FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Probiotic research priorities for the healthy adult population: A review on the health benefits of *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subspecies *lactis* BB-12

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Abstract: A diluted distribution of research efforts hampers probiotic innovation and curtails potential health benefits for the consumer market. Research priorities have been postulated to aid strategic planning, but it remains to be determined how probiotic strains currently pertain to these priorities. We therefore set out to review how probiotic research priorities are currently met by the two best-documented strains, *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium animalis* subspecies *lactis* BB-12 (BB-12), focusing on the needs of the healthy adult population. A literature search was conducted to retrieve clinical studies in adults, reporting on *in vivo* effects of BB-12, LGG, or LGG + BB-12. A framework of studies was created, with a separate emphasis on the potential of probiotics to prevent disease in healthy adults. A total of 76 papers were reviewed. Current evidence indicates that LGG and BB-12 supplementation may promote human health and support the daily wellness of consumers, although most (earlier) trials do not meet the stringent standards required for scientific substantiation of a health claim in Europe. To advance innovation and respond to unmet health needs, it is crucial that well-designed, appropriately scaled studies build on top of promising data, specifically in areas where strong associations are apparent.

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1. Introduction

While the potential health benefits of fermented foods have been acknowledged for centuries (Metchnikoff, 1908), the beneficial micro-organisms residing within them are playing an increasingly important role in contemporary culture (Saxelin, 2008). The majority of these micro-organisms, commonly referred to as “probiotics,” are lactic acid-producing bacteria such as bifidobacteria and lactobacilli. Probiotic bacteria are being incorporated into (food) matrices and may support the daily wellness of consumers, as the prevention of disease through probiotic intervention appears promising for a wide variety of indications. For instance, the consumption of certain probiotic strains is associated with a decreased risk for antibiotic-associated diarrhea (AAD) or upper respiratory infections (Hao, Lu, Dong, Huang, & Wu, 2011; Sanders et al., 2013). Research and development in the probiotic industry has therefore grown substantially over the past decades and continues to rise (Di Cerbo & Palmieri, 2015). However, there appear to be large discrepancies between regulatory authorities regarding the scientific substantiation of probiotic health claims. The European Food Safety Authority (EFSA) states, for instance, that no cause and effect relationship has been established between the consumption of *Lactobacillus rhamnosus* GG (LGG) and the maintenance of normal defecation during AAD (EFSA Panel on Dietetic Products, 2013) or the consumption of *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) and immune defense against pathogens (EFSA Panel on Dietetic Products, 2011). In contrast, health benefits of these strains are acknowledged by other regulatory authorities (He & Benno, 2011; Health Canada, 2015). The lack of substantiation in Europe leaves the consumer market and most medical professionals to question probiotic efficacy (Flach et al., 2017), forming a barrier to innovation (van den Nieuwboer, van de Burgwal, & Claassen, 2016) and curtail potential health benefits. The variety of probiotic strains (often with specific clinical effects (Hill et al., 2014) and the confounding effects of carrier matrices (Flach, van der Waal, van den Nieuwboer, Claassen, & Larsen, 2017) make it difficult to generalize conclusions. Moreover, the vast number of potential disease areas and the limited resources available (van den Nieuwboer et al., 2016) have led to a diluted distribution of research efforts. It is therefore vital that future research is focused and prioritized. In an attempt to aid strategic planning and to advance probiotic innovation, research priorities have previously been identified by Van den Nieuwboer and colleagues (2016). Their study, involving key opinion leaders within the probiotic industry, reports that AAD and irritable bowel syndrome (IBS) should be given the highest research priority in the adult population. Yet it remains to be determined how probiotic strains currently pertain to these priorities. Insight into the evidence base will help to identify opportunities for probiotic knowledge valorization, and ultimately serves to promote human health.

A large variety of probiotic strains have been studied for their potential health benefits, however, LGG and BB-12 represent the best-documented strains among them (Jungersen et al., 2014; Segers & Lebeer, 2014). We therefore set out to recapitulate how probiotic research priorities are currently met by LGG and BB-12, focusing specifically on the needs of the healthy adult population. To this end, a frame of reference is created of all clinical trials with BB-12 and LGG, categorized per probiotic research priority, with a separate emphasis on the potential of probiotics to prevent disease in healthy adults.

2. Methods

A literature search was performed in the scientific databases of PubMed, Embase, and Google Scholar in order to obtain an overview of BB-12’s and LGG’s clinical evidence base. All studies in adults published before September 2017 that report the *in vivo* effect of BB-12, LGG, or their combination (not in combination with other probiotics) were eligible for inclusion in the present review, except for papers solely reporting on gastrointestinal (GI) survival rates. The following search terms were used:
(BB 12 OR “BB-12” OR “BB 12” OR Bb12 OR “Bb-12” OR “bb-12” OR “bb 12” OR DSM15954 OR “DSM 15954” OR “LKM512” OR “FK120” OR “FK 120”) AND (Bifidobacter* OR Bifidobacterium animalis OR lactis) OR “Bifidobacterium animalis subsp. lactis (BB-12 OR BB12)” OR “Bifidobacterium animalis subsp. lactis strain BB-12” OR “Bifidobacterium animalis subsp. lactis str. BB-12”.

(LGG OR GG OR Lgg OR gg OR lgg OR ATCC53103 OR “ATCC 53103”) AND (Lactobacill* OR rhamnosus)) OR L. acidophilus GG OR Lactobacillus acidophilus GG OR L. rhamnosus GG OR L. rhamnosus GG.

Additional studies were retrieved using back- and forward-tracking methods within papers and databases. The selected studies were categorized per probiotic research priority, and studies pertaining to the clinical effects of probiotic intervention in healthy adults (or patient populations that are considered representative for effects in the general population) were subsequently reviewed with respect to study design (e.g. randomization and blinding, choice of study population, adequate size and duration, information on background diet, and appropriateness of outcome measures), outcome (e.g. statistical significance and appropriateness of statistical analysis), and quality.

3. Results and discussion

3.1. Results of literature search [clinical data]
The literature search resulted in more than 3000 publications considered potentially relevant for the present review. All papers were manually screened based on title and abstract, resulting in a final selection of 92 articles. Twenty-one papers were selected and read in full detail for BB-12, 58 papers for LGG, and 13 papers for BB-12 + LGG. An overview of the relative number of studies and their accompanying sample size (active treatment arm), as categorized per probiotic research priority, is presented in Figure 1 (van den Nieuwboer et al., 2016). The same study may be classified under multiple indications when two or more research objectives are addressed. Multiple papers reporting on the same clinical trial are portrayed as one study. A summary of trial characteristics and results is provided in Tables S1–S13 (Supplemental Online Materials).

As these studies report clinical effects that are representative of both healthy and diseased populations, a separate frame of reference was created to meet our research objective. In this framework, all studies pertaining to the clinical effects of probiotic supplementation in healthy adults (or patient populations that are considered representative for effects in the general population) (Figure 2) were portrayed, providing the basis of this paper. Forty-two studies are reviewed accordingly for LGG, 21 for BB-12, and 13 for BB-12 + LGG.

3.2. Potential mechanism of action
In order to facilitate a mechanistic understanding of the clinical effects of BB-12 and LGG, we provide here a brief overview of their potential mechanism of action. Adhesion to intestinal mucosa, epithelial barrier function enhancement, competitive exclusion of pathogens, inhibition of pathogen adhesion, production of antimicrobial substances, and modulation of the host’s immune system are suggested to be the key working mechanisms of probiotics (Gogineni, Morrow, & Malesker, 2013; Oelschlaeger, 2010).

3.2.1. Adherence to intestinal mucosa
Crucial for the induction of many proposed health effects is the ability of probiotic bacteria to adhere to the host’s intestinal mucosa. For instance, immunomodulatory- and pathogen-inhibitory effects are considered to be largely dependent on the adherent capacity of probiotic bacteria (Gogineni et al., 2013; Jungersen et al., 2014; Oelschlaeger, 2010). Preclinical studies suggest that BB-12 has high adherent properties. In an in vitro model with human fecal mucus isolates, BB-12 demonstrated adherence rates up to 30% (Rinkinen, Westermarck, Salminen, & Ouwehand, 2003). Polycarbonate-well plate adherence models, with mucin and Caco-2 and/or HT29-MTX cell cultures, also suggest that BB-12 adheres well to different combinations of plate wells (Laparra & Sanz, 2009). He et al.
(2001) furthermore reported that BB-12 has one of the highest adherences to immobilized human mucus glycoproteins (7.1%) out of the 24 *Bifidobacterium* strains they tested. Similarly, LGG adhered better to intestinal mucus than other *Lactobacillus* strains in an *in vitro* study by Tuomola, Ouwehand, and Salminen (1999). Genetic studies furthermore revealed that LGG possesses pili that enhance the adherent properties of the strain (Kankainen et al., 2009). LGG also produces mucus-binding proteins that may further explain the high colonization rates of the bacteria at the mucosal level (von Ossowski et al., 2011; Velez et al., 2010). Human intervention studies revealed that LGG persists longer in the feces of participants than closely related strains (Kankainen et al., 2009), being detectable from human feces until approximately one week after discontinuation of the intervention (Goldin et al., 1992). Together, these studies suggest that BB-12 and LGG have the properties required to (transiently) colonize the intestinal mucosal layer, a requirement for most health effects.

### 3.2.2. Function of the epithelial barrier

Maintaining epithelial integrity and protecting the host from the environment in order to prevent infection and inflammation are key functions of the intestinal barrier. A layer of dense mucus containing antimicrobial peptides and secretory IgA, together with epithelial junction complexes that regulate intercell permeability, constitute the main structural components of the epithelial barrier (Ohland & MacNaughton, 2010). In order to maintain fitness and health, it is crucial that the epithelial cell lining remains functional and intact. It is generally accepted that probiotics may promote health through the enhancement of the epithelial barrier function, although the exact mechanisms of action are not yet completely understood (Bermudez-Brito, Plaza-Diaz, Munoz-Quezada, Gomez-Llorente, & Gil, 2012). It has been reported that fermentation products of BB-12 significantly increased tight junction strength and that BB-12’s fermentation products yielded the greatest increase in electric resistance compared with other strains tested (Commare et al., 2005). In intestinal epithelial cell models, LGG was able to prevent cytokine-induced apoptosis by inhibiting tumor necrosis...
Lactobacillus species have furthermore been shown to increase mucin expression of intestinal epithelial cells in vivo (Mack, Ahrne, Hyde, Wei, & Hollingsworth, 2003; Mattar et al., 2002). In addition, it has been reported that the LGG strain was able to prevent inflammation and cell death of the intestinal epithelial cell lining (Gaudier, Michel, Segain, Cherbut, & Hoebler, 2005) and enhance mucosal regeneration (Caballero-Franco, Keller, De Simone, & Chadee, 2007), all hinting towards improved barrier function.

