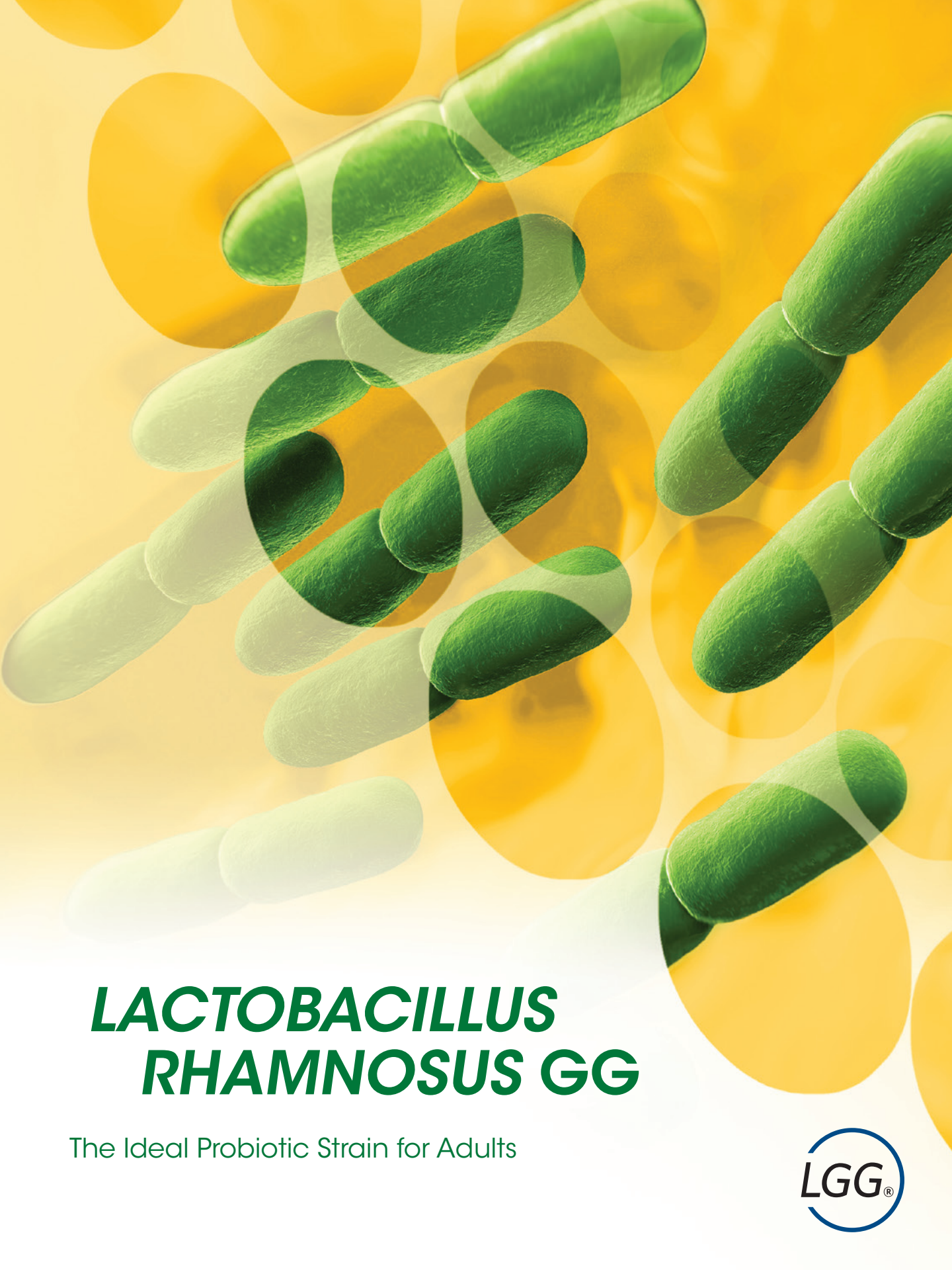


A 3D rendering of several Lactobacillus rhamnosus GG bacteria. The bacteria are depicted as green, rod-shaped cells with a textured surface, arranged in various orientations. They are set against a background of large, overlapping yellow and orange circles, creating a vibrant, abstract pattern.

# **LACTOBACILLUS RHAMNOSUS GG**

The Ideal Probiotic Strain for Adults





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# 1. The Intestinal Microbiota

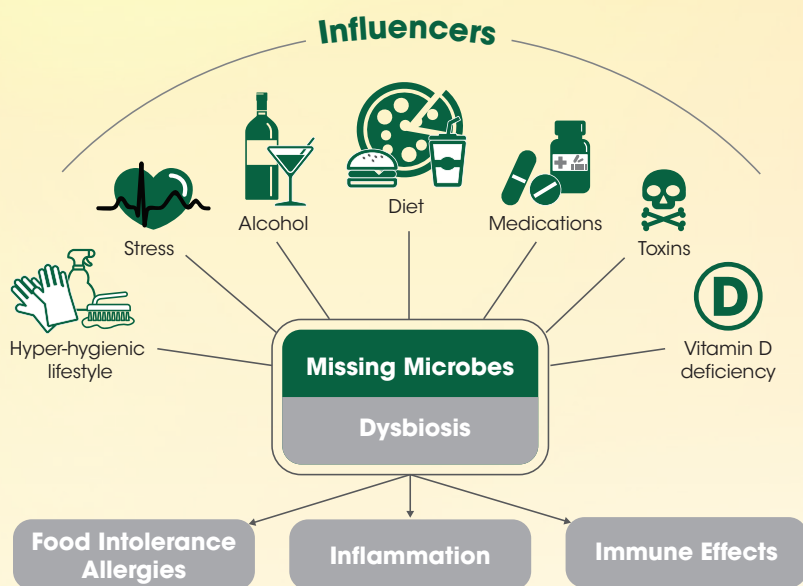
The microbiota consists of all the microorganisms that live inside and on the human body.<sup>1</sup> Having varied types (diversity) and quantities (abundance) of these microorganisms is significant; each person's microbiota is unique, like their fingerprints.<sup>1</sup> In exchange for a stable environment and adequate nutrients, the gut microbiota contributes to maturation of the gastrointestinal tract, provides the host with nutritional contributions, and helps safeguard the host from harmful microbes. The definition of what comprises a "healthy" microbiota remains to be determined, although low microbial diversity in the gut appears to be associated with disease.

