tremendous amount of evidence has strongly suggested that positive modulation of microbial composition by external approaches such as nutritional interventions with probiotics may have unprecedented health impacts to the host (Azad, Kalam, Sarker, Li, & Yin, 2018). Probiotics are defined as live microorganisms that, when ingested in adequate amounts, confer a health benefit to the host (Hill et al., 2014). Manipulation of the gut microbiota with probiotics intervention has been reported to exert both prophylactic and therapeutic effects on host gut and immune health (Scott, Jean-Michel, Midtvedt, & van Hemert, 2015). Nevertheless, as the use and diversity of probiotic products expand, choosing an appropriate type of probiotic has been challenging due to differences in the mechanisms of action, safety profile, origin, and efficacy of different strains. Recent research has pointed out that many properties of probiotics are strain-specific, and not all probiotics are equally safe and effective (McFarland, Evans, & Goldstein, 2018). In addition, therapeutic properties with substantial supporting *in vitro* and human data and mechanisms of action of many of the available probiotic strains are often lacking.

Bifidobacterium longum subsp. *longum* BB536 (designated as BB536) is one of the well-established probiotic strains with numerous profound health benefits in humans (Xiao, 2009). BB536 has been used as probiotic since its discovery for half a century and many studies have been conducted to clarify its effectiveness. Nonetheless, there is a lack of a detailed review about its beneficial properties that could provide both medical care professionals and the public more reliable health claims in helping them to make better-informed probiotics selection. In this review, we summarize the significant health benefits of BB536 from the most relevant clinical and animal studies and try to provide a fundamental framework for a better understanding of the functional health effects of BB536. We believe that a comprehensive knowledge of human substantiation of efficacy as well as the potential mechanisms of action involved is essential for a more tailored and targeted application of probiotics. Here, we highlight that modulation of the gut microbiome is the principal beneficial action of BB536 in humans.

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2. *Bifidobacterium longum* BB536

2.1. Origin and characteristics

BB536 was originated from the gut of a healthy breast-fed infant in 1969. BB536 has been incorporated in various products such as milk-based drink, yogurt, infant formula, and nutritional supplements as functional food ingredient and marketed in over 30 countries for more than 40 years. In 1996, yogurt products containing BB536 have been awarded the Food for Special Health Uses (FOSHU) status by the Japanese Ministry of Health as foods that bear enough scientific evidence for health claim substantiation of benefits in increasing intestinal bifidobacterial abundance as well as improving and conditioning intestinal environment (He & Benno, 2011).

BB536 is a Gram-positive, anaerobic, catalase-negative rod with irregular morphology. BB536 is highly accessible to human gut and highly stable in various finished products, including powdered formula, yogurt, and fermented milk (Abe, Miyauchi, Uchijima, Yaeshima, & Iwatsuki, 2009; Abe, Tomita, Yaeshima, & Iwatsuki, 2009; Ballongue & Grill, 1993; Odamaki, Xiao, Yonezawa, Yaeshima, & Iwatsuki, 2011; Yonezawa et al., 2010); with excellent stability during storage and high survivability in probiotic food until consumption.

2.2. Safety

BB536 is well-evaluated for safety and has been listed on Generally Recognized as Safe (GRAS) notice inventory (GRN No. 268) in the United States Food and Drug Administration (FDA) in 2009. The GRAS status was granted based on the evidence that BB536 is a non-pathogenic, non-toxigenic, non-haemolytic, and non-antibiotic resistant probiotic bacterium that does not contain any plasmids and does not display harmful metabolic activities (FDA, 2009; Momose, 1979; Toscano, De Vecchi, Gabrieli, Zuccotti, & Drago, 2015; Xiao, Takahashi, Odamaki, Yaeshima, & Iwatsuki, 2010). BB536 produces predominantly L-lactic acid, while production of D-lactic acid is negligible (FDA, 2009). In addition, BB536 was demonstrated to possess the conjugated bile salt hydrolase (BSH) enzyme that catalyses hydrolysis of bile salts (Grill, Schneider, Crociani, & Ballongue, 1995). BB536 was able to deconjugate 80–95% of the selected bile salts (taurocholic acid, glycocholic acid, taurochenodeoxycholic acid, glycochenodeoxycholic acid, taurodeoxycholic acid, and glycodeoxycholic acid) in which the production of deconjugated bile salts was concurrent with bacterial growth. It has long been known that high physiological levels of certain secondary bile acids, especially deoxycholic and lithocholic acids, can cause DNA damage and promote colon carcinogenesis (Bernstein, Bernstein, Payne, Dvorakova, & Garewal, 2005; M. J. Hill et al., 1975; Nagengast, Grubben, & Van Munster, 1995). In view of this, concerns may arise over the safety of administering a secondary bile acids-producing bacterium. Remarkably, deconjugated bile salt was the only compound produced by BB536 while secondary bile acids such as dehydrated or hydroxylated products (e.g. the hepatotoxic and carcinogenic lithocholic acid produced from the dehydroxylation of chenodeoxycholic acid) were not detected upon the complete biotransformation of bile salts (Grill, Manginot-Dürr, Schneider, & Ballongue, 1995).

Studies on the acute and chronic toxicological features of BB536 revealed that oral administration of BB536 did not cause death and any toxic symptoms in both mice and SD rat models, respectively (Momose, 1979). The results demonstrate the absence of acute and chronic toxicity by consumption of BB536. In addition to toxicity, questions and concerns have been raised about the safety of probiotic administration, for which bacterial translocation has been assumed to be a potential risk factor of probiotics to cause diseases such as bacteraemia, endocarditis, and sepsis. Therefore, the assessment of probiotic bifidobacteria on the translocation ability is an important safety parameter, particularly in infants, elderly people and immunocompromised persons. Towards this end, an *in vivo* study was conducted involving oral administration of BB536 at a high dose (9.3×10^{11} CFU/kg/day) to healthy 4-week-old mice for 7 days (Abe et al., 2010). The study revealed that BB536 did not translocate into blood, liver, spleen, kidney, and mesenteric lymph nodes and did not induce damages to the intestinal surfaces. Moreover, additional *in vitro* tests on mucin degradation activity of BB536 revealed that BB536 was not able to degrade mucin *in vitro* as the control type strains (Abe et al., 2010). Taken together, these studies provide a substantial support for the safe use of BB536 in foods as probiotics.

3. Clinical efficacy of BB536

3.1. Alleviation of gastrointestinal disorders

Probiotics have historically been used in the treatment and prevention of many forms of gastrointestinal disorders, for which BB536 has long been recognized as one of the most effective probiotic strains for improvement of gastrointestinal conditions (Table 1). Mounting clinical evidences have shown that consumption of dairy products containing BB536, including yogurts, yogurt drinks, and non-fermented milks, can improve the frequency of defecation and faecal characteristics in healthy adults with constipation (Ogata et al., 1997, 1999; Xiao, Kondo, Odamaki, et al., 2007; Yaeshima et al., 1997, 1998, 2001).

Constipation is one of the most common gastrointestinal disorders encountered in clinical practice that has a significant impact on health-related quality of life. The prevalence increases with age and it is more frequent among elderly residents of long-term care facilities (De Giorgio et al., 2015). BB536 has been reported to modulate gut motility and normalize defecation frequency in hospitalized elderly patients (Kondo et al., 2013). In two double-blind placebo-controlled human intervention trials involving 168 patients aged $>$ 65 years who were receiving enteral tube feedings, administration of BB536 (at both low and high doses of 2.5×10^{10} and 5×10^{10} CFU per day, respectively) for 16 weeks significantly increased the bowel movements of patients with infrequent defecation ($\leq$4 times/week) as compared to the placebo group (Kondo et al., 2013). Furthermore, significant decreases in the bowel movements of patients with a high frequency of defecation ($\geq$10 times/week) were observed in BB536 group but not the placebo group (Kondo et al., 2013). The prevalence of normally formed stools was also significantly increased in BB536 group as compared to the placebo group. Collectively, these findings have exemplified the functional properties of BB536 as an effective modulator in maintaining regular defecation frequency and conditioning the intestinal environment.