### 3.2.3. Inhibition of pathogens

The ability to inhibit pathogens is another fundamental characteristic of probiotic bacteria. It is suggested that pathogen inhibition is expedited through several mechanisms, including production and secretion of antimicrobial substances (e.g., organic acids, bacteriocins, or hydrogen peroxide), competition for nutrients, competition for binding/adherence sites, competitive depletion of vital nutrients, and induction of the host’s immune response. BB-12 was tested for its antagonistic properties in an in vivo study by Martins et al. (2009). A total of 12 pathogens were exposed to BB-12, including Shigella flexneri, Escherichia coli, and Enterococcus faecalis. Antagonistic properties of BB-12 were seen in 8 of the 12 pathogens, where the inhibitory zones of BB-12 were in general among the largest. BB-12 was also tested against two common pathogens (E. coli and Campylobacter jejuni), together with two prebiotics. Both pathogens were inhibited by the symbiotic preparation, where it was suggested that the production of acetate and lactate were the main mechanisms of action.

The ability of BB-12 to compete with pathogens and displace adhesion sites was also investigated in vitro by Collado and colleagues (2007). Several pathogens were tested, including E. coli. It was shown that BB-12 adhered to human mucus and inhibited all pathogens, except E. coli. The displacement of other pathogens, however, was high. The antagonistic properties of LGG have been studied...
in multiple *in vitro* studies. LGG was able to reduce the viability of several pathogens including *S. sonnei* (Zhang et al., 2011), *Staphylococcus* and *Streptococcus* strains (Silva, Jacobus, Denke, & Gorbach, 1987), and *Salmonella enterica* ssp. *enterica serovar typhimurium* (De Keersmaecker et al., 2006; Hutt, Shchepeleva, Loivukene, Kullisaar, & Mikelsaar, 2006; Makras et al., 2006; Marianelli, Cifani, & Pasqualli, 2010; Silva et al., 1987). Protection against *S. typhimurium* infection was also demonstrated *in vivo* in a mouse model (Hudault, Lievin, Bernet-Camard, & Servin, 1997).

Several studies sought to elucidate which antimicrobial compounds produced by LGG mediate the antagonistic effects, often focusing on *S. typhimurium*. One study suggested that the lowering of pH was responsible for the inhibition of *S. typhimurium* growth (Lehto & Salminen, 1997). Other studies postulate that lactic acid is the main antimicrobial compound of LGG (De Keersmaecker et al., 2006; Hutt et al., 2006; Makras et al., 2006). Lactic acid may facilitate the antimicrobial actions of other compounds, as the acid permeabilizes the gram-negative outer membrane (Alakomi et al., 2000), concurrent with the study by Mariannelli et al. (2010) that suggested that antagonistic properties of LGG are not solely mediated by lactic acid. BB-12 and LGG have also been tested *in vitro* together on the adhesion of pathogenic strain to intestinal mucus (animal) (Collado, Meriluoto, & Salminen, 2007). The tested pathogens demonstrated significant reduction in adhesion to the intestinal mucus when the probiotic strains were present, both alone and in combination. In conclusion, these studies demonstrate that LGG and BB-12 are capable of inhibiting pathogens, although the exact mechanism of action remains to be determined.

### 3.2.4. Modulation of the immune system

Probiotic bacteria may also exert immunomodulatory effects on the host mediated by their metabolites, cell wall structure, and DNA. As such, even nonliving bacteria can induce immune effects. Probiotic bacteria interact with macrophages and with epithelial and dendritic cells. When probiotic bacteria adhere to the epithelial cells of the host, a signaling cascade can be triggered, leading to changes in immune function. The release of soluble factors is another way through which a signaling cascade can be induced. The immunomodulatory effects of BB-12 have been studied in various preclinical settings. In the study by López, Gueimonde, Margolles, and Suárez (2010), BB-12 caused maturation of dendritic cells and induced interleukin (IL)-12, TNF-α, and IL-10. High levels of IL-10, interferon (IFN)-γ, and TNF-α were induced in peripheral blood mononuclear cells (PBMCs) (López et al., 2010). The induction of cytokine expression and cell maturation in dendritic cells was also studied by Latvala et al. (2008). All cytokines that were tested were induced by BB-12 (IL-1β, IL-6, IL-10, IFN-γ). The study by Matsumoto, Hara, and Benno (2007) furthermore showed that the consumption of BB-12 in elderly subjects caused the TNF-α response in murine macrophage-like cell lines to be higher than during nonprobiotic supplementation periods.

Regarding LGG, it is suggested that mucosal anti-inflammatory responses are mediated through a direct interaction with macrophages, CD4 + lymphocytes, and dendritic cells, leading to decreased pro-inflammatory cytokine production. The studies by Peña and Versalovic (2003), Braat et al. (2004), and Donkor et al. (2012) demonstrated that LGG decreased the production of TNF, IL-2, and IL-4 *in vitro*. In addition, soluble factors of LGG promote epithelial cells growth and survival through the inhibition of TNF-α–mediated cell apoptosis by activation of antiapoptotic Akt and protein kinase B. The proapoptotic p38 mitogen-activating protein kinase signaling pathway in epithelial cells is also activated through these soluble factors (Yan et al., 2007).

En masse, these studies demonstrate that BB-12 and LGG can adhere to intestinal mucosa, enhance epithelial barrier function, inhibit pathogens, and modulate the immune system of the host. These local and microbiological changes may result in health benefits for the consumer, although *in vitro* assays are not always predictive of potential health effects *in vivo*. 
3.3. Antibiotic-associated diarrhea

Approximately 5 to 39% of people using antibiotics develop diarrhea during the course of their treatment (McFarland, 2008). Although sometimes regarded as mere nuisance, AAD may increase healthcare expenditures, morbidity, mortality, and length of hospital stay (McFarland, 1998; Pilotto et al., 2008; Surawicz, 2003), and as such constitutes a significant societal and economic burden. Probiotic intervention may be a safe and cost-effective method of preventing AAD and has the highest research priority according to Van den Nieuwboer and colleagues (Figures 1 and 2) (van den Nieuwboer et al., 2016). It is suggested that probiotics may prevent diarrhea by maintaining the gut flora and carbohydrate fermentation, by interrupting the potential disease mechanism, and/or by competitively inhibiting the growth of pathogens (Hickson, 2011). The exact mechanism of action remains to be determined and may vary between strains. In this section, clinical studies with BB-12 and LGG are reviewed that investigate their potential role in AAD prevention. A summary of clinical trials is provided in Table S1 (Supplemental Online Materials).

3.3.1. Antibiotic-associated diarrhea—studies with BB-12

A single article was retrieved that reports the effects of BB-12 supplementation on AAD symptom severity (Merenstein et al., 2015). In this phase 1 safety study, 40 subjects received antibiotic therapy for a respiratory infection and were randomized to a dairy drink with BB-12 (4 × 10^9 colony forming units [CFU]/d) or placebo for a period of 10 days. Stool frequency was assessed as one of the secondary outcome parameters. Results indicate that subjects in the BB-12 group had (near-significant) lower stool frequencies compared with control (12.7 vs. 19.2%; p = 0.06). Subjects in the BB-12 group also had fewer reported loose stools compared with control (21 vs. 43%; significance not reported). However, no difference was observed on the incidence of self-reported diarrhea between groups (n = 2 vs. n = 2; significance not reported). Furthermore, the statistical underreporting and the relatively small number of participants enrolled diminish the overall quality of this study.

3.3.2. Antibiotic-associated diarrhea—studies with LGG

Six articles were retrieved that report the effects of LGG supplementation in the treatment/prevention of AAD. Siitonen et al. (1990) demonstrated that male subjects who received LGG yogurt (5 × 10^9 CFU/d) (n = 8) during a 7-day erythromycin acistrate course had less diarrhea than subjects who received regular yogurt (2 days vs. 8 days; p < 0.05) (n = 8). Antibiotic-associated side effects such as abdominal distress, stomach pain, and flatulence were furthermore less common in the LGG group compared with control. However, power calculations, baseline characteristics, and number of defecations per day were not reported. Moreover, diarrhea was not defined and multiple comparisons were not considered.

Four studies report the effects of LGG intervention during Helicobacter pylori eradication therapy. Armuzzi and colleagues (2001) showed in a unblinded pilot study with 120 participants that freeze-dried LGG intervention (6 × 10^9 CFU) reduced the most-frequent side effects of eradication therapy (bloating, diarrhea, and taste disturbances) compared with participants who received the same treatment without probiotic intervention (relative risk [RR]: 0.4; 95% confidence interval [CI]: 0.2, 0.8; RR: 0.3; 95% CI: 0.1, 0.8; RR: 0.3; 95% CI: 0.1, 0.7, respectively) (Armuzzi et al., 2001). Due to the open-label trial design, the risks of bias on self-reported outcomes are high. The results of this study were therefore confirmed in a second, randomized, double-blind trial by the same group (Armuzzi et al., 2001), where the probiotic intervention was compared with placebo (n = 60).

In a similar trial with 85 subjects and multiple probiotic formulations (including LGG: 6 × 10^9 CFU/d), diarrhea and taste disturbances associated with eradication therapy were also significantly reduced in all probiotic intervention groups compared with placebo (Cremonini et al., 2002). However, the studies by Armuzzi et al. (2001) and Cremonini et al. did not define diarrhea and did not report baseline characteristics, and only Cremonini et al. performed power calculations (Cremonini et al., 2002).