**Symbiosis between the microbiota and the host is a critical determinant for health or disease.**

The diversity and abundance of microorganisms generates millions of unique bacterial genes, meaning that there are millions of genes in the gut microbiome alone compared to approximately 22,000 genes contributed by our human genes. The genes of the microbiome are more unique to an individual than their human genes.<sup>2</sup>



Although humans are exposed to microbes *in utero*, colonization of the gastrointestinal tract largely begins during birth. Within a few days the microbiota established in a newborn is predominantly lactobacilli and bifidobacteria<sup>3</sup> and after one year of age the microbiota begins to stabilize, resembling that of a young adult by age three.<sup>4,5</sup> Medication, diet, environment, and stress can alter the microbial abundance and diversity as we age.<sup>6</sup>

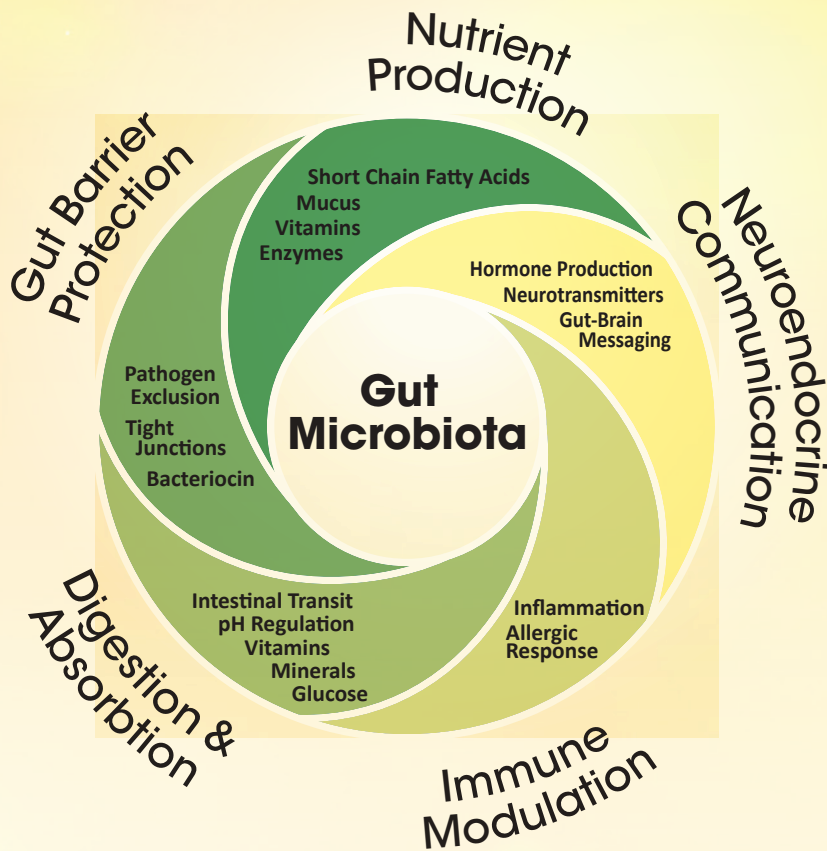


The gut microbiota is essential to our health in a number of ways. Adverse changes in the gut microbiota, known as dysbiosis, are associated with a variety of diseases.<sup>7</sup> The loss of microbial diversity in the gut is generally associated with increased frailty and a reduction in cognitive performance in the elderly.<sup>6</sup>

While an increase of clostridia is associated with increased frailty regardless of age, an increase in *Bacteroidetes* species is specifically associated with increased frailty in elderly populations, and has been found to be more prevalent in those living in long-term residential care facilities.<sup>6</sup> Diet is considered to be one of the largest contributors to these changes in diversity.

# 1. The Intestinal Microbiota

The gut microbiota directs or contributes to a variety of processes that can be organized into five categories.



**GUT BARRIER PROTECTION:** The integrity of the intestinal epithelial barrier heavily relies on the commensal microbiota, the mucus gel layer, and the intestinal epithelium that form the first line of defense, providing a physical and chemical barrier against the diffusion of toxins, antigens, and pathogens. Intestinal epithelial cells communicate extensively with the gut microbiota, which has been shown to regulate permeability of tight junctions, the paracellular space between adjacent epithelial cells, and the increased expression of structural proteins, such as claudins and occludins.<sup>8</sup> Additionally, the intestinal microbiota secrete bacteriocins, toxins that hinder the growth of other bacterial species.<sup>9</sup>

**DIGESTIVE & ABSORPTIVE FUNCTIONS:** The microorganisms in the gut impact its function, specifically digestion, intestinal transit, and nutrient absorption. These microorganisms modulate their own environment by secreting lactic acid, thereby lowering the pH near the intestinal wall, influencing microbial diversity and abundance. These microorganisms also affect lipid uptake and deposition,<sup>4</sup> provide the enzymes and biochemical pathways required for humans to obtain nutrients from polysaccharides,<sup>10</sup> and promote the absorption of other nutrients such as calcium, magnesium, and iron.