Moreover, consumption of BB536, which was demonstrated as an effective probiotic strain in normalizing defecation problems, was also able to reduce antibiotic-induced alterations of gut microbiota and alleviate gastrointestinal discomforts caused by antibiotic therapy (Colombel, Cortot, Neut, & Romond, 1987; Orrhage, Sjöstedt, & Nord, 2000). In addition to alleviation of constipation and antibiotic associated diarrhoea, consumption of BB536 has also been used to treat gastrointestinal diseases and conditions such as ulcerative colitis (Tamaki et al., 2016), radiation-induced gastrointestinal dysfunction (Demers, Dagnault, & Desjardins, 2014), and irritable bowel syndrome (Giannetti et al., 2017), though the strength of evidence is not robust. More clinical trials will be needed to disclose the effectiveness of BB536 in the treatment of gastrointestinal diseases. Taken together, these clinical findings, evaluated in double-blind placebo-controlled trials, have substantiated the health benefits of BB536 as an effective probiotic strain in improving gastrointestinal disorders in humans.

3.2. Impact on intestinal microecology

The gut microbiota is an important determinant of intestinal homeostasis and health. Some studies have indicated that perturbed gut microbiota, for example, reduced microbial diversity – a sign of a

Table 1
Summary from clinical studies of effect of BB536 on gastrointestinal disorders.

References	Type of Study	Country	Subjects	Intervention/Dose	Time	Main Outcomes
Ogata et al. (1999)	Non-RCT	Japan	6 healthy adults (21–42 years)	Yogurt (250 mL/d) $\geq 5 \times 10^9$ CFU	2 w	Increased defecation frequency
Ogata et al. (1997)	Non-RCT	Japan	40 healthy adults (20–28 years)	Non-fermented milk (200 mL/d) 2×10^9 CFU	3 w	Increased defecation frequency Improved faecal characteristics
Xiao, Kondo, Odamaki, et al. (2007)	Placebo-controlled Double-blind 2-way crossover	Japan	55 healthy adults (21–45 years)	Yogurt drink (100 g/d) $\geq 2 \times 10^7$ CFU/g	2 w	Increased defecation frequency
Yaeshima et al. (1997)	Non-RCT	Japan	39 adults –	Yogurt (100 g/d) $\geq 2 \times 10^7$ CFU/mL	3 w	Increased defecation frequency Improved faecal characteristics
Yaeshima et al. (2001)	Non-RCT	Japan	43 healthy adults (32–53 years)	Non-fermented milk (180 mL/d) $\geq 2 \times 10^9$ CFU	2 w	Increased defecation frequency Improved faecal characteristics
Yaeshima et al. (1998)	Non-RCT	Japan	41 healthy adults (28–52 years)	Yogurt (100 g/d) $\geq 2 \times 10^9$ CFU	2 w	Increased defecation frequency Improved faecal characteristics
Kondo et al. (2013)	Randomized Double-blind Placebo-controlled	Japan	168 elderly (> 65 years)	Powder (2 g/d) Low: 2.5×10^{10} CFU High: 5×10^{10} CFU	16 w	↑ Bowel movements of patients with infrequent defecation (≤ 4 times/week) ↓ Bowel movements of patients with a high defecation rate (≥ 10 times/week) ↑ Prevalence of normally formed stools
Colombel et al. (1987)	Placebo-controlled Double-blind	France	10 healthy adults (22–50 years)	Yogurt –	3 d	Improved faecal weight, stool frequency, and abdominal complaints during antibiotic treatment ↓ Antibiotic-induced clostridial spores
Orrhage et al. (2000)	Randomized Double-blind Parallel Placebo-controlled	Sweden	30 healthy adults (21–50 years)	Fermented milk (250 mL) 5×10^7–2×10^8 CFU/mL	3 w	Alleviate gastrointestinal discomforts caused by antibiotic therapy ↓ Antibiotic-induced clostridial spore count 63% of patients receiving BB536 showed clinical remission
Tamaki et al. (2016)	Randomized Double-blind Placebo-controlled	Japan	56 patients with ulcerative colitis (44 ± 14 years)	Powder (thrice/d) $2–3 \times 10^{11}$ CFU	8 w	↓ UCDAI scores, EI & Mayo subscore
Demers et al. (2014)	Prospective Single centre Randomized Double-blind Placebo-controlled	Canada	246 patients with pelvic cancers (mean = 61 years)	Bifilact probiotics (LAC-361& BB536) Std: 1.3×10^9 CFU Twice daily High: 1×10^{10} CFU Three times daily	60 d	May reduce radiation induced grade 2–3–4 diarrhoea at the end of the treatment of patients with pelvic cancer
Giannetti et al. (2017)	Randomized Double-blind Placebo-controlled Cross-over	Italy	48 with IBS (8–17.9 years) 25 with FD (8–16.6 years)	Powder (1 sachet/d) M-63: 1×10^{10} CFU M-16V: 1×10^{10} CFU BB536: 3×10^{10} CFU	6 w	Improved abdominal pain and QoL in children with IBS, but not in FD

RCT: randomized controlled trial; –: not described; CFU: colony forming unit; std: standard; w: weeks; d: days; ↑: increased; ↓: decreased; UCDAI: ulcerative colitis disease activity index; LAC-361: *Lactobacillus acidophilus* LAC-361; M-63: *Bifidobacterium longum* subsp. *infantis* M-63; M-16V: *B. breve* M-16V; IBS: irritable bowel syndrome; FD: functional dyspepsia; QoL: quality of life.

dysfunctional ecosystem that leads to instability of the microbiota – is associated with several diseases in humans including gastrointestinal and metabolic disorders (Gong, Gong, Wang, Yu, & Dong, 2016; Mosca, Leclerc, & Hugot, 2016). Diet is one of the major factors influencing the structure and activity of the gut microbiota. A remarkable study by David et al. (2014) has implicated that short-term consumption of animal-based or plant-based diets can alter the microbial profiles. It revealed that an animal-based diet but not the plant-based diet increased the abundance of bile-tolerant bacteria (*Alistipes*, *Bilophila* and *Bacteroides*) and decreased the levels of Firmicutes that metabolize dietary plant polysaccharide. These changes, particularly the increased abundance and activity of *Bilophila wadsworthia*, were found to be associated with the development of IBD (Devkota et al., 2012) and colorectal cancer (Han et al., 2018; Yazici et al., 2015).

It is noteworthy that the fluctuation of the gut microbiota caused by an animal-based diet was restored by the probiotic strain BB536. An open, randomised, parallel-group study involving 33 healthy Japanese subjects (aged 20–50 years) demonstrated that intake of yogurt supplemented with BB536 ($\geq 2 \times 10^9$ CFU/100 g) once a day could maintain a normal microbiota composition during the consumption of animal-based diet (Odamaki et al., 2016). The study consisted of 7-day baseline, 5-day feeding of animal-based diet, and 2-week feeding of balanced diet. The subjects were randomly allocated into three groups; (i) subjects ingested BB536 yogurt during both the animal-based and balanced diet periods as YAB group, (ii) subjects ingested BB536 yogurt only during the balanced diet period as YB group, and (iii) subjects who did not ingest yogurt throughout the intervention as the control (CTR) group. In line with the previous report (David et al., 2014), the animal-

based diet induced changes in the microbial community after five days of intake, with a significant increase in the relative abundance of *Bilophila*, *Odoribacter*, *Dorea*, and *Ruminococcus* (belonging to *Lachnospiraceae*) and a significant decrease in the level of *Bifidobacterium* in both the YB and CTR groups. In contrast, ingestion of BB536 yogurt re-established a healthy gut microbiota wherein the altered microbial profile caused by animal-based diet was not demonstrated in the YAB group, with the exception of *Ruminococcus*.