Padilla Ruiz, Fernandez Aguiar, Arce Nunez, and Polo Amorin (2013) found no significant differences on eradication of therapy symptoms between subjects (n = 59) who received LGG (6 × 10^9 CFU/..
BID) or placebo during a week of *H. pylori* eradication therapy, in contrast to the previous studies. Thomas and colleagues (2001) also found no effect of LGG supplementation (20 × 10⁹ CFU/d for 14 days) on diarrhea incidence in a large, well-designed study of 302 hospitalized patients receiving antibiotic treatment (compared with placebo, 29.3 vs. 29.9%; *p* = 0.93, respectively). Different classes of antibiotics were used for different indications, increasing the heterogeneity of the study.

### 3.3.3. Antibiotic-associated diarrhea—studies with LGG + BB-12

The effects of a combination of BB-12 and LGG in the prevention of AAD were addressed by a single study (Hauser, Salkic, Vukelic, JajacKnez, & Stimac, 2015). During this trial, 650 subjects (included in the analysis) received a probiotic formulation with both LGG and BB-12 (10⁶ to 10⁸ CFU per capsule) or placebo throughout the course of *H. pylori* eradication therapy. The results showed a significantly larger share of cured subjects in the probiotic arm compared with placebo (87.4 vs. 72.6%; *p* < 0.001). Furthermore, 7 out of 10 symptoms related to eradication therapy significantly improved in the probiotic group compared with placebo: epigastric pain, bloating, flatulence, taste disturbance, nausea, heartburn, and diarrhea (0.76 vs. 0.55; *p* < 0.001).

### 3.3.4. Antibiotic-associated diarrhea—conclusions

Current clinical evidence suggests that LGG alone, or in combination with BB-12, may be effective in reducing the incidence and severity of symptoms related to *H. pylori* eradication therapy. However, it remains to be determined whether LGG supplementation may prevent AAD in the general population of adults, as evidence is less convincing for other patient groups treated with antibiotics. Overall trial quality is furthermore deemed moderate to low (e.g. imprecise criteria for self-diagnosed diarrhea episodes, insufficient description of the statistical analyses, and insufficient description of the study population) and warrants additional research. No conclusions can be drawn on the efficacy of BB-12.

### 3.4. Irritable bowel syndrome

IBS is a chronic GI disorder that is characterized by a symptom complex of abdominal pain and abnormal bowel habits that present as diarrhea or constipation and general physical weakness, in the absence of abnormal morphological, histological, or inflammatory markers (Grundmann & Yoon, 2010). This prevalent functional GI disorder can significantly erode health-related quality of life and places a major cost burden on healthcare services (Corsetti & Whorwell, 2017). Probiotic intervention has been suggested to reduce IBS symptoms, with efficacy reported in a recent meta-analysis by Didari, Mozaffari, Nikfar, and Abdollahi (2015). However, we argue that grouping multiple probiotic species in such an analysis provides little information on the efficacy of individual strains as they tend to have specific clinical effects (Hill et al., 2014). In this section, clinical studies are therefore reviewed that report the effects of BB-12 and LGG supplementation on IBS symptom severity. Results are summarized in Table S2 (Supplemental Online Materials).

#### 3.4.1. Irritable bowel syndrome—studies with LGG

Two studies were retrieved that report the effects of LGG supplementation on IBS symptom severity. Pedersen and colleagues (2014) compared the effect of a low FODMAP diet (low fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols) on IBS symptoms with a normal Western diet, with and without LGG supplementation in capsules (12 billion CFU). One hundred twenty-three IBS patients were randomized in this nonblinded study, and the control group received no intervention. Although the articles’ conclusion is that both the low FODMAP diet and LGG are efficacious in IBS, that conclusion is not supported by the data. Symptom scores were reduced after six weeks in both the LGG supplemented and the normal diet group (*p* < 0.01), but the changes in the LGG group were not significantly different from changes in the normal diet group (*p* = 0.20). Patients with IBS with diarrhea (IBS-D) appeared to be more responsive to treatment than patients with IBS with constipation (IBS-C).
O'Sullivan and O'Morain (2000) demonstrated in a small-scale, crossover trial that administration of LGG tablets ($10^{10}$ CFU/d) in IBS patients ($n = 25$, both IBS-D and IBS-C) did not significantly improve symptoms of bloating, pain, or urgency compared with control. A nonsignificant trend was observed for reduction of diarrhea (defined as $\geq 3$ unformed bowel movements/d) in the IBS-D subgroup. Despite the small number of participants and absence of power calculations, the study appeared well-designed overall.

3.4.2. Irritable bowel syndrome—conclusions

No studies have been conducted with BB-12, and the in vivo evidence base of LGG in the treatment of IBS is limited. A small-scaled and an unblinded study suggest that LGG is not significantly efficacious in treating or preventing IBS symptoms in contrast to the conclusion on probiotic efficacy by Didari et al. (2015).

3.5. Bowel function

Reducing GI discomfort, including complaints of constipation and diarrhea, has been a strong focus in probiotic research over the past decades and is an important priority according to key opinion leaders (Figures 1 and 2). Chronic constipation, for instance, constitutes a major societal problem with a severe impact on patient quality of life and a hefty burden on our economy, particularly in a nursing home setting where the prevalence of constipation is estimated to be 63% (Larsen, Van den Nieuwboer, Koks, Flach, & Claassen, 2017), draining sizable portions of the institutions’ budgets in treatment and care expenditures. Probiotic intervention reportedly improves whole gut transit time, stool frequency, and stool consistency in constipated individuals (Dimidi, Christodoulides, Fragkos, Scott, & Whelan, 2014) and may offer a cost-effective solution. In addition, probiotics may prevent or reduce complaints of diarrhea by maintaining the gut flora and by competitively inhibiting the growth of pathogens (Hickson, 2011). In this section, clinical studies are therefore reviewed that report the effects of BB-12 and LGG supplementation on bowel function (excluding all trials on AAD and IBS, as they have been previously discussed above). Table S5 summarizes the findings (Supplemental Online Materials).

3.5.1. Bowel function—studies with BB-12

Six articles were identified that report the effects of BB-12 supplementation on bowel function improvement. Most studies have looked at the effects of BB-12 on defecation frequency. Both Eskesen et al. (2015) and Pitkala et al. (2007) found a clinically relevant benefit of BB-12 supplementation ($>10^9$ CFU/d) on defecation frequency ($p < 0.05$, both), and both used relevant study populations. Eskesen et al. (2015) studied 1248 healthy volunteers with low defecation frequency for a period of four weeks, whereas Pitkala et al. (2007) included 209 institutionalized elderly with an intervention period of six months. Pitkala et al. reported that participants in the BB-12 group (at least $1 \times 10^9$ CFU/d) had significantly higher stool frequencies than participants in the placebo group ($p = 0.03$). No significant difference between groups for diarrhea and soft stools was reported. BB-12’s effects on abdominal discomfort (such as abdominal pain and flatulence; measured by questionnaires) were addressed by Eskesen et al. (2015) as well, but no significant difference between BB-12 and placebo was found.

The other four studies originated in Japan. The study by Uchida et al. (2005) appeared well-designed, although they used a relatively short intervention period of two weeks. They showed that participants in the BB-12 group (at least $1 \times 10^9$ CFU/d) had significantly higher stool frequencies than participants in the placebo group ($p < 0.05$, $N = 50$, crossover). This study was performed in healthy subjects, but within this group a relatively sizeable proportion of subjects could be identified with a tendency to constipation. Stool consistency and fecal output volume did not differ between BB-12 and placebo groups. It should be noted that the control yogurt also had an effect, compared with no intake.

The other Japanese studies showed methodological concerns that may reduce the validity of the results. Matsumoto et al. (2001) used a relevant study population (30 healthy subjects with
defecation < 4 times per week), but the crossover design was not randomized and therefore probably not blinded either. They reported a significant difference ($p < 0.05$) between the BB-12 (4.5/week) and placebo period (3.9/week) for defecation frequency. The difference in defecation frequency is statistically significant, but relatively small in absolute terms. The treatment period of 2 weeks is furthermore shorter than recommended (Pravst et al., 2017).

Nishida et al. (2004a) performed a crossover study in 35 healthy young women, with washout periods. There is no report of blinding subjects or investigators. Again, the intake period of 2 weeks is relatively short. In our assessment, we focus on the comparison between BB-12 (4 $\times 10^9$ CFU/d) and placebo periods. The article also gives comparisons with the preintake (run-in) period, which we consider less relevant. Within the study population, a distinction was made between constipated and nonconstipated subjects based on bowel habits during the preintake period. In the constipated subgroup, defecation frequency was significantly increased during the BB-12 period ($p < 0.05$). This is a relevant comparison, but may have been performed post hoc, which is looked upon critically by regulatory authorities. Moreover, the numbers of subjects in these subgroups are relatively small, which reduces power. Nishida et al. (2004b) looked at the effect of two doses of BB-12 yogurt on stool frequency (80 gram and 150 grams, $4 \times 10^9$ CFU/g). No effect on defecation frequency was observed. However, effects were compared within dose group, not between dose groups. The groups were very small ($n = 8$, each) and there was no control treatment. They did find an effect on stool quantity in the high-dose group. However, this cannot be solely attributed to BB-12 intake, because the intake of the yogurt itself may have had an effect, as was shown in the study by Uchida et al. (2005).

3.5.2. Bowel function—studies with LGG

Eight articles report the effects of LGG on bowel habit improvement, excluding all trials that focus on AAD and IBS. Four studies included healthy volunteers with (self-reported) constipation problems. Of these studies, two Finnish trials (Holma, Hongisto, Saxelin, & Korpela, 2010; Hongisto, Paajanen, Saxelin, & Korpela, 2006) compared the effects of different diet groups (rye bread) alone or in combination with LGG. Holma and colleagues (2010) reported that LGG (2 $\times 10^{10}$ CFU/d) did not relieve constipation or significantly affect colonic metabolism. According to Hongisto et al. (2006), rye bread shortened total intestinal transit time (TITT) ($p = 0.007$), increased stool frequency ($p = 0.001$), softened feces ($p < 0.001$), and made defecation easier ($p < 0.001$), but also increased GI symptoms ($p < 0.001$) compared with the LGG and control groups. However, when LGG was administrated together with rye bread, fewer GI problems were reported compared with the group consuming solely rye bread (adjusted symptom score: 1.3; 95% CI: −2.4, −0.2; $p = 0.027$). LGG increased the effect of rye bread on all bowel function variables, but the interactions were not statistically significant. LGG yogurt did not have a significant independent effect on bowel function, but it did tend to shorten TITT. However, in these studies, the described effect of LGG yogurt might also have been due to components of yogurt/milk other than LGG, since a placebo dairy product was not used.