**NUTRIENT PRODUCTION:** The gut microbiota is responsible for the utilization of key energy and nutrient sources that are otherwise inaccessible to the human body. Through the fermentation activity of the microbiota, non-digestible carbohydrates are transformed into absorbable short chain fatty acids. The unique chemical capabilities of the human microbiota also play an important role in producing additional compounds that are essential for good health such as mucins, the primary proteins found in the mucus that line much of our digestive tract, and enzymes like lactase and bile acid hydrolase, as well as B vitamins and vitamin K.<sup>13-15</sup>

**IMMUNE MODULATION:** The microbiota helps to balance our bodies' immune responses. Approximately 80% of all immunoglobulin-producing cells in the body are located in the gastrointestinal tract, making it the body's largest site of immunological response.<sup>11,12</sup> The mucosal immune system relies on the integrity of the intestinal epithelial layer as well as the system's ability to readily discriminate commensal organisms from pathogens. The gastrointestinal microbiota is key to preventing foreign microbes, benign or otherwise, from becoming permanent residents. To this end, the microbiota are constantly competing for binding sites and food sources within the gastrointestinal tract and interfering with colonization by crowding out the adhesion of potential pathogens. **When the epithelial barrier is breached by an unwanted compound or microbe, the gut microbiota plays a role in activating the appropriate immune reaction, which includes inflammatory and allergic response pathways.**

# 1. The Intestinal Microbiota

**NEUROENDOCRINE COMMUNICATION:** The significance of the two-way communication between the gut and the brain is truly an emerging area of microbiota science. It is becoming clear that gut microbiota acts as a practical endocrine organ. Recent research has demonstrated the influence of the microbiota on behavior and mental health.<sup>16</sup> Dysbiosis has even been associated with stress, anxiety, and depression. The gut microbiota produces hundreds of different compounds that may enter the bloodstream and act in the brain and other organs.<sup>17</sup> Short chain fatty acids not only act as an energy source but also as signaling molecules. Various neurotransmitters, for example serotonin and noradrenaline, as well as precursors to neuroactive compounds, such as tryptophan, are produced by the microbiota which in turn impact behaviors like appetite regulation and stress response.<sup>17</sup> Elucidating the players and pathways of communication between the gut microbiota, the immune system, and the nervous system remains a key interest in *in vitro* and *in vivo* studies.

Genetics, birth method, early feeding practices and medications received in the first year of life affect the initial establishment of the gut microbiota. Later our diet, the environment, and medications influence the diversity and abundance of organisms in the microbiota, which impact its function, influencing all the processes for which the microbiota is responsible. **These relatively simple microorganisms are able to rapidly respond to changes in their environment in ways that human cells cannot.**



## The Intestinal Microbiota (pgs.4-8)

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## 2. Probiotics

One way to benefit the microbiota is through the use of probiotics. This is a way of supplementing the microbiota with “friendly live bacteria.” An official, and widely accepted definition of probiotics has been provided by the United Nations’ Food and Agriculture Organization and the World Health Organization:

“**Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host**”

Probiotics provide numerous health benefits, such as promoting balance of the intestinal microbiota, digestive assistance, and immune support. These health benefits are generated through many of the same mechanisms as the endogenous microbiota. By directly competing with pathogens for binding sites on the intestinal epithelial cells and mucus layer, probiotics support epithelial barrier integrity.<sup>1</sup> Probiotics also help digest compounds to provide various essential nutrients to the host, including short chain fatty acids that can modulate the luminal pH as well as influence apoptosis and cell diversity. Through mitigation of apoptosis and mucin production, probiotics influence aspects of the epithelial barrier function.<sup>2</sup> Probiotics have been shown to directly release antimicrobial substances and to induce host cells to express them.<sup>3</sup> All of this activity contributes to the strength of the epithelial barrier, which in turn provides a defense against potential toxins and pathogens. Adaptive immune responses are also sensitive to probiotic activity, influencing pathogen specific secretory IgA production as well as beneficial cytokine and chemokine production.<sup>4</sup>

**The World Gastroenterology Organization (WGO) Practice Guidelines on Probiotics and Prebiotics concludes that the potential probiotic health benefits “can only be attributed to the strain or strains tested, and not to the species or the whole group of lactic acid bacteria or other probiotics.”<sup>5</sup>** It

stands to reason that different organisms will have varying abilities to tolerate acid and bile, survive the gastrointestinal tract, adhere to gastrointestinal mucosa, produce diverse antimicrobial substances, and employ different mechanisms to compete with pathogens among other strain specific features that may lead to overall health benefits.<sup>6</sup>

## 2. Probiotics

Based on scientific research, we have learned some basic requirements for microbiota supplementation to qualify as probiotic and additional studies continue to define the characteristics of an ideal probiotic.

| The Ideal Probiotic                         |
|---------------------------------------------|
| Human derived                               |
| Resists the harsh upper GI tract conditions |
| Adheres to human intestinal cells           |
| Colonizes the human intestinal tract        |
| Inhibits illness-causing bacteria           |
| Balances immune responses                   |
| Supports fermentation                       |
| Clinically supported and safe               |

Scientific research led to the definition of these characteristics, and clinical research has further established safety and efficacy for several well studied strains. **Potential benefits of a probiotic strain should be indicated at a given dose based on clinical studies. The clinical evidence for probiotics boasting high colony-forming units (CFU) or multiple strains is largely unsupported.** *Lactobacillus rhamnosus* GG meets all the requirements of an ideal probiotic and is the most extensively studied probiotic strain in the world for people of all ages.

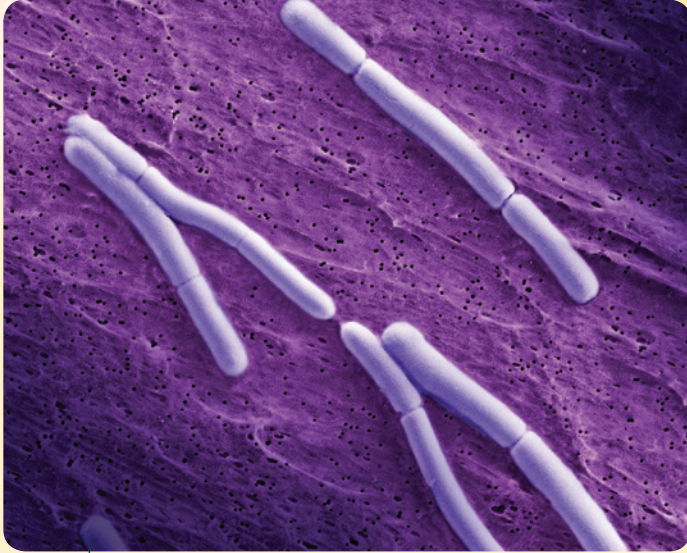


## Probiotics (pgs.10-12)

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### 3. ***Lactobacillus rhamnosus* GG**



*Lactobacillus rhamnosus* GG (*Lactobacillus* GG, deposited at ATCC 53103), is the most extensively studied probiotic strain since its identification in 1985 by Professors Sherwood Gorbach and Barry Goldin at Tufts University. Seeking naturally occurring, intestinal bacteria that could produce health benefits as a probiotic, *Lactobacillus* GG was the ideal candidate because of its ability to survive stomach acid and bile, adhere to human intestinal

epithelial cells, and produce an antimicrobial substance.

