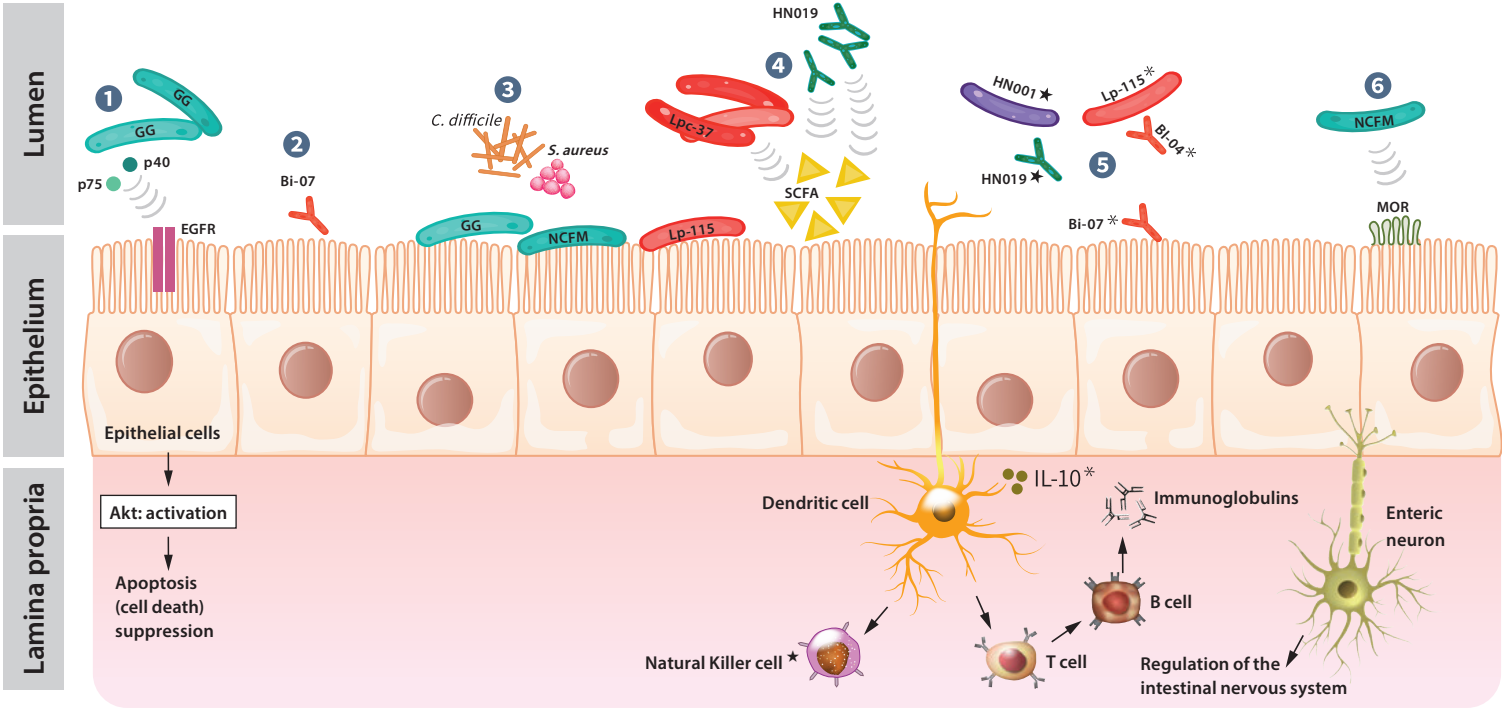


Key mechanisms of action

The mechanism of action of probiotics are complex and differ by strain. Mechanisms of action described below were determined through preclinical research unless otherwise indicated.



p40 and p75: Soluble proteins secreted by *L. rhamnosus* GG
MOR: μ -opioid receptor
EGFR: Epithelial Growth Factor Receptor
IL-10: Interleukin-10, an anti-inflammatory cytokine
Akt: a protein that works in the signaling pathway for cell survival