In constipated elderly, a significant reduction in constipation status was reported in subjects receiving a synbiotic product with LGG (Granata, Brandi, Borsari, Gasbarri, & Gioia, 2013). However, effects on constipation status were attributed to the prebiotic (fructooligosaccharides) present in the synbiotic product. In addition, the study population consisted of only 12 subjects, and no control group was used. Hence, no conclusions can be drawn from this study on the potential effects of LGG. The same can be concluded for the nonblinded study by Ling et al. (1992), where a 50% dropout rate resulted in data for only 6 subjects: too small to expect significant results, and none were observed.

In healthy marathon runners, LGG (4 $\times 10^{10}$ CFU/d) seemed to shorten the duration of GI-symptom episodes (Kekkonen et al., 2007). During a three-month training period, subjects received LGG in the form of a 65-ml bottle of milk-based fruit drink ($n = 70$) or a similar but inert drink without bacteria ($n = 71$), twice daily. The duration of GI-symptom episodes in the LGG group was 2.9 days vs. 4.3 days.
in the placebo group during the training period ($p = 0.35$) and 1.0 day vs. 2.3 days, respectively, during the 2 weeks after the marathon ($p = 0.046$), suggestive of a potential probiotic effect on GI discomfort recovery. No difference was observed in the number of GI-symptom episodes.

Oksanen et al. (1990) and Hilton, Kolakowski, Singer, and Smith (1997) studied the effect of LGG on the incidence of traveler’s diarrhea, which is typically caused by a bacterial infection. Both studies reported LGG to be efficacious. Oksanen and colleagues (1990) demonstrated that the total number of subjects with diarrhea during the trip was 331 (out of 756), of whom 153 (41.0%) were in the LGG group ($2 \times 10^9$ CFU/day) and 178 (46.5%) were in the placebo group (RR: 0.88; 95% CI: 0.75, 1.04; $p = 0.065$). However, different outcomes were reported per location, for which no good explanation could be given, which does not strongly support overall effectiveness. Hilton et al. (1997) report a significantly lower overall risk of diarrhea in the subjects taking LGG capsules ($2 \times 10^9$ CFU) among 245 travelers in various geographic areas (3.9 vs. 7.4%; $p < 0.05$). In patients with relapsing *Clostridium difficile* diarrhea, LGG intervention appeared to reduce the incidence of diarrhea, as 27 patients (84%) were reported cured after intervention with LGG $10^9$ CFU/d (Bennett et al., 1996). But again, because a before/after comparison was used, time effects may introduce bias.

### 3.5.3. Bowel function—studies with BB-12 + LGG

A single article was retrieved that reports the combined effect of BB-12 and LGG on bowel habit improvement. Dickerson et al. (2014) recruited 65 patients with schizophrenia symptoms who tend to have a high prevalence of GI problems, especially constipation. During 14 weeks of double-blind adjunctive probiotic (approximately $10^8$ CFU LGG and $10^9$ CFU BB-12) or placebo therapy, participants were asked to rate the difficulty in stool passage over the past weeks on a 4-point scale from “no difficulty” to “severe difficulty.” The probiotic group was less likely to report severe difficulty moving their bowels over the course of the trial (hazard ratio [HR]: 0.23; 95% CI: 0.09, 0.61; $p = 0.003$). However, the probiotic and placebo groups did not differ significantly in the use of laxatives or in the experience of diarrhea. Furthermore, an equal number of participants (12) in each group reported new onset of GI symptoms, which included constipation, diarrhea, heartburn, nausea, stomach cramps, and/or flatulence.

### 3.5.4. Bowel function—conclusions

Overall, these studies provide evidence that BB-12 positively affects stool frequency in populations with reduced stool frequency and without increasing diarrhea. The main studies to support this are those by Eskesen et al. (2015), Pitkala et al. (2007) and Uchida et al. (2005), although the use of less-well validated questionnaires and post hoc statistical analyses is looked upon critically by regulatory authorities. The evidence for a potential effect on stool consistency is still quite weak. Evidence from two large placebo-controlled studies suggests that LGG intervention may reduce the overall risk of travelers’ diarrhea, but not in every travel destination (Hilton et al., 1997; Oksanen et al., 1990). In addition, these studies did not comply with the rigorous criteria for design, conduct, and statistical analysis that are required for the substantiation of a health claim (i.e. high drop-out rate; statistics performed in the population of completers only; no data on symptoms accompanying diarrheal episodes). The evidence base is currently not considered strong enough to support consistent associations between LGG intervention and bowel habit improvement in these and other populations.

### 3.6. Microbiota balance

Probiotics are known for their potential to obstruct microbial communities and (transiently) induce changes in the intestinal microbiota (Butel, 2014). Altering the composition of the gut microbiome may ameliorate intestinal inflammation and promote human health (Hemarajata & Versalovic, 2013). Here we review the effects of BB-12 and LGG supplementation on the overall composition of the gut microbiota. Articles solely reporting on the survival or colonization of BB-12 and LGG were not included. Table S6 (Supplemental Online Materials) summarizes all studies that were reviewed with respect to the effects of BB-12 and LGG administration on gut microbiota balance.
3.6.1. Microbiota balance—studies with BB-12

In total, seven trials were identified that studied the effects of BB-12 supplementation on the composition of gut microbiota. The safety study by Merenstein et al. (2015) and the four Japanese trials (Matsumoto et al., 2001; Nishida et al. 2004a, 2004b; Uchida et al., 2005) suggest that the relative abundance of Bifidobacteria increases during BB-12 supplementation, which is to be expected. As the relative abundance of one class of bacteria increases, the abundance of another classes may decrease. The studies investigated high-level classes in relatively small populations, which gives little information on actual microbiota changes induced by BB-12 administration. Nevertheless, reported effects on Clostridium, Bacteroidaceae, and Streptococcus species by Nishida and colleagues (2004) may be of interest.

The study by Palaria, Johnson-Kanda, and O’Sullivan (2012) was the only trial specifically designed to investigate BB-12’s effects on gut microbiota. In this crossover study, 52 healthy volunteers were randomized to consume acidified placebo milk or drinkable yogurt containing 10^6–10^10 CFU of BB-12 (and 1 g of inulin) for three consecutive weeks. It should be noted that the confounding effects of standard yogurt starter cultures and the prebiotic inulin cannot be overlooked in this design. Total numbers of bifidobacteria increased and clostridia decreased at the end of the study for all subjects irrespective of when they consumed the study products. No significant differences in bifidobacteria, clostridia, or enterobacteria counts were observed between the probiotic and placebo groups during any of the feeding periods.

Alander et al. (2001), Satokari, Vaughan, Akkermans, Saarela, and De Vos (2001), and Malinen et al. (2002) all report on the same trial. Thirty healthy volunteers were randomized into three groups consuming: BB-12 (3 × 10^{10} CFU), galacto-oligosaccharides (GOS)-containing syrup, or GOS-containing syrup together with BB-12, in a plain low-fat, nonsugar yogurt twice a day for two weeks. Mean numbers of fecal bifidobacteria increased slightly in all study groups during the feeding period, where GOS-syrup together with BB-12 yielded the highest counts. No differences in the prevalence or numbers of isolates with BB-12 genotype could be observed between groups.

3.6.2. Microbiota balance—studies with LGG

LGG supplementation was studied in six clinical trials. Apostolou et al. (2001) demonstrated that LGG supplementation resulted in an increase in numbers of bacteroides, clostridia, and total bacteria in both healthy (n = 9) and milk-hypersensitive (n = 8) subjects after 4 weeks of LGG supplementation. In healthy subjects, the number of bifidobacteria increased as well. The authors interpret these findings as LGG having a nonspecific proliferation-enhancing effect on the anaerobic gut microbiota. They state that anti-inflammatory properties of LGG are likely to be due to factors other than modulation of the composition of the intestinal microbiota, for example the ability to degrade milk proteins to smaller peptides and amino acids and to directly down-regulate the inflammatory response. However, this cannot be directly concluded from the data.

Gueimonde et al. (2006) looked at the transfer of specific bifidobacteria from 53 mothers to their children after four weeks of maternal consumption of placebo or LGG. In the mothers, no effect of LGG on bifidobacteria composition was observed. In this study, the presence of specific bifidobacteria strains was determined, but not quantified. No shifts in overall microbiota composition were addressed. In a randomized, controlled trial in healthy adults (N = 25), a specific increase in the LGG-related bacteria was observed during the intervention, but no other changes in the composition or stability of the microbiota were detected compared with the placebo group, indicating that the probiotic intervention did not alter the overall microbial stability (Lahti et al., 2013). After the intervention, lactobacilli returned to their initial levels. In a recent substudy of Lahti et al., dominant functionalities of the gut microbiome were characterized by conducting a comprehensive fecal metaproteome analysis in 16 healthy adults (Kolmeder et al., 2016). No significant changes in the metaproteome were attributable to the probiotic intervention. The consumption of LGG (1.55 × 10^{10} CFU/d) did not lead to a systematic change of the identified peptides and their associated function. This was in line with the previous findings from the same cohort that the LGG
intervention did not change the overall composition of the fecal microbiota. In samples collected after receiving the intervention, LGG represents a tiny fraction (up to 0.1%) of the total fecal community (Kolmeder et al., 2016), so barely detecting LGG-specific proteins is not surprising.

Eloe-Fadrosh et al. (2015) reported on the effects of LGG supplementation (10^{10} CFU BID) on microbiota composition in 12 elderly volunteers. The overall community composition was stable as assessed by 16S ribosomal RNA profiling. The dominant microbial taxa were not modified by probiotic consumption, although the level of sequencing was not sufficient to resolve whether rare members were perhaps impacted. The transcriptional response of the gut microbiota, however, was modulated by probiotic treatment. Increased expression of genes involved in flagellar motility, chemotaxis, and adherence from *Bifidobacterium* and the dominant butyrate producers, *Roseburia* and *Eubacterium*, was observed during probiotic consumption, suggesting that LGG may promote interactions between key constituents of the microbiota and the host epithelium.