Another open, randomized, parallel-group study involving 32 healthy Japanese adults (mean age 39.58 ± 9.18 years) who were the carriers of enterotoxigenic *Bacteroides fragilis* (ETBF) also revealed a positive impact of BB536 on the intestinal microenvironment (Odamaki et al., 2012). The study consisted of a 4-week run-in period, 8-week feeding of control milk or BB536 yogurt, and 12-week washout period. Ingestion of yogurt containing BB536 ($\geq 10^8$ CFU/g, 160 g per day) had a discernible effect on the cell numbers of ETBF in gut microbiota of healthy adults. ETBF has been suggested to be associated with acute and persistent diarrheal disease in patients with IBD (Sears, 2009) and the development of colorectal cancer (Orberg et al., 2017; Wu et al., 2009). A significant decrease in the cell numbers of ETBF at week-8 as compared to baseline values was observed in subjects receiving BB536 yogurt but not in the control milk group, indicating the ability of BB536 to eliminate the opportunistic ETBF pathogens in the gut microbiota and to condition the intestinal microenvironment. Consequently, these results imply a potential role of BB536 in colorectal cancer prophylaxis and therapy and support the notion that BB536 could maintain a healthy microbiota in humans.

On this basis, it is imperative to note that improvement of intestinal environment could be one of the main reasons for the clinical efficacy of BB536 in alleviating gastrointestinal disorders. Numerous human intervention studies have shown that ingestion of BB536 can stimulate the growth of beneficial bifidobacteria and reduced the levels of faecal putrefactive substances, thereby improving defecation problems in constipated subjects (Kondo et al., 2013; Ogata et al., 1997, 1999; Yaeshima et al., 1997). For instance, in a non-randomized controlled trial, administration of milk supplemented with BB536 (2×10^9 CFU and 2×10^{10} CFU/200 mL) once a day for 7 days to twelve healthy Japanese adults (aged 21–57 years) resulted in significant decreases in the faecal ammonia content and the activity of beta-glucuronidase while the relative percentage of *Bifidobacterium* was significantly increased (Ogata et al., 1997). The numbers of *Enterobacteriaceae* and *Clostridium perfringens*, the pathogenic bacteria responsible for several intestinal infections and enteric diseases, were tended to be decreased in subjects consuming milk supplemented with BB536 (Ogata et al., 1997).

Furthermore, the intestinal health promoting traits of BB536 appear to be strain-specific. A study investigating the effects of *Bifidobacterium* fermented milks on human gut microbiota showed substantial superiority of BB536 over others (Ballongue & Grill, 1993). In a non-randomized controlled trial, administration of BB536 fermented milk ($\geq 10^7$ CFU/g, 125 g) three times daily for 3 weeks significantly improved the intestinal microbial balance of 45 human subjects with a remarkable increase in the abundance of *Bifidobacterium* and decrease in the levels of putrefying bacteria. Nonetheless, these changes were not observed in subjects consuming fermented milk containing the species of *B. animalis* and all the other strains of *B. longum* subsp. *longum* ($\geq 10^7$ CFU/g) for the same period of time (Ballongue & Grill, 1993). These discrepancies serve to emphasize further the strain- and host-specific effects of BB536 in improving intestinal microenvironment in humans. Taken together, these clinical trials provide proof of a health benefit of BB536 in maintaining microbial balance for better health (Table 2).

3.3. Immuno-modulation

It has long been demonstrated that probiotics can modulate immune function and may therefore be applied as intriguing alternatives to prevent or alleviate certain pathologies involving the host immune system (Yan & Polk, 2011). Nevertheless, it is becoming evident that even closely related probiotic strains might have different effects on the immune system (Klaenhammer, Kleerebezem, Kopp, & Rescigno, 2012). Probiotic bacteria may elicit a differential cytokine response, and different *Bifidobacterium* strains may induce distinct and even opposing immune responses (He et al., 2002). In other words, fine-tuned selection of biologically relevant probiotic strains is necessary for the modulation of host immunity. BB536 is one of the well-established probiotic strains with strong scientific evidence on their positive effects on the immune system. Supplementation of BB536 has been shown to confer health benefits by their immunomodulatory function and has been clinically evaluated for their application in the possible prevention and therapy of immune-mediated diseases (Table 3).

Early research showed that feeding of BB536 (10^8 CFU/g, 7–10 g per day) in milk to eight Japanese children (aged 4–12 years) suffering

Table 2
Summary from clinical studies of impact of BB536 on intestinal microenvironment.

References	Type of Study	Country	Subjects	Intervention/Dose	Time	Main Outcomes
Odamaki et al. (2016)	Open Randomised Parallel-group	Japan	33 healthy adults (20–50 years)	Yogurt (200 g/d) $\geq 2 \times 10^9$ CFU/100 g	YAB:19 d YB: 14 d	Re-established a healthy gut microbiota
Odamaki et al. (2012)	Open Randomised Parallel-group	Japan	32 healthy adults (39.58 ± 9.18 years)	Yogurt (160 g/y) $\geq 10^8$ CFU/g	8 w	↓Cell numbers of ETBF at week-8
Kondo et al. (2013)	Randomized Double-blind Placebo-controlled	Japan	168 elderly (> 65 years)	Powder (2 g/d) Low: 2.5×10^{10} CFU High: 5×10^{10} CFU	16 w	microbiota
Ogata et al. (1999)	Non-RCT	Japan	6 healthy adults (21–42 years)	Yogurt (250 mL/d) $\geq 5 \times 10^9$ CFU	3 w	↑ Bifidobacteria & lactobacilli levels ↓ Faecal putrefactive substances ↑ Short chain and volatile fatty acids
Ogata et al. (1997)	Non-RCT	Japan	12 healthy adults (21–57 years)	Non-fermented milk (200 mL/d) A: 2×10^9 CFU B: 2×10^{10} CFU	7 d	↓ Faecal ammonia content ↑ Abundance of bifidobacteria ↓ Number of Enterobacteriaceae and *Clostridium perfringens*
Yaeshima et al. (1997)	Non-RCT	Japan	11 adults –	Yogurt (100 g/d) $\geq 2 \times 10^7$ CFU/mL	2 w	↑ Abundance of bifidobacteria ↓ Faecal ammonia ↑ Faecal organic acids
Ballongue and Grill (1993)	Non-RCT	France	45 subjects –	Fermented milk (125 g thrice/d) $\geq 10^7$ CFU/g	3 w	↑ Abundance of bifidobacteria ↓ Levels of putrefying bacteria No changes were detected with other strains of *B. longum*

RCT: randomized controlled trial; -: not described; CFU: colony forming unit; w: weeks; d: days; ↑: increased; ↓: decreased.

Table 3
Summary from clinical and animal studies of immuno-modulatory effects of BB536.

References	Type of Study	Country	Subjects/Model	Intervention/Dose	Time	Main Outcomes
Clinical studies						
Sekine et al. (1985)	Non-RCT	Japan	8 children	Non-fermented milk	1 y	Elevated chemiluminescence of non-specific macrophages against monocytic cells
			(4–12 years)	(4–6 years: 7 g/d) (7–12 years: 10 g/d) 10^8 CFU/g		Reduced mean corpuscularvolume of peripheral RBC
Akatsu et al. (2013)	Single-centre Double-blind Placebo-controlled Randomized Parallel group	Japan	45 elderly patients (81.7 ± 8.7 years) Influenza infected (A/H1N1, A/H3N2, and B)	Powder (2 g twice/d) 5×10^{10} CFU/2 g twice daily	12 w Tended to increase IgA levels	Increased bifidobacteria numbers Tended to stimulate NK cell activity
Namba et al. (2010)	Randomized Placebo-controlled Double-blind	Japan	27 elderly patients (86.7 ± 6.6 years) Influenza infected (HA vaccine)	Powder (2 g/d) 1×10^{11} CFU	5 w	Reduced influenza & fever cases Stimulated NK cell activity and the bactericidal activity of neutrophils
Takeda et al. (2009)	Open-label Non-RCT	Japan	14 patients with ulcerative colitis (43 ± 5 years)	Powder 2–3 × 10^{11} CFU	24 w	Reduced Clinical Activity Index Induced remission of patients with ulcerative colitis
Mizuta et al. (2016)	Randomized Single-centre Single-blinded	Japan	60 patients with colorectal cancer (20–85 years)	Powder (2 g/d) 5×10^{10} CFU/2 g	Pre: 7–14 d Post: 14 d	Balanced intestinal microbiota Attenuated postoperative inflammatory responses
Arai et al. (2018)	Prospective Randomized	Japan	64 critically ill patients –	Transluminal preparation containing BB536	– procalcitonin and IL-6 levels	Reduced mean serum
Animal studies						
Sekine et al. (1994)	Mouse peritoneal cells	Japan	BALB/c male mice	Intraperitoneally injection 5.7×10^5 CFU	–	Promoted the production of IL-1β, IL-6 and TNF-α
Reddy and Rivenson (1993)	IQ-induced carcinogenesis	USA	F344 rats n = 156	Experimental diet 2×10^{10} CFU	58 w	Inhibited IQ-induced colon and liver tumours
Yamazaki et al. (1985)	Mono-association of BB536	Japan	GF BALB/c nude mice	Intragastrical administration 2.5×10^7 CFU	18 w	Stimulated cell-mediated immunity
Iwabuchi et al. (2009)	Intranasal injection of influenza virus (PR8)	Japan	BALB/c male mice	Intranasal administration –	3 d	Improved cumulative incidence and survival rate Increased IL-12p40 and IFN-γ production
Iwabuchi et al. (2011)	Intranasal injection of influenza virus (A/PR/8/34 H1N1)	Japan	SPF female BALB/c mice	Oral administration 2×10^9 CFU	2 w	Alleviated symptoms, reduced loss of body weight, and inhibited viral proliferation in the lungs Reduced IL-6 and IFN-γ levels
Takeda et al. (2009)	Toll-like receptor 2 knockout model	Japan	Mice resembling Th2 dominant stimulated with CD3/CD28	Heat-inactivated BB536 1.5×10^{10} CFU	–	Induced IL-12 & IFN-γ production Inhibited IL-4 & IL-13 production Upregulated expression of tight-junction molecules (claudin-1 and ZO-1)