1. Intestinal Barrier Support

Decrease in intestinal cell apoptosis by *L. rhamnosus* GG³³⁻³⁶

2. Suppression of intestinal hyperpermeability

Mitigation of bacterial translocation, intestinal permeability increases, and inflammatory biomarker increase by *B. lactis* Bi-07³⁷

3. Inhibition of pathogenic microorganisms

C. difficile and *Staphylococcus aureus* may attach to intestinal epithelial cells and cell receptors; displacement of these pathogens by *L. rhamnosus* GG and *L. acidophilus* NCFM, *L. plantarum* Lp-115³⁸

4. Production of Useful Metabolites

Production of short-chain fatty acids (SCFA) in children's feces by *L. paracasei* Lpc-37 or *B. lactis* HN019³⁹
Lactobacilli and bifidobacteria produce lactate and acetate^{28,40}

5. Immune activity

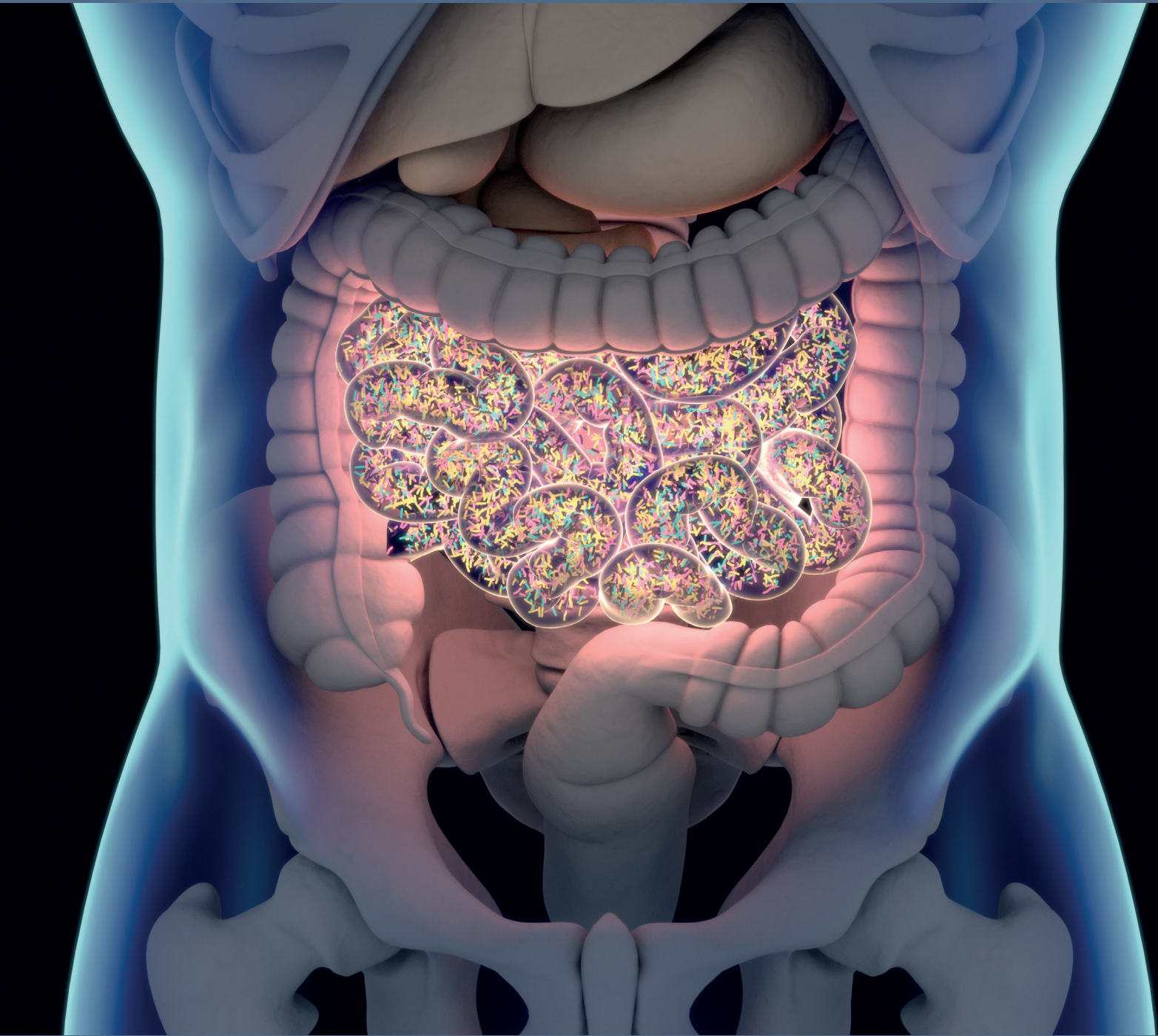
Increase in the activity of natural killer cells in elderly humans by *L. rhamnosus* HN001 or *B. lactis* HN019^{17,19,41-42}
Induction of secretion of anti-inflammatory cytokines by *B. lactis* BI-04 and Bi-07⁴³
Stimulation of immune response via increased immunoglobulins during vaccination in people by *L. plantarum* Lp-115 as well as *B. lactis* Bi-07 and BI-04²⁰