Mechanisms of action for *Lactobacillus* GG include interference with enteropathogen colonization through competition as well as secretion of antibacterial substances, stimulation of bowel epithelial cell proliferation, and production of protective mucins.<sup>1,2</sup> Physiologically this translates into improved epithelial barrier function delivered by *Lactobacillus* GG. The epithelial barrier is exposed to a broad spectrum of substances and organisms and is a critical control point for good health and appropriate immune responses.



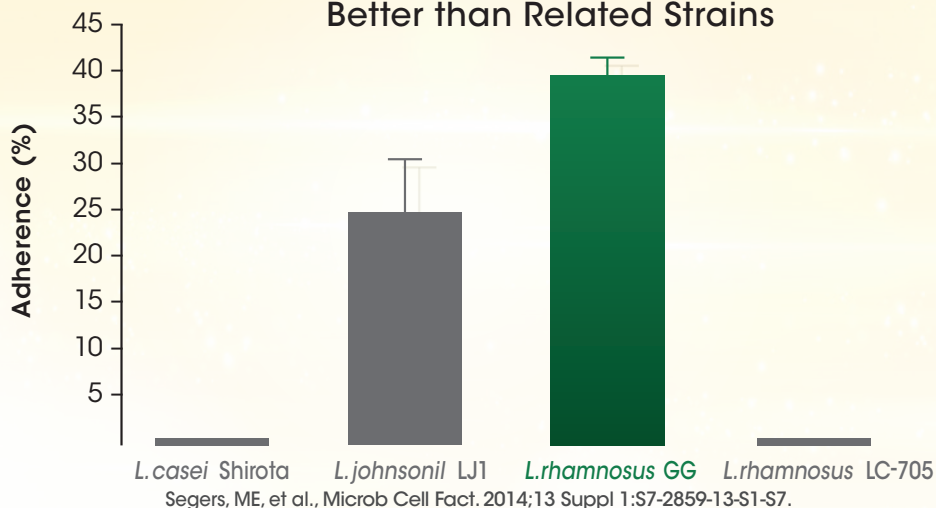
LGG® is a registered trademark in the United States of Chr. Hansen A/S.

The unique features of *Lactobacillus* GG have been recognized in over 1000 scientific studies including over 200 human clinical trials. *Lactobacillus* GG, a Gram positive bacillus, originally isolated from the intestine of a healthy adult, has been completely sequenced— revealing over 300 strain-specific proteins. It is these strain-specific proteins that give rise to the unique features of *Lactobacillus* GG as a probiotic. Adhesion is fundamental to competitive exclusion, particularly in the case of pathogen competition. ***Lactobacillus* GG was shown to adhere to mucus proteins far better than even closely related strains *in vitro* (Figure A).**<sup>3</sup>

The Saxelin laboratory examined the dose dependence of *Lactobacillus* GG survival and colonization<sup>4</sup> as well as the effects of the delivery matrix.<sup>5,6</sup> Doses of 10-100 billion CFU/d (but not 1-100 million CFU/d) colonized the intestine to meet the detection limit of 1,000 CFU/g feces. Other probiotics were also compared with respect to oral and fecal recovery demonstrating that *Lactobacillus* GG is superior to other strains, not only in fecal recovery, but in persistence as well, regardless of delivery matrix.<sup>6</sup> Additional clinical studies show that *Lactobacillus* GG survives the gastrointestinal tract via fecal recovery analysis following supplementation,<sup>7-11</sup> and through biopsy results,<sup>12</sup> further emphasizing its ability to bind to the mucus and cells lining the large intestine.

**Figure A**

### *Lactobacillus* GG Adheres to Mucus Proteins Better than Related Strains



### 3. *Lactobacillus rhamnosus* GG

One subset of the *Lactobacillus* GG-specific proteins is largely responsible for *Lactobacillus* GG's ability to adhere to the epithelial cells that line the intestines. These proteins are structural components of the pili, hair-like projections that physically attach the lactobacillus to specific sites on epithelial cells. These pili are visible in the electronmicrograph in Figure B. Tripathi and colleagues visualized the pili using a second method, atomic force microscopy<sup>13</sup> (Figure C).



Figure B.

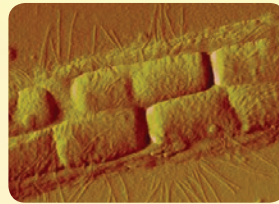


Figure C.

Tripathi P, Dupres V, Beaussart A, et al. Langmuir. 2012;28(4):2211-2216.

We now know that **pili are composed of strain-specific proteins, or pilins, that zip together holding the lactobacillus fast to the epithelium.**<sup>13</sup> SpaC, a pilin and the key adhesion protein of *Lactobacillus* GG, binds mucin and collagen and can interact with other SpaC pilins. SpaC acts like the teeth of a zipper—lying across and protecting the cell surfaces and junctions, while epithelial cells reciprocate binding, forming a network. Ultimately, adhesion of *Lactobacillus* GG to the intestinal epithelium gives rise to its health benefits.

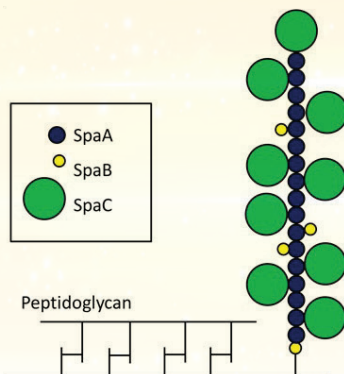


Figure D.

Tripathi P, Dupres V, Beaussart A, et al. Langmuir. 2012;28(4):2211-2216.