RCT: randomized controlled trial; -: not described; CFU: colony forming unit; y: year; w: weeks; d: days; RBC: red blood cells; NK: natural killer; IgA: immunoglobulin A; IL: interleukin; IQ: 2-amino-3-methylimidazo [4, 5-f] quinolone; GF: germ free; SPF: specific pathogen free; Th2: T helper 2 cells; TNF-α: tumour necrosis factor alpha; IFN-γ: interferon gamma.

leukaemia for one year, besides regular antineoplastic chemotherapy, resulted in a significant elevation in the chemiluminescence of non-specific macrophages against monocytic cells and a reduction of mean corpuscular volume of peripheral red blood cells, suggesting an immunostimulatory effect of BB536 on macrophage-mediated anti-tumour immunity (Sekine, Yoshihara, Homma, Hirayama, & Tonozuka, 1985). Several *in vivo* animal studies have also demonstrated that BB536 could enhance host immunity against cancer whereby BB536 promoted the production of inflammatory interleukins (IL-1β and IL-6) and tumour suppressive cytokine (TNF-α) in mouse peritoneal cells (Sekine, Kawashima, & Hashimoto, 1994), inhibited 2-amino-3-methylimidazo [4, 5-f] quinolone (IQ)-induced colon and liver carcinogenesis in F344 rats (Reddy & Rivenson, 1993), and stimulated cell-mediated immunity in germ-free nude mice mono-associated with BB536 (Yamazaki et al., 1985). These findings suggest that BB536 could act as an immunomodulator in stimulating immune response against tumour growth.

The immunostimulatory effects of BB536 have also been demonstrated in a model of influenza virus infections. Preclinical studies have indicated that intranasal and oral administration of BB536 to mice infected with influenza virus enhanced host cellular immunity, ameliorated influenza-like symptoms, and protected against influenza virus

infection (Iwabuchi et al., 2009; Iwabuchi, Xiao, Yaeshima, & Iwatsuki, 2011). Further clinical studies have shown that BB536 could improve waning immunity in the elderly. Administration of BB536 to elderly subjects with influenza vaccination in two different clinical trials (refer to details in Table 3) was shown to have a tendency to stimulate neutrophil phagocytic activity and natural killer (NK) cell activity, reduces the incidence of influenza and fever, and increases the levels of immunoglobulin A (IgA) in the elderly (Akatsu et al., 2013; Namba, Hatano, Yaeshima, Takase, & Suzuki, 2010). It has been reported that cellular immune responses are weaker in the elderly and the decline in immune system function has been suggested to contribute to impaired vaccine efficacy and increased risk of influenza virus infection. These results imply that BB536 may enhance resistance of elderly consumers to pathogenic viruses and could be applied as a potential adjuvant to improve the immune response to influenza vaccines in the elderly.

It is interesting to note that BB536 can not only augment host immunity against cancer and influenza virus infection, but can also restore immune balance and inhibit inflammation. An open-label pilot study revealed that administration of BB536 (2–3 × 10^{11} CFU) for 24 weeks was effective for inducing remission of patients with ulcerative colitis (n = 14; mean age 43 ± 5 years) (Takeda et al., 2009). Further investigation using mice models demonstrated that both alteration of T-helper type 1 (Th1)-dominant cytokine profile of splenocytes and enhancement of mucosal barrier function with upregulation of tight junction molecules could be the possible mechanisms of BB536 in abrogating inflammatory conditions in patients with ulcerative colitis (Takeda et al., 2009). Subsequently, the efficacy of BB536 in reducing postoperative complications of colorectal surgery was evaluated in a randomized, single-centred and single-blinded clinical trial (Mizuta et al., 2016). Sixty Japanese patients undergoing colorectal resection (aged 20–85 years) were randomized to receive BB536 (5 × 10^{10} CFU/2 g) or control once a day, preoperatively for 7–14 days and postoperatively for 14 days. BB536 intervention attenuated systemic postoperative inflammatory responses and improved recovery of haematological and nutritional status in patients undergoing colorectal surgery, suggesting administration of BB536 could promote healthy recovery after colorectal resection (Mizuta et al., 2016).

More recently, BB536 was reported to be able to prevent septic complications and could be beneficial for critically ill patients with non-infectious diseases (Arai, Mishima, Ohta, Yukioka, & Matsumoto, 2018). Sepsis is a commonly fatal disease and is characterized as an exaggerated inflammatory response (Nee, 2006). The prospective randomized study involved 64 critically ill patients receiving either conventional therapy alone as control or with a transluminal preparation containing BB536. It was demonstrated that upregulation of procalcitonin and IL-6, which are the specific markers of septicaemia in critically ill patients, was significantly suppressed in patients administered with BB536, but the effect was counteracted by antibiotics treatment (Arai et al., 2018). This study documents that BB536 could prevent complications resulting from sepsis in patients with critical illness and the use of broad-spectrum antibiotics may have inhibited the colonization of BB536 in the intestinal microbiota and its beneficial effect. Taken together, these studies have demonstrated the ability of BB536 to regulate host immune response and the encouraging results obtained in the clinical studies have strengthened the scientific evidence to support the health benefits of BB536.

3.4. Anti-allergy

Seasonal allergic rhinitis caused by Japanese cedar pollen is one of the most prevalent forms of allergic disease in Japan and is considered a national affliction (Yamada, Saito, & Fujieda, 2014). Japanese cedar pollinosis (JCPsis) is an immunoglobulin E (IgE)-mediated type I allergy triggered by exposure to the irritating cedar pollens. The prevalence of JCPsis has increased dramatically in the last two decades and it is currently affecting about 30% of the Japanese population (Fujimura & Kawamoto, 2015). Growing body of evidence suggests that probiotics could modulate the host immune system and may alleviate the symptoms of allergic disease (Yang, Liu, & Yang, 2013). In the past decade, a few strains of probiotic have been advocated for the treatment of JCPsis (Ishida et al., 2005; Kawase et al., 2009; Tamura et al., 2007; Xiao et al., 2006a). Nevertheless, the clinical efficacies of many of these probiotic strains have not been explored in detail and are inconclusive. For instance, the data from two studies addressing the effects of probiotic lactobacilli strains for improvement of the symptoms of allergic rhinitis are conflicting (Helin, Haahtela, & Haahtela, 2002; Tamura et al., 2007). The studies have highlighted that the beneficial effects of probiotics on allergic diseases are dependent on the strain used and the timing of treatment.