6. Regulation of the intestinal nervous system

Regulates the expression and activity of intestinal opioid receptors in humans with abdominal pain: *L. acidophilus* NCFM⁴⁴

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How Specific Strains of Lactobacilli and Bifidobacteria Work Together

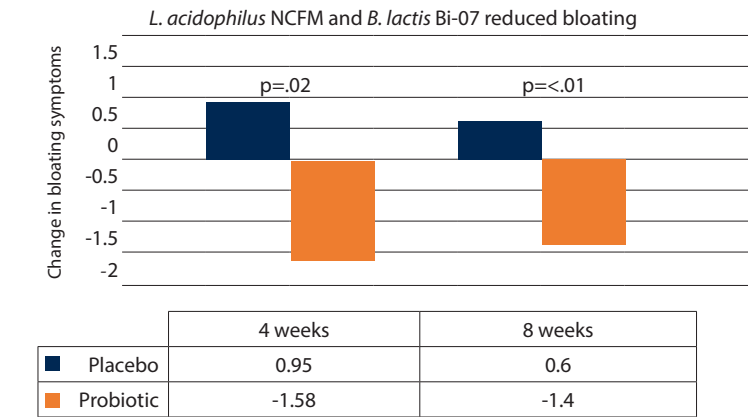
Probiotics have a long history of safe¹ and effective use, with clinical studies demonstrating strain-specific effects on specific conditions.² The early identified probiotic *Lactobacillus acidophilus* NCFM[®] (*L. acidophilus* NCFM) has been used in foods and supplements since 1970³ and has demonstrated clinical efficacy alone and even more so with other strains. *Lactobacillus rhamnosus* GG (*L. rhamnosus* GG) is one of the most widely studied lactobacilli, patented in 1989⁴ and has been widely adopted for acute gastroenteritis in children.⁴⁻⁷ These strains as well as others highlighted here can work together in particular dose and strain combinations to impact gut microbiota, gastrointestinal (GI) function, and immune function.



Probiotic strain combinations for gastrointestinal health

Bloating:

In a double-blind, randomized, placebo-controlled trial (RCT), *L. acidophilus* NCFM® and *Bifidobacterium animalis* subspecies *lactis* Bi-07® (*B. lactis* Bi-07) reduced bloating symptoms by 15% in patients with functional bowel disorders (FBD).⁸