In a small open-label study by Benno et al. (1996), eight healthy subjects were given LGG yogurt (1.4 × 10^8 CFU/mL) for four weeks and five subjects were given LGG on a single occasion. In subjects receiving LGG for four weeks, lecithinase-negative clostridia decreased significantly (*p* < 0.05), whereas no significant differences were observed in the total counts of *Bacteroidaceae*, *Eubacterium*, *Peptostreptococcus*, *Veillonella*, and *Megasphaera*. *Bifidobacterium* and *Lactobacillus* increased significantly (*p* < 0.05).

### 3.6.3. Microbiota balance—studies with BB-12 + LGG

A single trial was identified that studied the combined effect of LGG and BB-12 on the gut microbiota balance. Rafter et al. (2007) and Roller, Clune, Collins, Rechkemmer, and Watzl (2007) reported on the same trial and randomized 40 patients who had been diagnosed with adenomatous polyps (and 34 cancer patients, not discussed here) to a synbiotic product with BB-12 and LGG (log10 CFU) or control. The relative numbers of *Bifidobacterium* and *Lactobacillus* increased in the active treatment group, whereas other groups of bacteria (*Bacteroides* and *Enterococcus*) were not affected. In polyp patients, the number of *C. perfringens* decreased significantly. However, it should be kept in mind that this study tested not just LGG and BB-12, but also a combination with a specific prebiotic, which in itself may have affected the gut microbiota composition, as has been demonstrated in previous research (Femia et al., 2002; Kruse, Kleessen, & Blaut, 1999; Tuohy, Kolida, Lustenberger, & Gibson, 2001).

### 3.6.4. Microbiota balance—conclusions

The seven studies reviewed here provide little information on changes in gut microbiota composition, whether transient or sustained, induced by BB-12 consumption. No changes have been reported that could be considered harmful. Larger and more-targeted studies are required to investigate whether BB-12 ingestion can influence the gut microbiome over a longer period of time and whether such changes could have a biologically relevant health benefit. The six LGG trials indicate LGG supplementation does not seem to alter the overall composition of the fecal microbiota in healthy adults. However, it cannot be excluded that LGG has an impact on specific microbial taxa with low abundance, and it may affect interactions of other microbiota within the gut epithelium. Studies so far have mainly addressed fecal microbiota composition; potential effects of LGG on the small intestinal microbiome and potential consequences of such an effect have not been addressed yet.

### 3.7. Immune support

Dysbacteriosis of the GI system is associated with several human diseases, including inflammatory bowel disease, colorectal cancer, and infections (Artis, 2008; Karin, Lawrence, & Nizet, 2006). Probiotics may promote human health by modulating the immune system (Hemarajata & Versalovic, 2013), producing soluble factors and metabolites, such as vitamins and short-chain fatty acids. These compounds alter the function of intestinal epithelium and mucosal immune cells, resulting in the production of cytokines and related factors (Preidis & Versalovic, 2009). Clinical implications of
probiotic immunomodulation are broad and may depend on the consumed strain. Here we provide an overview of the immunomodulatory effects of BB-12 and LGG. Useful markers to interpret the modulation of immune function in the general population have been described in a guidance paper by Albers et al. (2013). These include selected *ex vivo* markers of immune functions (e.g. natural killer (NK) cell activity, phagocytosis, and responsiveness of specific T-cell subpopulations) and selected basal markers essential in the exertion of critical immune functions, such as mucosal immunoglobulin (Ig)A for infection resistance, C-reactive protein (CRP), and inflammatory mediators to indicate low-grade inflammation (Albers et al., 2013). A summary of clinical trials is provided in Table S7 (Supplemental Online Materials).

### 3.7.1. Immune support—studies with BB-12

A total of seven studies were identified that report the immunomodulatory effects of BB-12. The study by Rizzardini et al. (2012) with 211 healthy volunteers appeared well designed and evaluated relevant parameters. Significant effects were observed on vaccine-specific antibody responses in plasma and saliva and non-specific circulating antibodies after six weeks of BB-12 supplementation (1 x 10^9 CFU) compared with placebo. No effect was seen on markers of innate immune function or cytokines.

The study by Kekkonen et al. (2008) randomized 16 healthy subjects to a milk-based fruit drink with added BB-12 (3.5 x 10^10 CFU) and 16 subjects to placebo for three weeks. The only effect observed for BB-12 was a lower *ex vivo* influenza virus-stimulated IL-2 production. No effect was seen on other mechanistic markers or on the most clinically relevant marker, salivary secretory IgA (SIgA). The study population was relatively small, no information was given on required sample size, and given the large intra- and interindividual variations in immune response markers, statistical power may have been insufficient to detect significant differences.

Schiffrin, Brassort, Servin, Rochat, and Donnet-Hughes (1997), Schiffrin, Rochat, Link-Amster, Aeschlimann, and Donnet-Hughes (1995) also used a fermented milk drink as carrier for BB-12 (1 x 10^9 CFU BB-12). Twenty-eight healthy volunteers consumed regular milk during a three-week run-in period before consuming six weeks of BB-12 milk. An effect was reported on phagocytic activity (innate immune function), whereas there was no effect on *ex vivo* adaptive immune function. Both Schiffrin papers were discussed in an EFSA Scientific Opinion on the health benefits of BB-12 (EFSA Panel on Dietetic Products, 2011). The panel considered that no conclusions can be drawn from this study for the scientific substantiation of the claimed effect (immune defense against pathogens).

The study by Meng et al. (2016) was reported to be a randomized crossover design, but all values were compared with baseline (which is not randomized) and not with the reference group. Healthy adults (n = 30) received the following treatments in random order: (1) yogurt smoothie alone; smoothie with BB-12 (log10 ± 0.5 CFU/d) added (2) before or (3) after yogurt fermentation, or (4) BB-12 in capsule form. For BB-12 in capsules, no appropriate control group was included. Outcome parameters were *ex vivo* cytokine secretion, NK cell function, and gene expression in PBMCs in a small subset of subjects. Except for yogurt smoothies with BB-12 added postfermentation, results from the 30 participants included in the analysis demonstrated elevated *in vitro* stimulated IL-2 secretion and NK cell cytotoxicity. Participants in the postfermentation group had significantly lower expression of toll-like receptor (TLR)-2 on CD14 + HLA-DR + cells (p < 0.02) and reduction in TNF-α secretion (p < 0.05) compared with baseline. This may suggest that the yogurt with BB-12 added after fermentation has some effects that are not seen in the other groups. However, this direct comparison between groups was not made.

The safety study by Merenstein et al. (2015) compared a yogurt drink containing BB-12 to placebo. In a subset of the population (n = 15), changes in gene expression associated with regulation and activation of immune cells were measured. In the BB-12 group, changes were most visible on day 14. However, the study was not specifically designed to test effects on immune response. Subjects
furthermore suffered from upper respiratory infections and received antibiotic treatment both of which will have affected immune responses. Therefore, results can therefore not be directly translated to a healthy population or a population not receiving medication.

Kabeerdoss et al. (2011) administered 200 mL of probiotic yogurt containing BB-12 (10^9 CFU in 200 mL) to 26 healthy women for three weeks after a week-long run-in with control yogurt. Compared with baseline, fecal IgA increased during the probiotic intervention period (p = 0.0184) and returned to normal afterward. Ouwehand et al. (2008) administered BB-12 (10^6 CFU/d) to 55 elderly nursing home residents, in a fermented oat drink for six months. The paper reports changes in three serum cytokine groups. Serum IL-10 and TNF-α were lower in the BB-12 group than in the placebo group, but this difference existed already at baseline. No changes were observed during the study period. These data contribute little to mechanistic understanding and do not support clinical efficacy.

3.7.2. Immune support—studies with LGG
Sixteen articles were identified that report on the immunomodulatory properties of LGG. Four of these studies described immune responses following vaccination and may therefore provide the most useful information for the interpretation of immune changes (Albers et al., 2013). In healthy volunteers receiving an oral S. typhimurium vaccine, no difference was observed between the LGG (4 × 10^10 CFU/d) and placebo groups in the number of specific antibody-secreting cells (Fang, Elina, Heikki, & Seppo, 2000). However, LGG stimulated specific IgA-secreting cells in a greater number of subjects compared with placebo. As this was a very small study (10 subjects per group), these effects are highly speculative and would need to be confirmed in other studies. No other studies on the effect of LGG on Salmonella vaccination have been reported since then.

Davidson, Fiorino, Snydman, and Hibberd (2011) used a live attenuated influenza vaccination (LAIV), in a relatively small (N = 42) but well-designed proof-of-concept study. For the H3N2 strain, but not the other two strains, significantly more subjects receiving LGG (10^1 CFU/d) compared with subjects receiving placebo had a protective titer 28 days after vaccination (84 vs. 55%; p < 0.05). In this study, LGG was first administered on the day of vaccination. The authors state to have had insufficient power to detect small and moderate effects on vaccine responsiveness. Subjects previously vaccinated with an inactivated trivalent influenza vaccine (TIV) may have had lower antibody responses to subsequent LAIV, and this may have impacted the immune responses in 49% of the subjects who had previously received TIV. In addition, a more profound immune adjuvant effect may be demonstrated in groups that traditionally have a poor response to the influenza vaccine, such as the elderly. Notwithstanding these limitations, a statistically significant temporary effect was observed for protection against one of the influenza strains. Administration of LGG (10^10 CFU/d) one week prior to an oral polio booster was associated with increased poliovirus neutralizing antibody titers and poliovirus-specific IgA and IgG in a double-blinded study with 66 healthy volunteers (de Vrese et al., 2005). The response was most pronounced for poliovirus serotype-1-specific neutralizing antibodies (p = 0.083).