BB536 has been extensively studied for the treatment of JCPsis with substantial clinical data and reproducible results (Xiao et al., 2006a, 2006b; Xiao, Kondo, Takahashi, et al., 2007; Xiao, Kondo, Yanagisawa, et al., 2007). Intakes of BB536 yogurt or lyophilized powder have been shown to potentially alleviate nasal and ocular allergic symptoms and modulate allergic immune response in patients sensitive to Japanese cedar pollens. A remarkable randomized, double-blind, placebo-controlled trial involving 44 JCPsis subjects (aged 26–57 years) receiving BB536 powder at a dose of 5 × 10^{10} CFU/2 g twice daily for 13 weeks during the pollen season in 2005, which was the heaviest season within the past 10 years, showed a significant improvement in all symptoms associated with JCPsis and tended to improve immune functions (Xiao et al., 2006b). Moreover, BB536 significantly improved the T-helper type 2 (Th2)-skewed immune response that was occurred along with pollen dispersion whereby the plasma levels of thymus and activation-regulated chemokine (TARC) were remarkably normalized in subjects receiving BB536 powder (Xiao et al., 2006b; Xiao, Kondo, Takahashi, et al., 2007). It was noted that the elevated plasma TARC levels during the pollen season is positively correlated with disease severity of JCPsis. In addition, supplementation of BB536 could also ameliorate allergic reactions whereby reduced levels of cedar pollen-specific IgE and eosinophils and increased level of interferon gamma (IFN-γ) were detected in BB536 group from baseline at week-4 (Xiao et al., 2006a). These studies consistently show a positive impact of BB536 consumption on important markers of allergic reactions in subjects sensitive to Japanese cedar pollens.

Another double-blind, placebo-controlled, randomized trial involving 40 Italian children (4–17 years) treated with probiotics mixture containing three bifidobacterial strains, BB536 (3 × 10^{9} CFU), *B. longum* subsp. *infantis* M-63 (1 × 10^{9} CFU), and *B. breve* M-16V (1 × 10^{9} CFU), for 4 weeks has also revealed a significant improvement in pollen-induced allergic rhinitis (Del Giudice et al., 2017). Administration of probiotic mixture protected the children against pollen-induced allergic reactions and improved their quality of life, for which these parameters were worsened in the placebo group. This study suggests that BB536 could also possess a remarkable beneficial effect when combined with other *Bifidobacterium* strains.

Furthermore, the superior protective effects of BB536 against allergic rhinitis have been demonstrated in an *in vivo* study using a mouse model of poly-sensitization to major birch and grass pollen allergens (Schabussova et al., 2011). The immuno-suppressive properties of two probiotic strains, *L. paracasei* NCC2461 and BB536 (denoted as *B. longum* NCC3001 in the study) at a dose of 5 × 10^{8} CFU applied at different time points were compared. Treatment with both probiotic strains significantly suppressed the allergen-specific immune responses. However, when applied prior to the sensitization and challenge, only BB536 had a long lasting protective effect, indicating BB536 can be applied for both prevention and treatment of allergic rhinitis. Collectively, the findings from the clinical and animal studies support the concept of probiotic strain selectivity and have pointed out the prominent beneficial effects of BB536 in improving allergic conditions and immune responses (Table 4).

Table 4
Summary from clinical and animal studies of effect of BB536 on allergic disorders.

References	Type of Study	Country	Subjects/Model	Intervention/Dose	Time	Main Outcomes
Clinical studies						
Xiao et al. (2006a)	Randomized Double-blind Placebo-controlled	Japan	40 adults with JCPsis (23–61 years)	Yogurt (2×100 g/d) $\geq 2 \times 10^7$ CFU	14 w	Alleviated eye symptoms Reduced cedar pollen-specific IgE & Eosinophils and increased level of IFN-γ at week-4 from baseline
Xiao et al. (2006b)	Randomized Double-blind Placebo-controlled	Japan	44 adults with JCPsis (26–57 years)	Powder 5×10^{10} CFU/2 g twice daily	13 w	Decreases in rhinorrhea, nasal blockage and composite scores Suppressed increases in plasma TARC
Xiao, Kondo, Takahashi, et al. (2007)	Randomized Double-blind Placebo-controlled	Japan	44 adults with JCPsis (26–57 years)	Powder 5×10^{10} CFU/2 g twice daily	13 w	Significant increases in plasma TARC in placebo but not in BB536 group Positive correlation between elevated plasma TARC level & disease severity
Xiao, Kondo, Yanagisawa, et al. (2007)	Double-blind 2-way crossover Randomized	Japan	24 adults with JCPsis (25–56 years)	Powder 5×10^{10} CFU twice daily	4 w × 2	Reduced ocular symptom scores Reduced prevalence of medical use
Del Giudice et al. (2017)	Randomized Double-blind Placebo-controlled	Italy	40 children with pollen-induced AR (4–17 years)	Powder M-63: 1×10^9 CFU M-16V: 1×10^9 CFU BB536: 3×10^{10} CFU	4 w	Improved allergic symptoms and QoL
Animal studies						
Schabussova et al. (2011)	Intraperitoneal poly-sensitization	Austria	Female BALB/c mice	Intranasal administration 5×10^8 CFU	–	Significant suppression of airwayinflammation and down-regulated allergen -specific immune responses

JCPsis: Japanese cedar pollinosis; AR: allergic rhinitis; CFU: colony forming unit; w: week; IgE: immunoglobulin E; IFN-γ: interferon gamma; TARC: thymus and activation-regulated chemokine; QoL: quality of life.

Table 5
Summary from animal and clinical studies of effect of BB536 on various infectious diseases.

References	Type of Study	Country	Subjects/Model	Intervention/Dose	Time	Main Outcomes
Animal studies						
Matsumoto et al. (2008)	Gut-derived *P. aeruginosa* sepsis	Japan	SPF male ICR mice	Oral administration 1×10^9 CFU	10 d	Decreased viable cells of *P. aeruginosa* in the liver, blood and intestinal contents Suppressed *P. aeruginosa* adherence to intestinal epithelial cells
Namba et al. (2003)	Intragastrical administration of IPH9	Japan	GF mono-associated mice	10^7 CFU	–	Protected against lethal effect of IPH9 and inhibited the growth of IPH9
Clinical studies						
Kageyama et al. (1984)	Non-RCT	Japan	56 leukaemia patients (20–60 years)	Milk (200 mL/d) 10^7 CFU/mL	3m	Inhibited *Candida* overgrowth Reduced levels of plasma endotoxin and urine indican
Tomoda et al. (1988)	Non-RCT	Japan	49 leukaemia patients (47 ± 16)	Milk (200 mL/d) 10^7 CFU/mL	3m	Inhibited *Candida* overgrowth
Chitapanarux et al. (2015)	Randomized Double-blind Placebo-controlled	Thailand	63 patients with *H. pylori* infection	Two capsules twice daily –	4 w	Improved *H. pylori* eradication and anti-*H. pylori* antibiotherapy-associated complications
Lau et al. (2018)	Randomized Double-blind Parallel Placebo-controlled	Malaysia	219 children (2–6 years)	Powder 5×10^9 CFU/g	10m	Improved duration for sore throat Protected against the incidence of respiratory illnesses

P. aeruginosa: *Pseudomonas aeruginosa*; IPH9: *Escherichia coli* O157:H7 IPH9; RCT: randomized controlled trial; SPF: specific pathogen free; GF: germ free; *H. pylori*: *Helicobacter pylori*; CFU: colony forming unit; d: days; m: months; w: weeks.

3.5. Anti-infections

BB536 has also been shown to be effective for the treatment and control of microbial infections. Several *in vitro* and animal studies have demonstrated that BB536 can inhibit the growth of Gram-negative pathogens (e.g., *Escherichia coli*, *Klebsiella pneumonia*, *C. perfringes*, *Ba. fragilis*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) (Araya-Kojima, Yaeshima, Ishibashi, Shimamura, & Hayasawa, 1995; Inturri, Stivala, Furneri, & Blandino, 2016; Matsumoto et al., 2008; Namba, Yaeshima, Ishibashi, Hayasawa, & Yamazaki, 2003; Yamazaki, Kamimura, Momose, Kawashima, & Ueda, 1982). Competitive exclusion is one of the main reasons for the effectiveness of BB536 against pathogenic infections. BB536 interferes the adhesion of pathogenic bacteria to the epithelial cells, which has been shown *in vitro* with *E. coli*, *S. enteritidis*, and *S. typhi* (Inturri et al., 2016) and in mice model infected with *P. aeruginosa* (Matsumoto et al., 2008). BB536 also may inhibit lethal activity of pathogenic bacteria via the release of metabolites such as lactic and acetic acids, as demonstrated in BB536-monoassociated germ-free mice with *E. coli* O157:H7 infections (Namba et al., 2003).