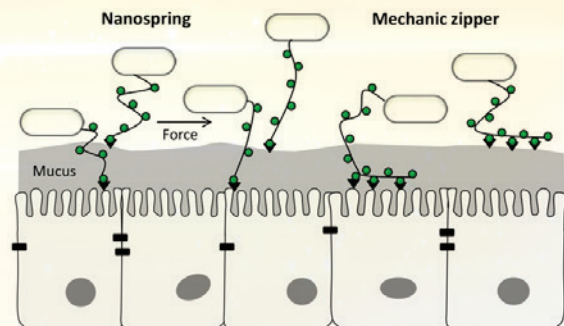


Figure E.

Tripathi P, Dupres V, Beaussart A, et al. Langmuir. 2012;28(4):2211-2216.



*Lactobacillus* GG has targeted and systemic effects on the immune system that appear to be mediated via the gastrointestinal tract. By influencing such processes of the humoral and cell-mediated immune response, along with important features of innate immunity, *Lactobacillus* GG impacts immune regulation.<sup>14-16</sup> *In vitro* studies have shown that *Lactobacillus* GG attachment is required for attenuation of the immune response.<sup>17</sup> This firm, strain-specific attachment allows *Lactobacillus* GG to exert its benefits where they are needed. Beyond adhering to and fortifying the gut barrier, ***Lactobacillus* GG has been shown to stimulate processes that improve:**

- **The activity of macrophages and other immune cells**<sup>18-20</sup>
- **Production of immunoglobulins and cytokines**<sup>15</sup>
- **The balance of inflammatory processes including the response to allergens, such as food antigens**<sup>21,22</sup>

| The Ideal Probiotic Strain                  | <i>Lactobacillus rhamnosus</i> GG |
|---------------------------------------------|-----------------------------------|
| Human derived                               | X                                 |
| Resists the harsh upper GI tract conditions | X                                 |
| Adheres to human intestinal cells           | X                                 |
| Colonizes the human intestinal tract        | X                                 |
| Inhibits illness-causing bacteria           | X                                 |
| Balances immune responses                   | X                                 |
| Supports fermentation                       | X                                 |
| Clinically supported and safe               | X                                 |

## 3. *Lactobacillus rhamnosus* GG

### 3.1 SAFETY OF *LACTOBACILLUS RHAMNOSUS* GG

Practical experience from over 30 years of use in 90 countries confirms the large-scale safety and tolerability of *Lactobacillus rhamnosus* GG. Extensive epidemiological studies show that rapidly increasing consumption of this strain in Finland did not increase the incidence of *Lactobacillus* or *Lactobacillus* GG isolates in blood culture samples.<sup>23</sup> The safety of *Lactobacillus* GG is further supported by surveillance studies<sup>24,25</sup> that evaluated potential increases in clinical infections with increased probiotic consumption. Such studies showed that during a nine-year period, despite a notable increase in *Lactobacillus* GG consumption (~10-fold) in Finland, the number of infections involving lactobacillus species reported to Helsinki health authorities remained at a constant background level of 10-20 cases per year. Cases of bacteremia have occurred in patients with underlying immune compromise, chronic disease or debilitation.<sup>26</sup> No reports have described sepsis related to probiotic use in otherwise healthy persons.<sup>27,28</sup>

Dozens of clinical studies have demonstrated no adverse impact on nutritional, metabolic, or immune parameters due to *Lactobacillus* GG administration. The *Lactobacillus* species has achieved Qualified Presumption of Safety (QPS) status from the Scientific Committee of European Food Safety Authority and Generally Recognized as Safe (GRAS) status from the US Food and Drug Administration.

The complete genome sequence of *Lactobacillus* GG is documented, MCB1 RefSeq: NC\_013198.1. The fully annotated sequence was published in 2009<sup>29</sup>. In *Lactobacillus* GG, the antibiotic resistance genes are distinct from the transferable genes, and *Lactobacillus* GG does not carry the plasmids that can spread transferable genes<sup>30</sup>.

Taking together, the hundreds of clinical studies in healthy and vulnerable populations including adults, children, and infants, along with the above assertions, the totality of the evidence shows that the use of *Lactobacillus rhamnosus* GG is both safe and effective and is the gold standard in probiotic supplementation.

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## 4. ***Lactobacillus* GG Clinical Support**

*Lactobacillus rhamnosus* GG has been extensively studied in adults for a variety of outcomes. Here we highlight some of this clinical data that represent the ideal features of *Lactobacillus* GG.

### 4.1 **GASTROINTESTINAL BENEFITS**

Numerous studies have shown that supplementation with *Lactobacillus* GG reduces the incidence and duration of diarrhea resulting from dysbiosis due to viral and bacterial, including nosocomial, intestinal infections,<sup>1-3</sup> travel to foreign countries,<sup>4,5</sup> and side effects of antibiotic therapy.<sup>6-10</sup>

#### **LACTOBACILLUS GG REDUCES THE DURATION AND SEVERITY OF ANTIBIOTIC ASSOCIATED DIARRHEA**

Antibiotics kill many infection-causing bacteria but also disturb the balance of the gut microbiota, potentially allowing certain pathogenic bacteria to become overactive while impeding the function of beneficial microorganisms in the intestines.<sup>11,12</sup> This imbalance, or dysbiosis, can result in side effects that lead some patients to discontinue their regimen, causing a high risk of treatment failure and contributing to the development of antibiotic resistance.<sup>13</sup> Figure F shows the impact of some commonly prescribed antibiotics on the abundance and diversity of normal intestinal bacteria and the emergence of antibiotic resistant bacteria.<sup>14,15</sup>