The study reported by Kumpu et al. (2015) and Tapiovaara et al. (2016) looked at the response of healthy volunteers (N = 59) to an experimental infection with rhinovirus type 39 (RV-39) three weeks after starting intervention with live or heat-inactivated LGG (10^9 CFU/d) or a control drink. Kumpu et al. (2015) reported no significant differences in symptom scores or high-sensitivity CRP (hsCRP) between the groups. Tapiovaara et al. (2016) observed a nonsignificant difference in human rhinovirus (HRV) load. This study was reported to be a pilot study and the first to use an experimental rhinovirus model to study probiotic efficacy in viral infections. The study was designed to collect pilot data for potential studies, and formal sample size calculations were not performed. Based on the pilot results, the authors state that, with a power of 80% and a significance level of 0.05, 102 subjects per group would be needed to detect a difference between the live LGG and the control group in the number of infected subjects, and 103 subjects to detect a difference in the symptom scores during the five days following the virus challenge.
Pelto, Isolauri, Lilius, Nuutila, and Salminen (1998) performed challenges with milk in milk-hypersensitive and healthy adults ($N = 17$) with or without LGG ($2.6 \times 10^8$ CFU/d) in a crossover design. The challenge-induced immunoinflammatory response was recorded by measuring the expression of phagocytosis receptors on neutrophils and monocytes prior to and after the challenge. LGG was shown to down-regulate the expression of phagocytosis receptors after milk consumption in milk-hypersensitive adults, whereas it stimulated receptor expression in the healthy subjects. A weakness in the design and the reporting of this study is that the distinction between milk-hypersensitive and milk-tolerant subjects was made based on results obtained in the study, and it is not clear whether this subgroup analysis was intended from the start, or decided on post hoc. Although the subgroup analysis may be quite relevant, it is an approach that is looked upon very critically by EFSA when evaluating the value of studies in supporting a health claim.

Kekkonen et al. (2008) observed in a study with 68 healthy subjects that LGG intervention ($3.5 \times 10^{10}$ CFU/d) lowered hsCRP compared with placebo ($p = 0.014$), and that in the LGG group pro-inflammatory TNF-$\alpha$ production in the gram-positive bacteria-stimulated PBMC was reduced. However, there were no significant effects for a range of other cytokines and ex vivo stimulations, nor for serum immunoglobulins and immune cells, saliva SIgA, and serum cytokines. Schultz et al. (2003) did find decreased ex vivo secretion of pro-inflammatory cytokines (TNF-$\alpha$, IL-6, IFN-gamma), as well as an increased secretion of suppressive cytokines (IL-10, IL-4), following LGG intervention ($2 \times 10^9$ CFU/day). This small-scale study in healthy volunteers ($N = 10$) also demonstrated that oral administration of LGG leads to an increased response of peripheral CD4 + T-lymphocytes to intestinal bacterial components. However, due to the unrandomized and unblinded design of this study, and due to the limited number of participants, results should be interpreted cautiously. On the other hand, in a similar study by Boyle et al. (2008), a reduction in CD4 + T-cell-proliferative response was observed in PBMC exposed to heat-killed LGG after LGG administration ($1.8 \times 10^{10}$ CFU/d) in vivo ($N = 11$). An increase in the plasmacytoid DC (pDC) phenotype of dendritic cells was also observed. In this small sub-study ($N = 11$), no control group was used. The main study focused on differences in immune markers in cord blood cells of 73 mother-child pairs in a double-blind randomized study. Results in the small adult group were consistent with antigen-specific tolerance induction. In a similar study with 68 mother-child pairs (Kopp et al., 2008), PBMCs were collected from the mothers. No difference in proliferative capacity of PBMCs in response to any of the stimuli, including inactivated LGG ($5 \times 10^9$ CFU/d), was found between the LGG and placebo groups for mothers giving birth.

Ou et al. (2012) also studied pregnant women. One hundred ninety-one women were randomized to a placebo or LGG intervention ($10^{10}$ CFU/d) in this study, beginning at 24 weeks’ gestation. No significant effect of probiotic supplementation on maternal IgE levels was observed between the placebo and LGG groups. No conclusions can be drawn from the small pilot study performed by Amati et al. (2010) in free-living elderly. The study was inadequately controlled, used a symbiotic product with relatively low numbers of LGG ($10^7$ CFU), and was performed in only 10 subjects. Differential expression of genes after consumption of LGG gives insights into the mechanisms of action that may result in specific health benefits.

Gene expression was determined in duodenal biopsies taken from otherwise healthy patients with esophagitis ($N = 6$) in the study by Di Caro et al. (2005). All patients received medication, but half of them also ingested LGG ($6 \times 10^6$ CFU/d, for 30 days). The biopsies were taken from a healthy part of the GI tract. Patients treated with anti-inflammatory drugs, medications interfering with the immune response, or laxatives in the 30 days preceding enrollment were excluded. Therefore, the subjects in this study are considered representative for the general population. LGG affected expression of genes involved in the immune response and inflammation (transforming growth factor-$\beta$ and TNF family members, cytokines, nitric oxide synthase 1, defensin-$\alpha1$), apoptosis, cell growth and cell differentiation (cyclins and caspases, oncogenes), cell–cell signaling (intercellular adhesion molecules and integrins), cell adherence (cadherins), and signal transcription and transduction. As the authors state in their discussion, the list of genes resulting from a microarray analysis needs biological validation and comprehension of the biological meaning of specific genes and pathways alterations. To
understand the clinical value of these data, it is crucial to compare the probiotic effects in different segments of the GI tract, especially in the colonic mucosa, and in different clinical settings to evaluate the regulatory network governing their activity in order to scientifically support clinical decisions.

In another study (van Baarlen et al., 2011) duodenal biopsies were taken from seven healthy volunteers in a crossover design. These subjects were exposed continuously to the probiotic ($1.68 \times 10^{-1}$ CFU of LGG) or placebo intervention for a period of 6 h, after which biopsies were taken. Consumption of LGG appeared to lead to differential expression of genes participating in signaling networks involved in wound repair and healing, angiogenesis, IFN response, calcium signaling, and ion homeostasis. Treatment-specific response pathways were identified in all volunteers despite the large variation between transcriptomes obtained from the individual volunteers. Moreover, regulatory genes with central roles in networks showed markedly less variable expression between persons than genes that occurred less centrally in networks and that could be modulated directly and indirectly by multiple networks.

The effect of LGG on gene expression in whole blood cells was investigated in an open-label study by Solano-Aguillar et al. (2016) with 15 elderly subjects before and after administration of LGG ($2 \times 10^{-1}$ CFU/d) for 28 days. Down-regulation of overlapping genes involved with cellular movement, cell-to-cell signaling interactions, immune cell trafficking, and inflammatory response were observed. Pro-inflammatory cytokine IL-8 decreased during LGG consumption and returned to baseline one month after discontinuation ($p = 0.038$). No difference in other pro- or anti-inflammatory plasma cytokines was observed by Hibberd et al. (2014), reporting on the same trial. Piirainen et al. (2008) randomized 38 adolescents and young adults with birch pollen allergy (combined with oral allergy syndrome) to LGG ($2 \times 10^{10}$ CFU/d) or placebo for 5.5 months. All subjects received an oral apple challenge before, during, and after the pollen season. After 5.5 months, rBet v1- and rMal d1-specific IgA levels had increased from baseline in the LGG group compared with placebo ($p = 0.02$ for each comparison). rBet v1-specific IgE serum levels did not differ between the groups. Publishing on the same trial as Kekkonen et al. (2007), Moreira, Kekkonen, Korpela, Delgado, and Haatela (2007) reported that no significant difference on serum eosinophil cationic protein (ECP) or total IgE was observed between marathon runners ($N = 141$) who received LGG ($1 \times 10^{10}$ CFU/d) or placebo during pollen season.

3.7.3. Immune support—studies with BB-12 + LGG

Three studies report on the effects of multispecies formulation containing both BB-12 and LGG. Two were performed in patient populations that still may be considered representative for the general population. Some mechanistic information may be derived from a study by Tomasik, Yolken, Bahn, and Dickerson (2015) in 65 schizophrenic patients. Five out of 47 immune markers changed ($p < 0.10$) in the probiotic group ($10^9$ CFU) compared with placebo after two weeks of intervention. In silico pathway analysis revealed that probiotic-induced alterations were related to regulation of immune and intestinal epithelial cells through the IL-17 family of cytokines. Rafter et al. (2007) and Roller et al. (2007) performed a study in 40 patients who had been diagnosed with adenomatous polyps (and 34 cancer patients). The symbiotic intervention (BB-12 & LGG; $> \log_{10}$ CFU/g) prevented an increased ex vivo secretion of IL-2 by PBMCs in the polyp group, which seems to be in line with findings for BB-12 (Kekkonen et al., 2008). However, it should be kept in mind that this study tested not just LGG and BB-12, but a combination with a specific prebiotic, which in itself may have affected immune function through effects on gut microbiota composition. Hoppu, Isolauri, Laakso, Matomäki, and Laitinen (2012) randomized 125 pregnant women to a dietary intervention with or without probiotic supplementation ($10^{10}$ CFU/d) or a control intervention. The breast milk concentrations of TNF-α, IL-10, IL-4, and IL-2 were higher in both dietary intervention groups compared with control. However, no apparent effect of BB-12 was observed.
3.7.4. Immune support—conclusions
More than 20 studies have evaluated the effects of either BB-12 or LGG supplementation on immune function, but the interstudy differences on quality are vast. BB-12 has been shown to be capable of modulating immune function, but results for various markers are inconsistent. Differences between studies could in part be attributed to the inadequate study design utilized by some research groups. There is one study that supports an effect of BB-12 on clinically relevant markers of immune defense against pathogens in the upper respiratory tract (vaccine-specific immune response and non-specific circulating antibodies (Rizzardini et al., 2012). LGG clearly modifies immune response and inflammation markers, but biological relevance of many of the biomarker changes is difficult to interpret. and therefore these changes are not considered direct evidence for a health benefit. Direct evidence may be obtained from studies with experimental infection models or in vaccination trials. Results for LGG in such type of trials so far are promising, but need confirmation in larger, appropriately powered studies.

3.8. Vaginal/urogenital health
Vaginosis and urinary tract infections (UTIs) affect the lives of millions of people each year and pose a significant burden on patient quality of life (Foxman, Barlow, D’Arcy, Gillespie, & Sobel, 2000). A growing body of evidence suggests that probiotics may protect against urogenital infections, including UTIs (Antonio, Hawes, & Hillier, 1999; Falagas, Betsi, Tokas, & Athanasiou, 2006; Hawes et al., 1996). In this section, all clinical trials that report the effects of BB-12 and LGG supplementation on vaginal/urogenital health are reviewed. An overview of clinical studies is provided in Table S8 (Supplemental Online Materials).