These results documenting anti-infection activity of BB536 were extended in several clinical studies (Chitapanarux, Thongsawat, Pisespongsa, Leerapun, & Kijdamrongthum, 2015; Kageyama, Tomoda, & Nakano, 1984; Lau et al., 2018; Tomoda, Nakano, & Kageyama, 1988). It was reported that administration of BB536 prevented *Candida* overgrowth in patients with leukaemia (Kageyama et al., 1984; Tomoda et al., 1988). Invasive fungal infection is a major cause of morbidity and mortality in patients with leukaemia in which perturbation of gut microbiota and *Candida* overgrowth are the notable phenomena in patients undergoing anti-leukemic chemotherapy (Bhatt, Viola, & Ferrajoli, 2011). Strikingly, administration of BB536 (10^7 CFU/mL, 200 mL per day) for three months restored the intestinal balance and normalized the levels of plasma endotoxin (a known virulence factor) and urine indican (a measure of intestinal putrefaction) in 56 patients (aged 20–60 years) receiving anti-leukemic therapy (Kageyama et al., 1984). The data support the notion that BB536 could restore the natural balance of gut microbiota and prevent the invasion of opportunistic pathogenic bacteria.

Another randomized, double-blind, placebo-controlled trial examining the effect of BB536 on *Helicobacter pylori* infection revealed that administration of BB536 twice daily for 4 weeks in combination with the standard triple therapy (esomeprazole, amoxicillin, clarithromycin) improved the eradication rate of *H. pylori* infection in 63 patients (mean age 52.61 ± 11.30) (Chitapanarux et al., 2015). *H. pylori* bacterium is a major cause of chronic gastritis and it is considered as an important risk factor of gastric cancer (Uemura et al., 2001). Antibiotic therapy is commonly recommended to eradicate *H. pylori*; however such regimen is often associated with poor compliance, side effects, and resistance emergence (Deltenre, Ntounda, Jonas, & De, 1998). Notably, supplementation with BB536 significantly improved anti-*H. pylori* antibiotic therapy-associated diarrhoea in patients with non-ulcer dyspepsia as compared to placebo control (Chitapanarux et al., 2015). Several randomized controlled trials showed that probiotics treatment seems to be able to reduce *H. pylori* therapy associated complications, but a significant improvement in the eradication rate of *H. pylori* was not detected in most studies, suggesting the inhibitory activity of probiotics on *H. pylori* growth is extremely strain-specific (Lionetti et al., 2010). These data have therefore exemplified that BB536 is superior to combat against *H. pylori* infection and protect against antibiotic-associated complications.

More recently, BB536 was reported to be able to ameliorate upper respiratory infections in healthy pre-school children aged 2–6 years old (Lau et al., 2018). The study was a randomized, double-blind, parallel and placebo-controlled study involving 219 subjects who were administered with lyophilized powder of BB536 (5×10^9 CFU/g; n = 109) or placebo (n = 110) for 10 months, during the high-prone season of upper respiratory illnesses in Malaysia. It was noted that

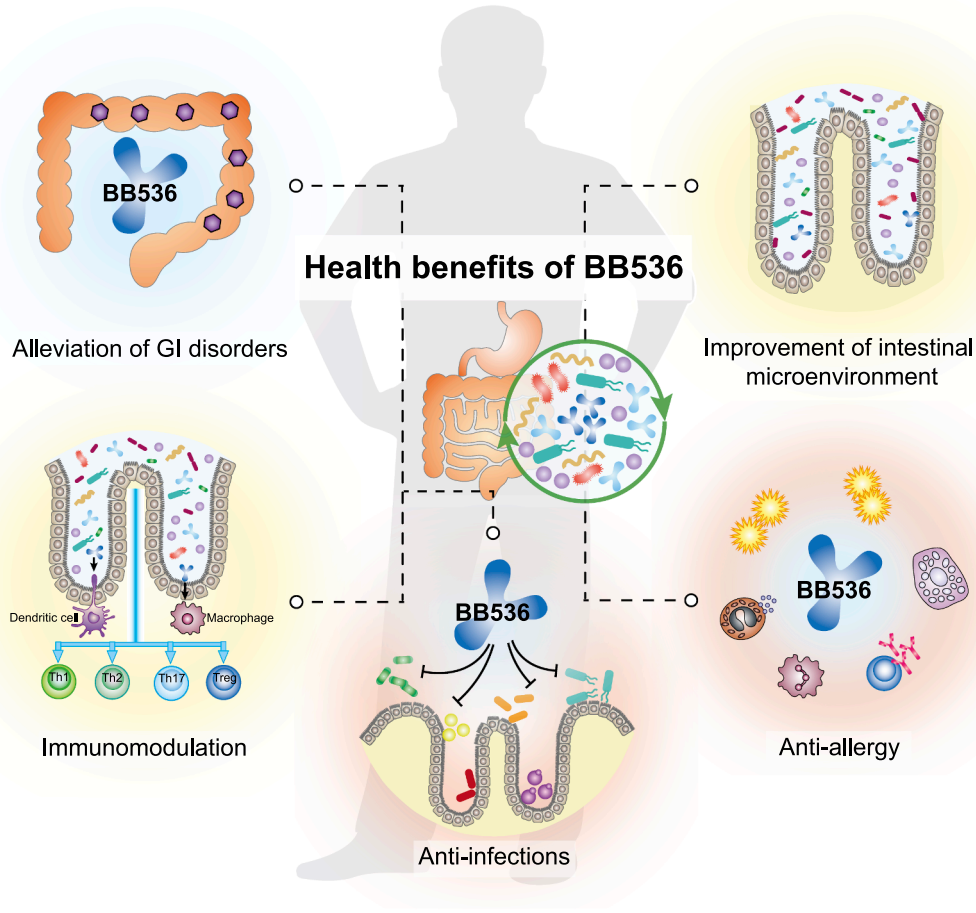

Fig. 1. Modulation of gut microbiome is the principal beneficial action of *Bifidobacterium longum* subsp. *longum* BB536 in promoting human health. BB536 acts in concert with the gut microbiota to improve gastrointestinal health, modulate host immune homeostasis, and alleviate allergic disorders and infectious conditions.

BB536 exerted a more prevalent anti-infection effect in protecting children against the incidence of respiratory illnesses wherein the duration for sore throat was significantly improved in children administered with BB536 as compared to the placebo group. Meanwhile, children supplemented with BB536 also had a marginal reduction in the number of days for other symptoms (fever, runny nose and cough) as compared to placebo control. Taken together, these clinical studies imply that BB536 could be used as a promising anti-infection agent against Gram-negative pathogenic and viral infectious diseases in humans (Table 5).

4. Modulation of gut microbiome as the principal action of BB536

Studies over four decades revealed that BB536 confers multiple health benefits to humans and the results suggested that the multifunctional activities of BB536 are attributed principally to the interplay between BB536 and host gut microbiota (Fig. 1).