**Figure F.**

## Impact of Certain Classes of Antibiotics on the Normal Intestinal Microbiota

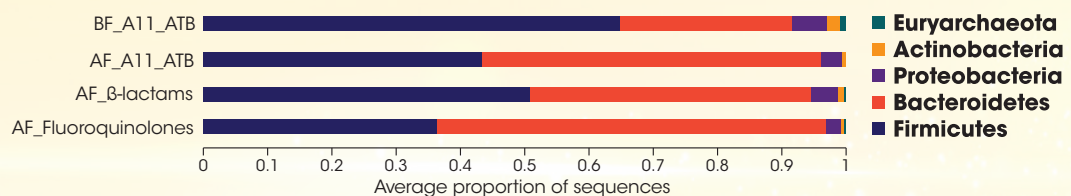
**Figure F1:**  $\Downarrow$ = strong suppression;  $\downarrow$ = moderate suppression;  $\uparrow$ = increase in number;  
 $\updownarrow$ = positive and negative effects seen in different studies, **NC**= no change detected.  
 $+$ = resistant strains detected

| Antibiotic                            | Impact on:   |                             |                | Emergence of resistant strains in: |                |
|---------------------------------------|--------------|-----------------------------|----------------|------------------------------------|----------------|
|                                       | Anaerobes    | Aerobic Gram positive cocci | Enterobacteria | Enterococci                        | Enterobacteria |
| Amoxicillin/clavulanic acid           | <b>NC</b>    | $\uparrow$                  | $\uparrow$     | <b>NC</b>                          | <b>NC</b>      |
| Ciprofloxacin (high conc. in faeces)  | <b>NC</b>    | <b>NC</b>                   | $\Downarrow$   | <b>NC</b>                          | $+$            |
| Clarithromycin/metronidazole          | $\downarrow$ | $\uparrow$                  | $\downarrow$   | $+$                                | $+$            |
| Cephalosporins (high conc. in faeces) | <b>NC</b>    | $\uparrow$                  | $\Downarrow$   | <b>NC</b>                          | $+$            |
| Clindamycin                           | $\Downarrow$ | $\uparrow$                  | $\uparrow$     | $+$                                | $+$            |
| Vancomycin                            | $\downarrow$ | $\updownarrow$              | <b>NC</b>      | $+$                                | $+$            |

Jernberg, et al., Microbiology, 2010;156:3216-23. Adapted from Sullivan, A., et al., Lancet Infect Dis. 2001;1:101-14.

## Microbial Composition at the Phylum Level Based on 16S rRNA Gene Sequences in Response to Antibiotics.

**Figure F2:** BF=before treatment; AF=after treatment; ATB=antibiotics. For all antibiotics, N=21; for  $\beta$ -lactams, N=11; for fluoroquinolones, N=10.

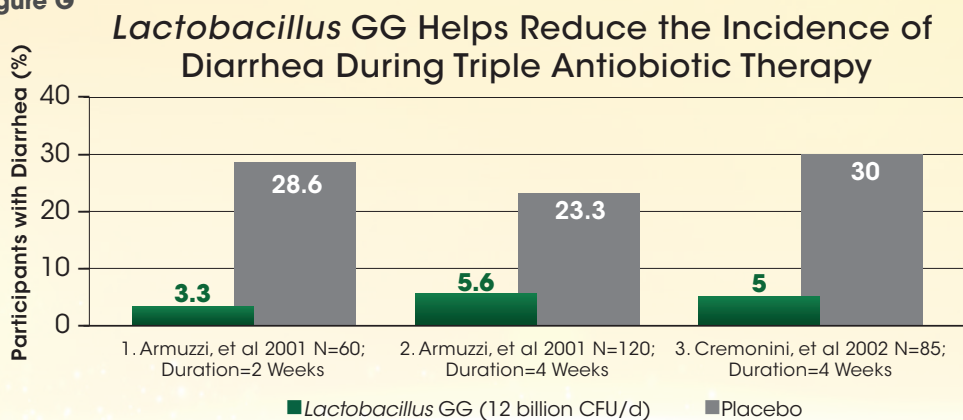


Panda, et al., PLOS One. 2014.9(4):e95476

## 4. *Lactobacillus* GG Clinical Support

*Lactobacillus* GG helps to protect adults against antibiotic-associated diarrhea (AAD) by competing with pathogens for resources and binding sites on the intestinal mucosa, forming a protective barrier, and producing an antibacterial substance that protects against pathogens.<sup>16,17</sup> Here we highlight three clinical trials that demonstrate the efficacy of *Lactobacillus* GG (all at 12 billion CFU/d) in reducing the incidence and severity of antibiotic associated diarrhea. Antonio Gasbarrini and colleagues conducted a series of clinical trials to study the potential for *Lactobacillus* GG to ameliorate side effects of antibiotic cocktails prescribed for *H. pylori* infection (Figure G1). In the first study, all participants followed a one week triple antibiotic regimen of rabeprazole, clarithromycin, and tinidazole.<sup>7</sup> These subjects were randomized to *Lactobacillus* GG or placebo during the antibiotic regimen plus an additional week. The study showed the incidence and severity of diarrhea, nausea, and taste disturbances were significantly reduced in the probiotic group compared to the placebo group.

**Figure G**



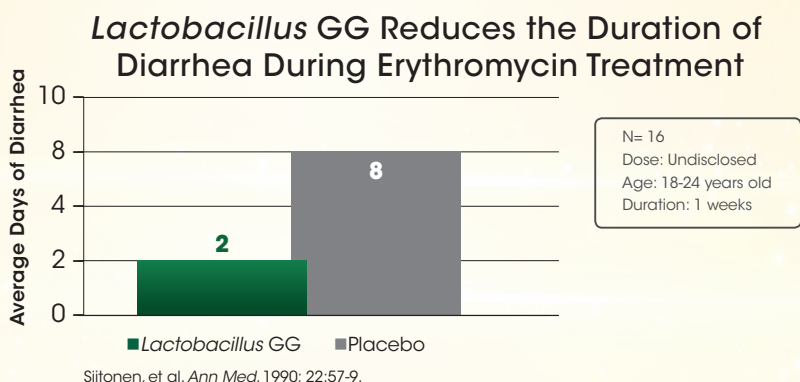
In the second study, adults taking the *H. pylori* antibiotic cocktail of pantoprazole, clarithromycin, and tinidazole for one week were less likely to experience diarrhea when concurrently beginning supplementation with *Lactobacillus* GG and continuing this probiotic treatment for an additional week.<sup>6</sup> Follow up was continued for two additional weeks. *Lactobacillus* GG supplementation resulted in a significant reduction in the risk of bloating, diarrhea (Figure G2), and taste disturbances compared to placebo.