3.8.1. Vaginal/urogenital health—studies with LGG
Two studies report that LGG has no clinically significant effect on the incidence of UTI or vaginosis. Reid, Beuerman, Heinemann, and Bruce (2001) demonstrated that treatment with L. rhamnosus GR-1 + L. fermentum RC-14 (8 × 10^8 and 6 × 10^9, respectively), but not LGG (10^10 CFU/d), reduced the number of patients with bacterial vaginosis (N = 42, total). Kontiokari and colleagues (2001) showed that treatment with cranberry juice, but not LGG (4 × 10^10 CFU), significantly reduced the cumulative rate of first UTI recurrence in 150 women with UTIs caused by E. coli (16 vs. 39% had at least one occurrence after six months respectively, p < 0.005). One trial suggests that LGG may be effective in the treatment of vaginosis (Hilton, Rindos, & Isenberg, 1995). Vaginal suppositories impregnated with LGG (10^9 CFU) were administered twice daily for 7 days to 28 women with recurrent vaginosis (>5 per year). All women reported subjective improvement of symptoms after the LGG intervention. Furthermore, all the women showed decreased erythema and discharge on repeat examination. However, the trial was nonrandomized and uncontrolled; only five women had considerable colonies of C. albicans prior to the trial and 15 women used antifungal agents just before the intervention, which probably caused the low number of women with considerable colonies of C. albicans.

3.8.2. Vaginal/urogenital health—conclusions
It appears that an oral LGG intervention is not effective in preventing UTIs, in contrast to other probiotic strains which have shown potential (no prior study with BB-12). However, only two clinical trials have been performed with oral LGG, with relatively few participants (Kontiokari et al., 2001; Reid et al., 2001), warranting cautious interpretation. Vaginal suppositories impregnated with LGG, on the other hand, did appear to reduce UTI symptoms and severity in one study. But the methodological limitations inherent to the study design of this trial are of concern.

3.9. Respiratory infections
Acute respiratory infections, such as rhinopharyngitis (the common cold) in the upper respiratory tract or bronchitis in the lower respiratory tract, represent the most common class of illnesses in humans, with due impact on health-related quality of life (Lehtoranta et al., 2014; Smith, Rigassio-Radler, Denmark, Haley, & Touger-Decker, 2013). As the immune response of the host plays a key role in the pathogenesis of respiratory infections (Turner, 1997) and probiotics have been ascribed immunomodulatory properties, there is increasing interest in the therapeutic potential of specific
probiotic species and strains with regard to respiratory infections (Kekkonen et al., 2007). Clinical trials in specific target groups suggest potential beneficial effects of probiotics on the incidence, duration, or severity of respiratory infections (Fujita et al., 2013; Garaiova et al., 2015; Hao et al., 2011). Probiotic therapy may therefore provide an alternative to conventional preventive or therapeutic interventions targeting acute respiratory infections, which may lack in efficacy (e.g. over-the-counter drugs for viral respiratory infections, antibiotics for bacterial respiratory infections that prove resistant). An overview of the trials that were retrieved is provided in Table S9 (Supplemental Online Materials).

3.9.1. Respiratory infections – studies with BB-12
A single clinical trial regarding BB-12 and respiratory infections was retrieved. In this four-period crossover study, Meng et al. (2016) investigated the effects of yogurt smoothies and capsules containing BB-12 (log 10 ± 0.5 CFU/d) on NK cell and T-cell function (as discussed above in “Immune support”) along with self-reported cold/flu outcomes in healthy adults. Subjects received yogurt smoothies with BB-12 added prefermentation, with BB-12 added postfermentation, without BB-12, or a capsule containing BB-12. Treatment periods lasted four weeks each and were separated by two-week washout periods. Elevated in vitro stimulated IL-2 secretion and NK cell cytotoxicity observed in all groups (see “Immune support” above), except for the postfermentation group, were associated with a decreased incidence of upper respiratory infections (URTIs) as measured by self-reported questionnaires. But again, all values were compared with the (nonrandomized) baseline instead of with controls (e.g. yogurt without BB-12). Direct comparisons between groups were not conducted. Moreover, no appropriate control group was included for the BB-12 capsule treatment, adding to the suboptimality of the study design.

3.9.2. Respiratory infections—studies with LGG
Two clinical trials were retrieved pertaining LGG and respiratory infections. Tapiovaara et al. (2016) and Kumpu et al. (2015) both reported on the same trial involving 59 healthy volunteers, as previously discussed above. Subjects were randomized to receive 100 ml fruit juice containing either live or heat-inactivated LGG (10⁹ CFU) or control fruit juice once a day for six weeks. Subjects were challenged with HRV immunotype 39 by intranasal inoculation after three weeks. As previously mentioned, a nonsignificant difference in HRV load was reported by Tapiovaara et al. (2016) (due to the limited power of this study). However, the HRV load positively correlated with clinical symptom scores on days 2 and 5 (p < 0.001 and p = 0.034, respectively). Kekkonen et al. (2007) investigated the effect of LGG on respiratory infections, the number of healthy days, and GI symptom episodes in marathon runners. The investigators included 141 subjects in their randomized, double-blind, placebo-controlled, parallel-group intervention study. No significant difference in the number of respiratory infections was observed between the LGG and control group in this study (nor on serum ECP or total IgE) (Moreira et al., 2007).

3.9.3. Respiratory infections—studies with BB-12 + LGG
Two studies were retrieved pertaining to the combined effect of BB-12 and LGG on respiratory infections. Reporting on the same randomized, placebo-controlled intervention study, Kalima et al. (2016) and Lehtoranta et al. (2014) investigated whether chewable tablets containing BB-12 and LGG (5 × 10⁹ CFU and 2 × 10⁹ CFU, respectively) could decrease the nasopharyngeal presence of respiratory viruses in Finnish military conscripts. A total of 983 subjects were randomized to receive either BB-12/LGG-containing tablets (n = 50.2%) or control chewing tablets (n = 49.8%) twice daily for 150 days or 90 days (recruits or reserve officer candidates, respectively). Nasopharyngeal swab samples were collected whenever conscripts sought treatment for symptoms of URTI. A total of 239 samples were collected; 90 from the active treatment group and 102 from control subjects (Lehtoranta et al., 2014). Lehtoranta et al. reported that fewer viruses were detected in the probiotic intervention group compared with the control group but that these differences were, however, not statistically significant. Furthermore, BB-12/LGG-containing tablets did not decrease the total number of picornaviruses, rhinoviruses, or enteroviruses in recruits, reserve officer candidates, or in both groups combined. A monthly investigation of samples testing positive for picornavirus did
demonstrate a significant difference between probiotics and controls; In September, picornavirus samples were three times fewer in the probiotic group when compared with controls \( (p = 0.007) \). No significant differences between the two groups were demonstrated for other months, however, and neither were any differences identified between placebo and controls for any of the other respiratory viruses.

Looking at symptom incidence and duration, Kalima et al. (2016) reported no overall statistical differences. Probiotic intervention was associated with a reduction of specific symptoms for respiratory infection in military recruits (i.e. less eye redness and dyspnea), but not in reserve officer candidates. The number of drop-outs during the study was very large, and the compliance was relatively low. Statistical power may not have been sufficient to detect an existing difference.

The combination of BB-12 and LGG was also used in a well-designed study among college students where compliance was better (Smith et al., 2013). This study investigated the effects of probiotic powder containing BB-12 and LGG (minimum daily dose of \( 1 \times 10^9 \) CFU each) or placebo on health-related quality of life outcomes in those who developed URTI. A total of 231 healthy college students were randomized to receive probiotic intervention \( (n = 101 \text{ [in final analysis]} \) or placebo \( (n = 97 \text{ [in final analysis]} \) for 12 weeks in a double-blinded fashion. A validated questionnaire (Wisconsin Upper Respiratory Symptom Survey-21 [WURSS-21]) was used for the assessment of symptoms. Median duration of URTI proved to be significantly shorter \( (2 \text{ days, } p = 0.001) \) and median self-reported severity scores proved to be significantly lower \( (34\%, p = 0.0003) \) in the probiotic intervention group compared with control, indicating higher health-related quality of life with probiotics during URTI. URTI symptoms result from the inflammatory response of the host towards the virus, not from the viruses themselves. Therefore, these findings may be partially explained by modulation of the inflammatory response. However, in this study no mechanistic immune markers were assessed to confirm this. This study supports a causal relation with reduction of URTIs, but, although likely, immune mediation of this effect has not directly been shown.

### 3.9.4. Respiratory infections—conclusions

Intervention with BB-12 + LGG is suggested to reduce the incidence of upper respiratory infections and improve health-related quality of life, according to the two clinical studies reviewed here. Changes in immune markers were furthermore associated with reduced respiratory infections in the study by Meng et al. (2016). Combining clinical outcome parameters with such changes in immune parameters may provide the most valuable information to support a causal relationship. Nevertheless, changes on respiratory infection incidence are not consistently observed and most studies presented methodological limitations (e.g. inadequate controls).

### 3.10. Metabolism regulation in pregnancy

The gut microbiota are seen as a key organ in regulating host energy homeostasis, representing a crucial factor in the energy harvest from diet and energy storage in the host. Microbiota are suggested to modulate plasma concentrations of lipopolysaccharides and to affect insulin sensitivity and, thereby, the risk of metabolic syndrome (Cani & Delzenne, 2007; Turnbaugh et al., 2006). During pregnancy, a balanced metabolism has been shown to reduce the risk of pregnancy-related complications, with long-term health benefits for both mother and infant (Crowther et al., 2005). Furthermore, it is widely acknowledged that maternal nutrition affects the nutritional and immunological environment of the fetus, in turn affecting the infant’s health (Barger, 2010; Martin-Gronert & Ozanne, 2006). In this section however, we primarily focus on clinical effects on the mother. An overview of the retrieved clinical trials is provided in Table S10 (Supplemental Online Materials).