4.1. Microbial crosstalk on gut microbial metabolism

In an attempt to unravel the molecular basis underlying the effects of BB536, gnotobiotic mice harbouring 15 strains of predominant human gut-derived microbiota (HGM) were employed (Sugahara et al., 2015). Humanized gnotobiotic mice model is a powerful tool that offers the possibility to study the mechanisms by which probiotic strains improve the intestinal environment; with better control over confounding factors in human studies, including the inter-individual gut microbiota variations. It is also noted that there could have pitfalls in translating gut microbiome research results from conventional murine models to humans, for which the gut microbiota composition of murine models differs greatly from those of humans. To this end, HGM-associated gnotobiotic mice were administered with BB536 or PBS daily for 14 days and the impact of BB536 on the gut environment were assessed using multifaceted approaches, including metabolome, metagenome and meta-transcriptome analyses (Sugahara et al., 2015). Significant increases in faecal levels of butyrate and pimelate, a precursor of biotin, were observed in BB536-HGM mice but not in BB536-mono-associated mice, suggesting BB536 is able to modulate gut metabolism through microbial crosstalk with HGM. Moreover, the bacterial transcripts of *Bacteroides caccae* involved in the biotin synthesis pathway, which proceeds through pimelate metabolism, were significantly higher in the BB536-HGM group than in the HGM group, indicating BB536 modulated biotin biosynthesis through interaction with *Ba. caccae* (Sugahara et al., 2015). BB536 promoted the production of the precursor pimelate and enabled *Ba. caccae* to metabolize it further into biotin, thereby contributing to host gut homeostasis (Fig. 2A).

Interestingly, BB536 may also influence the metabolic activity of other species of HGM through cross-feeding mechanisms (Fig. 2B), as demonstrated by the increased levels of intestinal butyrate and the prevalence of butyrate-producing *Eubacterium rectale* (Sugahara et al., 2015). Butyrate is a primary energy source for colonocytes and it plays an important role in maintaining gut health, enhancing epithelial barrier integrity, and inhibiting inflammation (Hamer et al., 2008). Bifidobacteria are capable to breakdown complex carbohydrates and

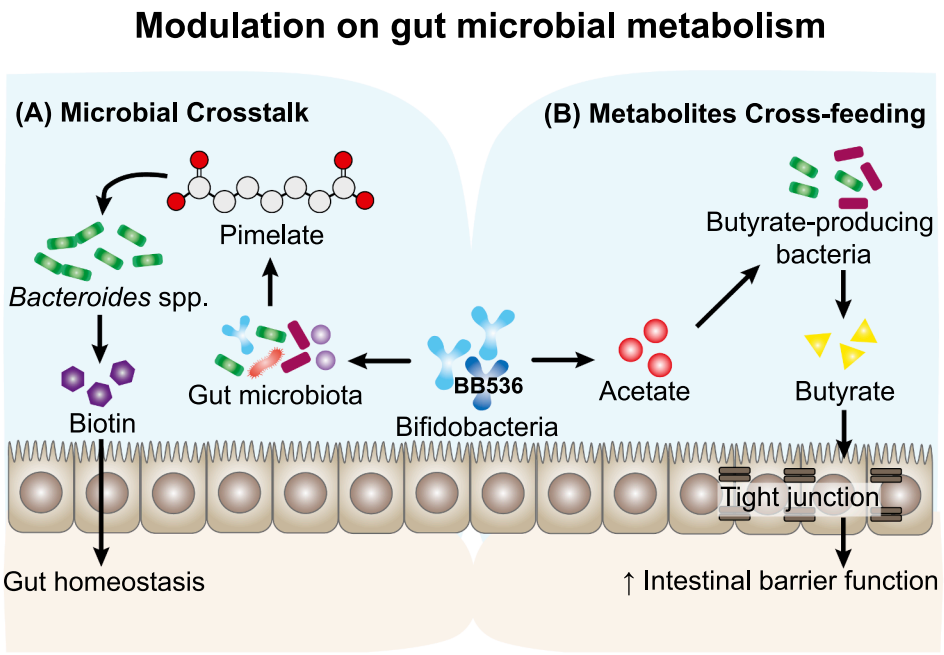

Fig. 2. Modulation of gut metabolism by *Bifidobacterium longum* subsp. *longum* BB536 via microbial crosstalk with human gut microbiota. (A) BB536 modulates biotin biosynthesis by promoting the production of the precursor pimelate and enables *Bacteroides caccae* to metabolize it further into biotin, thereby contributing to host gut homeostasis. (B) BB536 influences the metabolic activity of the commensal butyrate-producing bacteria (e.g. *Eubacterium rectale*) through cross-feeding mechanisms. Acetate produced by BB536 in carbohydrate fermentation acts as substrate to sustain the growth of *Eu. rectale* and stimulates the production of butyrate.

produce metabolic end products, such as acetate and lactate. Subsequently, these fermentative end products may act as substrates to sustain other microbial gut inhabitants, particularly the butyrate-producing enteric bacteria (Barcenilla et al., 2000). Nevertheless, it is noteworthy that certainly not all members of *Bifidobacterium* genus possess such capability as a primary degrader and to establish trophic relationships between members of the gut microbiota (Turroni et al., 2017). Notably, BB536 can positively influence the fitness of other gut commensals through cross-feeding. For instance, acetate produced by BB536 in carbohydrate fermentation has been shown to stimulate the growth of butyrate-producing colon bacteria and the *in vitro* production of butyrate (Falony, Vlachou, Verbrugghe, & De Vuyst, 2006). This study suggests that BB536 improves gut microbiota homeostasis through its cross-talk with other members of the gut microbiota (*Ba. caccae* and *Eu. rectale*). Collectively, these findings provide scientific proof that the interaction of BB536 with human gut microbial community in gut luminal metabolism seems to be the key element of the health-promoting activity of BB536 in human gut.

4.2. Restoration of gut microbiota balance

The diversity and balance of gut microbiota have been suggested to be closely linked to many aspects of human health including immune, metabolic, and neurological functions (Valdes, Walter, Segal, & Spector, 2018). Fluctuations in the population of gut microbiota have been strongly associated with many health disorders. For instance, loss of diversity and an imbalance of gut microbiota, particularly reduced levels of beneficial organisms such as *Lactobacilli* spp., *Bifidobacterium* spp., *Akkermansia municiphila* and *Faecalibacterium prausnitzii*, might confer a risk of developing gastrointestinal disorders, allergies, metabolic syndrome and other chronic diseases (Clemente, Ursell, Parfrey, & Knight, 2012). Of note, it has been evident that probiotic intervention of BB536 can promote the abundance of beneficial taxa in the gut microbiota and stimulate the prevalence of bifidobacteria in subjects with various health complications (Akatsu et al., 2013; Ballongue & Grill, 1993; Mizuta et al., 2016; Ogata et al., 1997). The increase abundance of bifidobacteria and presumably their associated activity, as stimulated by BB536 intervention, may act as a selective pressure on microbiota composition. On this basis, BB536 is believed to exert its positive effects on human host by restoring the gut microbiota balance.

A striking example of the impact of BB536 on microbial homeostasis has been shown in the human trial of animal-based dietary intake (Odamaki et al., 2016). The fluctuation of gut microbiota, particularly an increase abundance of *Bilophila wadsworthia*, caused by an animal-based diet was rebalanced upon ingestion of BB536 yogurt (Odamaki et al., 2016). *Bil. wadsworthia* is a Gram-negative sulphite-reducing and hydrogen-sulphide-producing bacterium that is commonly recovered from patients with appendicitis (Bernard, Verschraegen, Claeys, Lauwers, & Rosseel, 1994). It was reported that increased abundance of *Bil. wadsworthia* may promote intestinal inflammation, intestinal barrier defect, bile acid dysmetabolism, and changes in microbiome functional profile (Natividad et al., 2018). Over-representation of *Bil. wadsworthia* was found to be associated with animal-based diet (David et al., 2014). It is speculated that increased production of taurine-conjugated bile acids from saturated animal-derived fats contributes to the elevated availability of organic sulphur which stimulates the expansion of sulphite-reducing bacteria such as *Bil. wadsworthia* (Devkota et al., 2012). Remarkably, BB536 was efficient in suppressing the outgrowth of *Bil. wadsworthia* and attenuating the fluctuation of gut microbiota (Odamaki et al., 2016). How the probiotic intervention of BB536 protects against animal-diet induced detrimental effects and improves human health remains an open question, but it is possible that BB536 ameliorates numerous health disorders by delimiting the expansion of *Bil. wadsworthia* and restoring the gut microbiota balance. Taken together, these data suggest that restoration of gut microbial balance when it is in an imbalance state (gut dysbiosis) may be one mechanism by which the probiotic BB536 drives microbial fitness and improves human health.