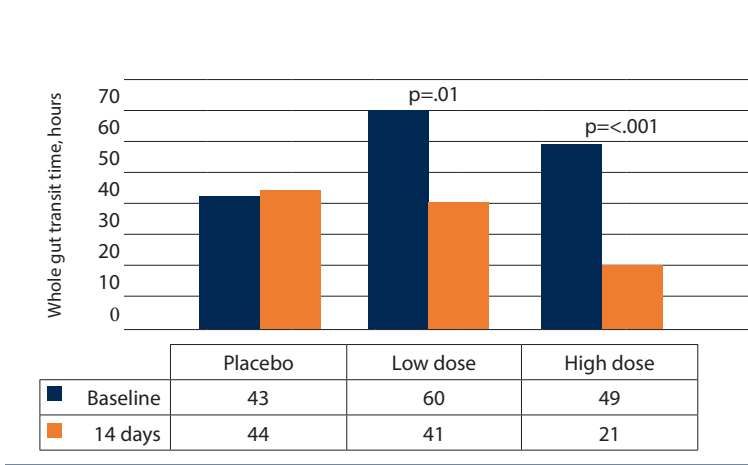
Study ran 8 weeks at 200 billion CFU/day, with 60 subjects.

Pain post-colonoscopy:

In a RCT of 320 patients, 12.5 billion colony forming units (CFU) *L. acidophilus* NCFM and 12.5 billion CFU *B. lactis* Bi-07 per day for two weeks reduced pain time 28% (19 hours) for patients following colonoscopy, a significant change (p<.033).⁹

Constipation:

In a RCT of 100 adults, a 30-50% reduction in whole gut transit time was achieved with 1.8 and 17.2 billion CFU *Bifidobacterium animalis* subspecies *lactis* HN019 (*B. lactis* HN019) as shown in the graph below. Consumption of *B. lactis* HN019 led to a reduction in constipation, irregularity, and flatulence about two times greater than the placebo.¹⁰

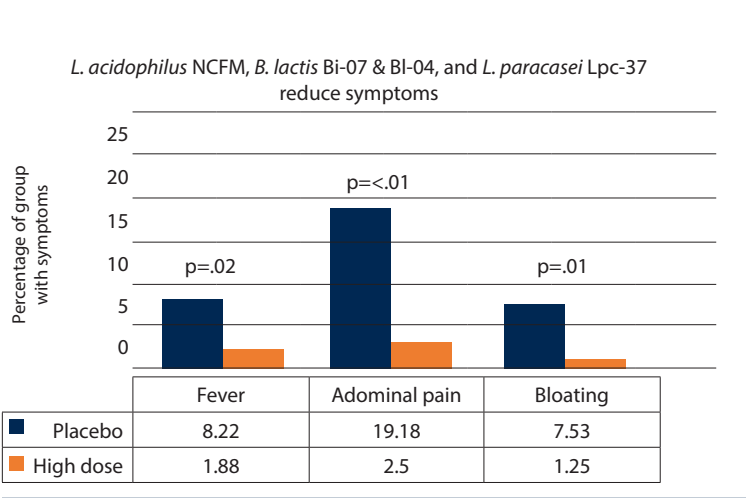
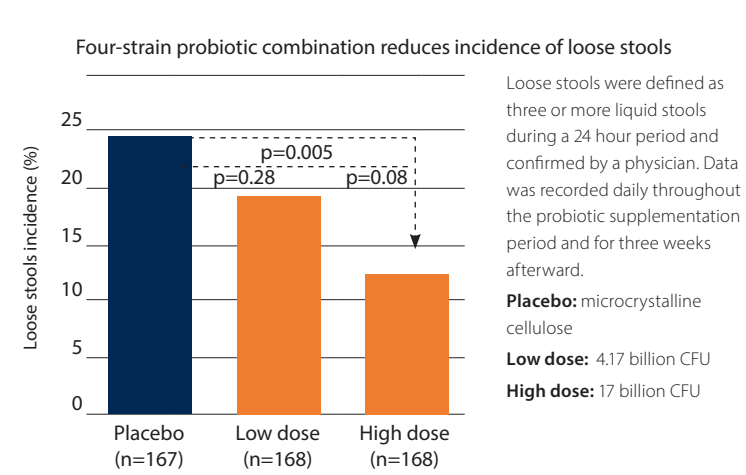


Flatulence:

When *B. lactis* HN019 was added to *L. acidophilus* NCFM, *B. lactis* Bi-07, *Bifidobacterium animalis* subspecies *lactis* BI-04 (*B. lactis* Bi-04), and *Lactobacillus paracasei* Lpc-37 (*L. paracasei* Lpc-37) (the four-strain combination was cited by the World Gastroenterology Organisation's Global 2017 guidelines for antibiotic-associated diarrhea⁷), the five-strain combination showed a statistically significant reduction in flatulence. In addition, less digestive discomfort was observed. The treatment included consumption of the combination for two weeks for a total of 156 constipated people in the RCT.¹¹

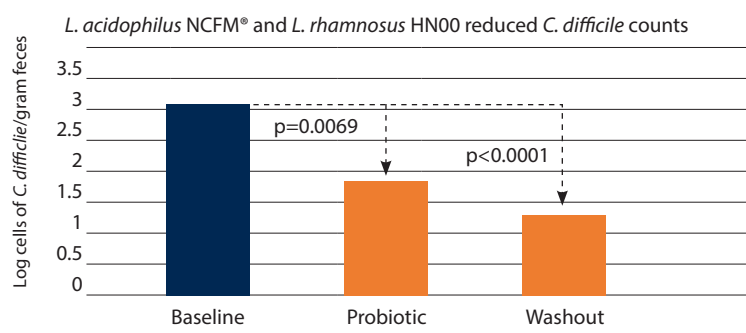
Antibiotic-associated diarrhea:

L. acidophilus NCFM, *B. lactis* Bi-07 and BI-04, and *L. paracasei* Lpc-37 at 17 billion CFU reduced antibiotic-associated symptoms by more than 50% fewer days of diarrhea and more than 80% less bloating and abdominal pain in a large RCT. *Clostridium difficile*-associated diarrhea was significantly lower (p=0.02) comparing the high dose group to the placebo group. The capsule was taken two hours after the antibiotic and breakfast. Probiotic usage continued one week after the antibiotic course completed.¹²



Clostridium difficile:

In a randomized crossover trial, *L. acidophilus* NCFM and *Lactobacillus rhamnosus* HN001 (*L. rhamnosus* HN001) eaten in cheese reduced *Clostridium difficile* as measured in feces of *C. difficile*-positive elderly after four weeks. An additional four weeks without the probiotic showed a continued reduction in *C. difficile* counts.¹³ This study was cited by the World Gastroenterology Organisation 2017 guidelines.⁷



Probiotic strain combinations for immune health

Upper respiratory tract infections:

A meta-analysis of probiotics concluded that in general, probiotic use was associated with an 8% reduction in relative risk of developing a cold.¹⁴ In particular, 2 billion CFU *B. lactis* BI-04 was found to reduce risk of a cold by 27% (with statistical significance), while the combination of *L. acidophilus* NCFM and *B. lactis* Bi-07 also reduced the risk (19%) compared to placebo in a RCT of healthy, active adults.¹⁵

Natural killer cell activity:

L. rhamnosus HN001 at 5 billion CFU/day increased cellular phagocytosis and natural killer cell activity in blood for healthy elderly who consumed it in milk for three weeks in two trials.^{16,17} *B. lactis* HN019 supported immune cell function in the elderly with an increase in natural killer cell activity, also at 5 billion CFU/day.^{16,18,19}

Immune response:

Vaccination can be improved with adjuvants, which are additional components that may help stimulate the immune response to the offending compound (antigen). Probiotics have been tested *in vitro* for their ability to modify the immune system and therefore are potential candidates to help with improving the efficacy of vaccination. Measuring serum and salivary immunoglobulins may be an indicator of immune response. Specifically, IgG is involved in immune memory.