#### 3.10.1. Metabolism regulation in pregnancy – studies with BB-12 + LGG

Three clinical trials focusing on metabolism regulation during pregnancy were identified that met the current review’s inclusion criteria, all of which were conducted by investigators associated with the University of Turku, Finland, and applying similar study designs. In a double-blind, placebo-controlled trial, Kaplas, Isolauri, Lampi, Ojala, and Laitinen (2007) randomized 30 healthy pregnant...
women to receive capsules containing BB-12 and LGG at a daily dose of 10^9 CFU each as well as dietary counseling (“diet/probiotics,” n = 10), dietary counseling with placebo (“diet/placebo,” n = 12), or placebo alone (“control,” n = 8). A comparison between the diet/probiotics and diet/placebo groups in this small-scale study demonstrated that the probiotic intervention with BB-12 and LGG increased concentrations and proportions of precursor fatty acids for eicosapentaenoic acid and arachidonic acid, 20:4n-3 and dihomo-γ-linolenic acid, respectively (p < 0.05). Furthermore, it demonstrated that probiotic intervention increased the concentration of linoleic acid (p < 0.05). Fatty acids may trigger a cascade of events, potentially resulting in health benefits for both mother (maternal risk of pregnancy-related complications) and child (e.g. improved neurological development and alleviation of inflammatory response) (Kaplas et al., 2007).

In a larger trial (N = 256) with similar treatment and control groups (i.e. “diet probiotics,” “diet/placebo,” and “control”), Laitinen, Poussa, and Isolauri (2009) examined the effect of probiotic intervention with capsules containing BB-12 and LGG at a daily dose of 10^{10} CFU on glucose metabolism in pregnant women. Glycemic concentrations were lowest in the diet/probiotics group (n = 64) compared with the diet/placebo group (n = 64) and control group (n = 66), both during pregnancy and over a 12-month postpartum period (4.5, 4.6, and 4.6 mmol/L, respectively; and 4.9, 5.0, and 5.0 mmol/L, respectively; both significant at p = 0.025). In the third trimester, furthermore, the diet/probiotics group demonstrated a significantly reduced risk of elevated glucose concentration compared with the control group (odds ratio [OR] 0.31; 95% CI: 0.12, 0.79; p = 0.012), while the diet/placebo group did not (OR: 1.26; 95% CI: 0.59, 2.69; p = 0.553). Similarly, over the postpartum period, the risk of elevated plasma glucose concentration remained lower in the diet/probiotics group, although not significantly, but not in the diet/placebo group. Pathological glucose test results, in addition, were lowest in the diet/probiotics group (37% of subjects) compared with diet/placebo (58%) and control (57%) groups, and the results indicated improved insulin sensitivity in the diet/probiotics group. However, neither of these results demonstrated statistical significance.

Studying the same cohort and applying the same design and interventions, Luoto, Laitinen, Nermes, and Isolauri (2010) furthermore reported a significantly reduced risk of gestational diabetes mellitus in the diet/probiotics group when compared with the control group (OR: 0.27; 95% CI: 0.11, 0.62; p = 0.002), while the risk reduction demonstrated no statistical significance when comparing the diet/placebo group with controls (OR: 1.08; 95% CI: 0.55, 2.12; p = 0.823). In the Hoppu et al. study (2012) that randomized pregnant women (N = 125) into three study groups (i.e. diet/probiotics, diet/placebo, and control/placebo groups), the effects on dietary intake and on breast milk fatty acids and cytokines were also investigated. No statistically significant differences were demonstrated between diet/probiotics and diet/placebo groups regarding dietary intake. Regarding breast milk fatty acids and cytokines, however, the content of γ-linolenic acid (18:3n-6) was significantly higher in the diet/probiotics group than in the diet/placebo group (p < 0.05).

3.10.2. Metabolism regulation in pregnancy—conclusions

The results of three clinical trials, originating from the same university, indicate that intervention with LGG + BB-12 in pregnant women may positively influence blood glucose levels, insulin sensitivity, breast milk fatty acid precursors, and cytokines. Probiotic intervention may hence play a key role in managing the risks of gestational diabetes mellitus (Luoto et al., 2010), and this hypothesis should to be further explored and confirmed by other research groups.

3.11. Oral health

Oral diseases such as caries, periodontal disease, or edentulism are an ongoing public health and economic burden worldwide. Collectively, they represent the most common chronic diseases in humans and significantly impact quality of life and overall health and well-being (Ng & Leung, 2006). While lactobacilli have long been of interest to dental research for their cariogenic properties (Caglar, Kargul, & Tanboga, 2005), conversely it has also been suggested that specific probiotic strains may be useful for the prevention of oral disorders (Teughels, Looszen, & Quirynen, 2011). This section
reviews all identified clinical trials investigating the effects of BB-12 and LGG on oral health. An overview of the studies is provided in Table S11 (Supplemental Online Materials).

3.11.1. Oral health—studies with BB-12

Two clinical trials examining effects of BB-12 on oral health were retrieved, both focusing on different outcome parameters. Gueimonde et al. (2016) found no statistically significant differences between treatment and placebo groups when randomizing 54 adults to receive either placebo chewing gum ($n = 19$), chewing gum containing LGG ($n = 18$), or chewing gum containing other probiotic strains for 12 weeks. Subjects were instructed to chew for 30 min twice daily, totaling the daily dosage at $2.87 \times 10^8$ CFU. Emphasizing saliva flow rate, saliva IgA levels, and saliva pH as main outcome parameters, the trial demonstrated increases for all three parameters in both groups. The lack of statistically significant differences between the groups may be partly due to the fact that both groups received gum containing xylitol, making the placebo not completely inert.

Caglar et al. (2008) focused on the effect of BB-12 on the number of salivary *mutans streptococci* and lactobacilli, examining whether short-term consumption of ice cream containing $1 \times 10^7$ CFU/gram of BB-12 affected those parameters. In a double-blind, randomized, crossover study involving 24 young adults, they found that daily consumption of 53 grams of BB-12 ice cream provided a statistically significant reduction of salivary *mutans streptococci* after 10 days ($p < 0.05$), while lactobacilli levels were unaltered. The study’s sample size was limited, and it should furthermore be noted that the study’s main outcome parameter, salivary *mutans streptococci* levels, is only an intermediate endpoint for which it remains to be investigated whether lower levels provide actual benefit to the patient in terms of caries reduction.

3.11.2. Oral health—studies with LGG

A single study was retrieved investigating the effects of LGG on acid production and the levels of *mutans streptococci* in dental plaque. In their randomized, double-blind, crossover trial, Marttinen et al. (2012) found no effect on either outcome parameter after enrolling 13 students who were randomized to receive tablets containing LGG ($1.96 \times 10^8$ CFU) or *Lactobacillus reuteri* twice a day for two weeks. Though the authors argue that the use of sensitive paired comparisons would have detected changes in the variables, the small number of subjects has an apparent diminishing effect on trial power and quality.

3.11.3. Oral health—studies with BB-12 + LGG

A single study was retrieved investigating the effects of BB-12 and LGG combined on oral health. In a randomized, double-blind, placebo-controlled trial, Toiviainen et al. (2015) evaluated whether orally administered LGG and BB-12 affected the number of salivary *mutans streptococci*, amount of plaque, gingival inflammation, and the oral microbiota. Twenty-nine healthy young adult volunteers used lozenges containing both LGG and BB-12 at doses of $4.4 \times 10^8$ and $4.8 \times 10^8$, respectively, while 31 controls used lozenges without probiotics. The well-designed, reasonably well-powered trial reported a significant decrease ($p < 0.05$) in both plaque index and (consequent) gingival index for the test group, while no changes were reported for the controls. No changes were found in the microbial composition of saliva in either group.

3.11.4. Oral health—conclusions

While the outcomes of two clinical trials hint at a possible beneficial effect of BB-12 (with and without LGG) on oral health-related parameters (i.e. reduction of salivary *mutans streptococci*, plaque index, and gingival index), currently available evidence on the impact of BB-12 on oral health is not yet sufficient to fully support this hypothesis. With a total of only four identified clinical trials pertaining oral health, more evidence resulting from larger, well-designed clinical trials seems warranted.

4. Conclusion

LGG and BB-12 are the best-documented probiotic strains to date, with over 92 clinical trials combined in the adult population. Our results indicate that supplementation with these strains may
promote human health and support the daily wellness of consumers in high priority areas. For instance, there is evidence that BB-12 beneficially affects stool frequency in populations with reduced stool frequency, without increasing diarrhea. LGG, furthermore, appears to prevent AAD in patients treated for *H. pylori* infection. It is also suggested that both LGG and BB-12 (separately and in combination) support immune defense against pathogens in the upper respiratory tract. Despite these apparent associations, however, no probiotic health claim has been approved in Europe for these strains (EFSA Panel on Dietetic Products, 2011, 2013), while their potential health benefits have been acknowledged by other regulatory authorities (He & Benno, 2011; Health Canada, 2015). It appears that the European criteria for the scientific substantiation of a health claim are particularly stringent (Binnendijk & Rijkers, 2013), and although this has expedited improved probiotic research quality over time, most (earlier) trials do not yet meet these stringent standards. The current lack of substantiation therefore forms a barrier to innovation and curtails potential health benefits for the consumer market. In order to advance probiotic innovation and respond to the unmet health needs, it is crucial that well-designed, appropriately scaled studies build on top of promising data, specifically in areas where strong associations are apparent. In this regard, prioritization of research efforts into managing constipation, AAD prevention, or immune defense against pathogens (in the upper respiratory tract) may be an expedient strategy to provide unassailable evidence of probiotic efficacy, potentially reducing skepticism and resolving a dominant innovation barrier.

**Supplementary material**

Supplementary material for this article can be accessed at https://doi.org/10.1080/23311932.2018.1452839.

**Acknowledgements**

Editorial support was provided by Dennis Stancavish of Peloton Advantage, LLC.

**Funding**

This work was funded by the Pfizer.

**Competing interests**

JF is employed as clinical research scientist at CR2O, a full-service contract research organization, and associated with the Athena Institute, Vrije Universiteit Amsterdam, through his doctoral research.

AFMK, JS, and MAJR are employed by NIZO, which received a financial contribution from Pfizer Consumer Healthcare to perform a literature review on health benefits of BB-12 and LGG.

MBvdW has no financial or personal interests that may be perceived as competing for this submission. He is employed by CR2O, a full-service clinical contract research organization, and associated with the Athena Institute of Vrije Universiteit Amsterdam. Furthermore, he is affiliated with Sovalac BV and acts as a consultant for FFUND BV.

EC is a consultant for several parties on probiotics, none of which are in direct conflict with the subject matter of this paper.

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**Citation information**

Cite this article as: Probiotic research priorities for the healthy adult population: A review on the health benefits of *Lactobacillus* rhamnosus GG and *Bifidobacterium animalis* subspecies *lactis* BB-12. JF, AM, HC, and EAC.

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