The third clinical trial investigated the administration of *Lactobacillus* GG, a different strain of probiotic, and a combination of probiotics, during and for one week after, triple antibiotic therapy with rabeprazole, clarithromycin, and tinidazole.<sup>8</sup> The subjects were followed for an additional two weeks. Probiotic administration, including *Lactobacillus* GG, led to a lower incidence of diarrhea (Figure G3) as well as reduced taste disturbance compared to placebo control.<sup>8</sup> Eradication of *H. pylori* was similar between the probiotic and placebo groups in all three of these studies.<sup>6-8</sup>

This series of clinical trials demonstrates that *Lactobacillus* GG supplementation improves the treatment tolerability by reducing several side effects associated with triple antibiotic regimens, including diarrhea (Figure G).

Additional support for the efficacy of *Lactobacillus* GG for antibiotic-associated diarrhea is provided by Siitonen and associates who administered yogurt supplemented with *Lactobacillus* GG (dose undisclosed) or plain yogurt placebo to adults taking erythromycin for two weeks (Figure H).<sup>10</sup> By the end of the intervention they found a reduction in the duration of diarrhea in the group supplemented with *Lactobacillus* GG compared to those in the placebo group, and reported less abdominal distress, stomach pain, and flatulence as well.

**Figure H**



**Taken together, these studies indicate the role of *Lactobacillus* GG in reducing the risks of antibiotic-associated side effects.**

## 4. ***Lactobacillus* GG Clinical Support**

### **LACTOBACILLUS GG SUPPLEMENTATION HELPS REDUCE RELAPSING *C. DIFFICILE*-ASSOCIATED DIARRHEA**

A very serious type of AAD, *C. difficile*-associated diarrhea, refers to a wide spectrum of diarrheal illnesses due to toxins produced by this organism. Initial antibiotic therapy of *C. difficile* colitis has a high rate of success but the frequency of relapse is 15-20%, irrespective of the drug used (metronidazole, vancomycin, bacitracin, or cholestyramine). Multiple relapses, involving diarrhea and reappearance of the organism, with its cytotoxin in the stool, can occur and have devastating consequences on overall health. *Lactobacillus* GG has been shown to reduce the relapse rate of *C. difficile*. Resolution of recurrent *C. difficile*-associated diarrhea has been demonstrated by Gorbach and colleagues through *Lactobacillus* GG supplementation at a dose of 10 billion CFU/day.<sup>1</sup> Bennet and associates confirmed this finding using lower doses, between 1 and 4 billion CFU/day.<sup>3</sup>

### **LACTOBACILLUS GG REDUCES THE RISK OF TRAVELER'S DIARRHEA**

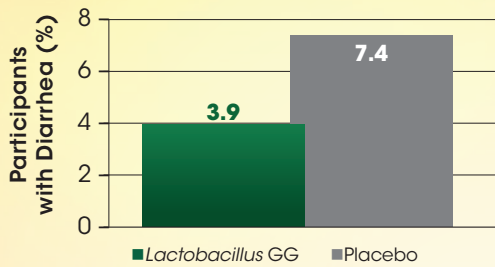
An example of dysbiosis resulting from a change in one's diet and environment is Traveler's diarrhea which affects 20-50% of travelers to tropical and subtropical destinations. Bacterial enteropathogens cause approximately 80% of Traveler's diarrhea, the source of which is predominantly contaminated food. *Lactobacillus* GG protects against Traveler's diarrhea as demonstrated by Hilton and Oksanen.<sup>4,5</sup>

Hilton and colleagues recruited hundreds of worldwide travelers and demonstrated that *Lactobacillus* GG administration, 2 billion CFU/day, significantly reduced the incidence of Traveler's diarrhea compared to placebo.<sup>4</sup> Travelers in the *Lactobacillus* GG arm of the study were half as likely to experience diarrhea as compared to those in the placebo arm (Figure 1). The study showed that for patients with a prior history of Traveler's diarrhea, the benefit of *Lactobacillus* GG administration was even greater.



Figure 1

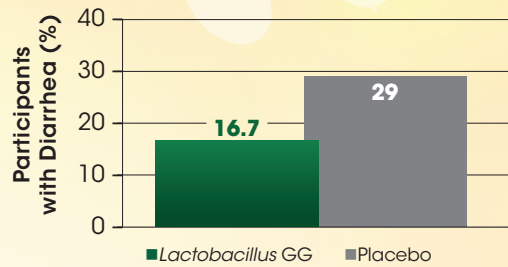
***Lactobacillus* GG Reduces the Incidence of Traveler's Diarrhea in Adults**



N= 24  
Dose: 2 x 10<sup>9</sup> CFU  
Age: 17-80 years old  
Duration: 1 or 2 weeks

Hilton et al. *J Travel Med* 1997; 4:41-43.

**Subgroup Analysis:  
Adults with Prior History  
Effect Even More Pronounced**



**The overall risk of Traveler's diarrhea was significantly reduced for the *Lactobacillus* GG cohort. For patients with a prior history of Traveler's diarrhea, the benefit of *Lactobacillus* GG administration was even greater.**

This Hilton study supported previous findings by Oksanen and associates who studied 820 overseas travelers to two Turkish cities.<sup>5</sup> Travelers self-administered *Lactobacillus* GG, at a dose of 2 billion CFU/d, or placebo and during trips of either one or two weeks. They found that the cohort provided with *Lactobacillus* GG, who traveled to the city of Alanya, had a significantly lower incidence of diarrhea than the cohort provided with placebo. These investigations indicate that colonizing the gastrointestinal tract with *Lactobacillus* GG prior to travel (at a dose as low as 2 billion CFU/d), and continuing this supplementation throughout the duration of travel to various regions of the world, may reduce the incidence of Traveler's diarrhea.