A study evaluated the changes in immunoglobulins, various proteins that act as antibodies in the immune system response, in blood as well as in saliva in response to one of seven probiotic strains (20 billion CFU each) or a placebo. In this eight-arm RCT using probiotic consumption over three weeks during two vaccinations, *B. lactis* BI-04 was one of two strains that showed a significant change to serum IgG; *Lactobacillus plantarum* Lp-115 as well as *B. lactis* Bi-07 also increased serum IgG compared to placebo although the changes were not significant.²⁰

Combining benefits: a novel, multi-strain formula

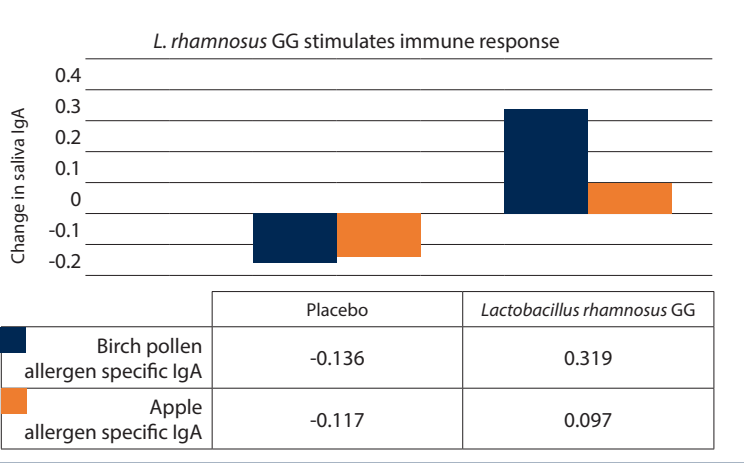
When combining multiple strains of probiotics for health benefits, even though each strain has a demonstrated health benefit and has been safely consumed, the combination may behave differently. The eight strains in these studies were combined together into a unique and novel combination. An IRB-approved clinical study on the combination resulted in no serious adverse events and no withdrawal of participants.

Stool testing showed that *Lactobacillus* species increased significantly and *Bifidobacterium* species also increased, but the change was not statistically significant. Unexpectedly, once-daily consumption over 10 days resulted in higher levels of *Faecalibacterium prausnitzii* (*F. prausnitzii*) and *Akkermansia muciniphila*. These findings were statistically significant, suggesting that the novel multi-strain combination impacts gut microbial composition.²⁷

The possible mechanism may be that acetate produced by the lactobacilli²⁸ and bifidobacteria²⁹ may encourage the growth of *Faecalibacterium prausnitzii*. *F. prausnitzii* produces butyrate as a metabolite, which supports the growth of intestinal goblet cells, which produce mucin, a food source for *Akkermansia muciniphila*.³⁰ These two cohabitants of the intestinal mucosa are lower in many disease conditions of the intestine associated with metabolism and

Allergy, gut-skin axis:

Some evidence has shown that *L. rhamnosus* GG demonstrated improved tolerance to milk in children with a milk allergy.^{21–23} In young adults with birch pollen and apple allergies, a RCT tested consumption of 20 billion CFU per day of *L. rhamnosus* GG for five and a half months during the birch pollen season. The probiotic-treated group was shown to stimulate secretion of birch pollen- and apple-specific IgA.²⁴ Increases in allergen-specific IgA levels suggest support for an immune response that tolerates the allergen.²⁵



Stress, gut-brain axis:

In a randomized, double-blind placebo-controlled trial, 120 healthy adults between the ages of 18 and 25 consumed either the probiotic *L. paracasei* Lpc-37 or the placebo once daily for 5 weeks and took the Trier Social Stress Test (TSST) before and after the intervention. Participants who received Lpc-37 reported significantly lower perceived stress levels compared to the placebo group by the end of the study (p=0.048).²⁶

inflammation.^{31, 32} Additional research on this novel multi-strain combination is warranted to evaluate published benefits on the strains as well as how these strains work together to provide additional benefits.

Genus	Species	Strain*	Billion CFU per capsule
<i>Bifidobacterium</i>	<i>lactis</i>	Bi-07	12.5
	<i>lactis</i>	BI-04	20
	<i>lactis</i>	HN019	5
<i>Lactobacillus</i>	<i>acidophilus</i>	NCFM	12.5
	<i>paracasei</i>	Lpc-37	10
	<i>plantarum</i>	Lpc-115	20
	<i>rhamnosus</i>	GG	20
	<i>rhamnosus</i>	HN001	5
Total			105**

*The blend of strains was supplied by DuPont

**Total CFU per formulation to be present at expiration date

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