4.3. Immune homeostasis

Maintenance of immune homeostasis is crucial to host survival. The gut microbiota is a critical regulator of host immune system (Belkaid & Hand, 2014). Alteration of gut microbiota balance, a state termed dysbiosis, is inextricably linked to a number of diseases marked by aberrant immune responses (e.g. inflammatory diseases and allergic disorders) (Kosiewicz, Dryden, Chhabra, & Alard, 2014). Recent studies have revealed the association of gut microbiota with the development of particular T-cell subtypes (Th1, Th2, Th17 and regulatory T (Treg) cells) (Lee & Kim, 2017). It has become clear that aberration in the gut

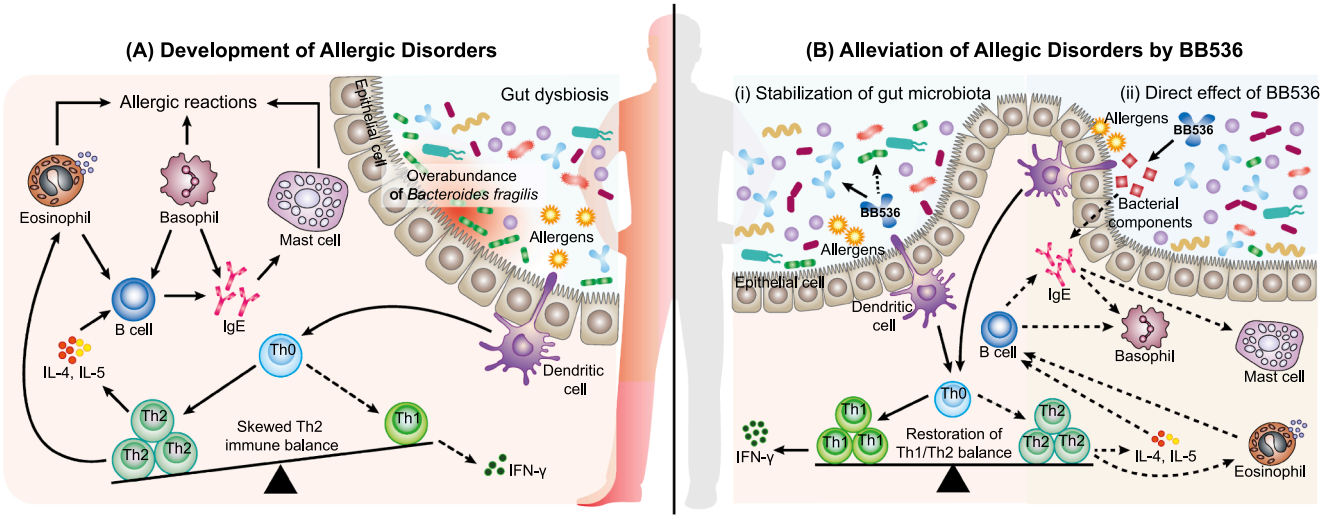

Fig. 3. Immunomodulatory effect of *Bifidobacterium longum* subsp. *longum* BB536. (A) Fluctuation of intestinal microbiota, particularly overabundance of *Bacteroides fragilis*, contributes to perturbation of host immunity and development of allergic disorders. In allergic reactions, an allergen is taken up by dendritic cells, and presented to naïve T cells (Th0) which then transforms into T-helper type 2 (Th2) cells. Th2 cells secrete interleukin (IL)-4 and IL-5 and subsequently stimulated memory B cells to switch to an allergen-specific humoral response that is predominated by the production of immunoglobulin E (IgE) antibodies. These IgE antibodies attach to mast cells and basophils thereby sensitizing them to subsequent exposure and development of allergic symptoms. (B) BB536 modulates immune homeostasis within the host-microbiome interaction and alleviates allergic disorders via both indirect and direct mechanisms. (i) BB536 promotes the stabilization of intestinal microbiota by rectifying the prevalence of *Bacteroides fragilis* and consequently restores Th1/Th2 balance and alleviates allergic symptoms. (ii) BB536 elicits a direct effect on antigen-induced IgE-mediated Th2 skewed immune balance via its bacterial component. Solid arrow line: stimulation; dashed arrow line: inhibition.

microbiota is closely related to skewed T cell responses and the induction of allergic diseases (Hong, Kim, & Surh, 2017). For instance, the seasonal allergic rhinitis, JCPsis, was found to be characterized by a loss of tolerance to gut commensals, as illustrated by the observations that exposure to cedar pollens contributes to fluctuation of intestinal microbiota in allergic subjects, but not healthy subjects. In particular, the abundance of *Ba. fragilis* was significantly increased within the microbial consortium in JCPsis subjects (Odamaki et al., 2007a, 2007b). Such increase in the prevalence of *Ba. fragilis* was positively correlated with the levels of Japanese cedar pollen-specific IgE (Odamaki et al., 2008), implying that gut microbiota disturbance might contribute to perturbation of host immunity and development of allergic disorders (Fig. 3A).

Remarkably, the fluctuated intestinal microbiota, particularly the overabundance of *Ba. fragilis*, was largely rectified in JCPsis subjects administered with BB536 (Odamaki et al., 2007b). Further *in vitro* study using peripheral blood mononuclear cells from JCPsis subjects demonstrated that *Ba. fragilis* possesses a capacity to induce T cell-associated cytokines. It was found that strains of *Ba. fragilis* significantly stimulated a higher level of Th2 cytokine (IL-6) and lower levels of Th1 cytokines (IL-12 and IFN-γ) than that of bifidobacteria (Odamaki et al., 2007a). This result could pave the way to an understanding on the association between *Ba. fragilis* abundance and Th2 skewed immune balance in the development of allergic disorders. It is implicated that supplementation of BB536 promotes the stabilization of intestinal microbiota and increases the abundance of bifidobacteria, which consequently restores Th1/Th2 balance and alleviates allergic symptoms (Fig. 3B(i)) (Odamaki et al., 2007a; Xiao et al., 2006b; Xiao, Kondo, Takahashi, et al., 2007). In addition, BB536 has also been reported to have a direct effect on Th2 skewed immune balance (Fig. 3B(ii)). Both *in vitro* and *in vivo* studies have shown that a novel immunostimulatory sequence oligodeoxynucleotides (ODN) BL07S identified from genomic DNA of BB536 inhibited IgE production (Takahashi et al., 2006a, 2006b, 2006c). Further studies using an ovalbumin-sensitized mice model revealed that the immunostimulatory ODN BL07S of BB536 significantly suppressed Th2 cytokine production (IL-4 and IL-5) and increased the levels Th1 cytokine (IFN-γ) in splenocyte cultures, indicating BB536 prevented antigen-induced Th2 skewed immune responses via its bacterial component (Takahashi et al., 2006a, 2006b). Taken together, these findings have shed light into the mechanism by which BB536 is able to reduce the prevalence of pollen sensitization and alleviate allergic symptoms. It is evident that BB536 improved immune dysfunction by driving a fine-tuned homeostatic balance within the host-microbiome interaction.

5. Conclusions

Bifidobacterium longum subsp. *longum* BB536 has become one of the clinically effective documented probiotic strains that can provide consistent beneficial health effects to human host. One of the clear advantages of BB536 is that this probiotic is a well characterized human origin strain that is widely used for human health with a proven track record of safety and clinical efficacy. Even though these beneficial health effects might not be unique for BB536, evidence on the functional benefits of BB536 stands in contrast to the many undocumented strains being marketed speciously as probiotics. The encouraging results obtained from both animal and clinical studies, despite still limited in scope, have provided an overview of the potential mechanisms underlying the effects of BB536 on human health. It has become clear that modulation of gut microbiome is likely to be the key element of the health-promoting activity of BB536 in human gut. BB536 acts in concert with the gut microbiota to modulate host homeostasis, improve gastrointestinal health, and alleviate allergic disorders. Given these facts, BB536 may serve as a useful and worthy probiotic candidate in the treatment and management of human health. Further investigations with large and longitudinal integrative and mechanistic studies are critical to gain new insights into the molecular basis of how the interaction between BB536, gut microbiota and the host influence human health and to realizing the full potential of BB536 as human probiotic.

Ethics statements

This is a review article. It has not involved any human subjects and animal experiments.

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Conflict of interests

There are no conflicts of interests.

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