## 4. **Lactobacillus GG Clinical Support**

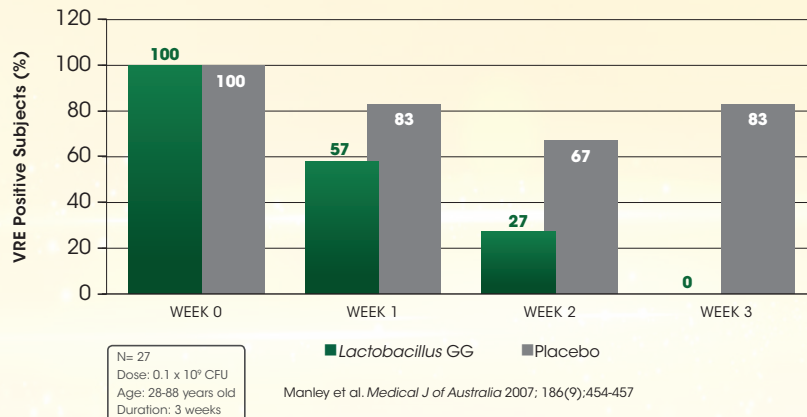
### **LACTOBACILLUS GG ELIMINATES VANCOMYCIN-RESISTANT ENTEROCOCCI IN EFFECTED PATIENTS**

Vancomycin-resistant enterococci (VRE) represent a class of enteropathogens that are of special concern in healthcare settings. VRE colonize the gastrointestinal tract and can lead to infection, which is associated with a mortality rate of at least 37%.<sup>19,20</sup> It is estimated that 25% of all enterococci associated with nosocomial infections are vancomycin resistant.<sup>18</sup> Elimination of the VRE carrier state is desirable to reduce transmission and infection.

In a clinical study published by Manley and co-workers (Figure J), *Lactobacillus* GG-containing yogurt was significantly more effective at eliminating vancomycin-resistant enterococci in renal patients by week three compared to plain yogurt as placebo.<sup>2</sup> This further supports the role of *Lactobacillus* GG in inhibiting the presence of harmful pathogens and encouraging desirable microorganisms in the gut.

**Figure J**

***Lactobacillus* GG Helps Eliminate Vancomycin – Resistant Enterococcus (VRE) in Adults**



***Lactobacillus* GG administration helps eradicate gastrointestinal carriage of VRE in renal patients.**

These clinical findings are supported by pre-clinical research using the VRE *Enterococcus faecium* E1165, which has high sequence and antigenic homology between its pili and that of *Lactobacillus* GG.<sup>21</sup> Tytgat and colleagues showed that antibodies raised against the SpaC pilin of *Lactobacillus* GG interfered with the mucus-binding capacity of *E. faecium* E1165, suggesting that *Lactobacillus* GG may induce cross-immunity against VRE.<sup>21</sup>

## GASTROINTESTINAL BENEFITS: ADDITIONAL RESOURCES

### **Systematic review with meta-analysis: *Lactobacillus rhamnosus* GG in the prevention of antibiotic-associated diarrhea in children and adults.**

Szajewska, H. & Kolodziej, M. *Aliment Pharmacol Ther.* 2015 42(10):1149-57.

This meta-analysis discusses twelve clinical trials that investigated the efficacy of *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in both children and adults, and concludes that *Lactobacillus* GG is indeed effective in its prevention, however their analysis indicates the data is most significant for children.

### **Probiotics in the gastrointestinal diseases of the elderly.**

Malaguamnera, G., et al. *J Nutr Health Aging.* 2012 16(4):402-10.

This review discusses the potential for probiotics to protect against age-related changes to the microbiota and resulting gastrointestinal conditions. While specific probiotic recommendations are made for conditions such as antibiotic-associated diarrhea, the authors admit more research is needed to evaluate the effect of long-term probiotic consumption as well as the effects on more serious diseases, such as colon cancer.

## 4. ***Lactobacillus* GG Clinical Support**

### **Probiotics and prebiotics in the elderly**

Hamilton-Miller, J. *Postgrad Med J.* 2004;80:447-51.

This review describes the potential for probiotics and prebiotics to benefit ailments commonly experienced in elderly populations including constipation, malnutrition, and weakened immunity. Although existing clinical data is encouraging, the author points out that larger scale trials are necessary to establish probiotics as a benchmark in elderly supplementation with regard to these outcomes.

### **4.2 IMMUNE BENEFITS**

*Lactobacillus rhamnosus* GG has been clinically proven to help improve overall health. *Lactobacillus* GG supports immune defenses by contributing to the integrity of the intestinal epithelial barrier and stimulating the innate and adaptive immune responses.<sup>22</sup> *Lactobacillus* GG has been shown to balance cytokines, generate antimicrobial substances, and elicit phagocytic activity in response to potential attacks. This immune system support has been demonstrated in numerous *in vitro* and animal studies.<sup>23-34</sup> *In vitro* studies have shown that *Lactobacillus* GG influences the maturation of dendritic cells, induces differentiation of helper T cells, and modulates cytokine and immunoglobulin production.<sup>35-39</sup> The observed effects of *Lactobacillus* GG on the down-regulation of interleukin-8 appears to be linked to the bacterium's attachment to intestinal epithelial cells.<sup>37,38</sup> Miettinen *et al.* showed that tumor necrosis factor-alpha and interleukin-6 are induced by *Lactobacillus* GG.<sup>40</sup> Both molecules participate in the inflammatory/anti-inflammatory balance. In addition to these preclinical findings, the benefits of *Lactobacillus* GG in support of natural defenses and overall health has been demonstrated in dozens of clinical trials involving thousands of healthy participants. Although the effects of *Lactobacillus* GG on upper respiratory tract infections haven't been studied in adults, a few studies have



shown that *Lactobacillus* GG helps reduce the incidence and severity of such infections in children.<sup>41-43</sup> Additionally, Morrow and associates investigated the effects of *Lactobacillus* GG on ventilator-associated pneumonia (VAP) in 146 mechanically ventilated participants.<sup>44</sup> *Lactobacillus* GG supplementation, at a dose of 2 billion CFU/d, significantly reduced the incidence of developing VAP compared to placebo, and fewer days of antibiotics were prescribed for those participants that did develop VAP. **Taken together, these studies indicate that *Lactobacillus* GG shows promise in promoting upper respiratory health in adults.**

## LACTOBACILLUS GG IS EFFECTIVE AS AN IMMUNE ADJUVANT

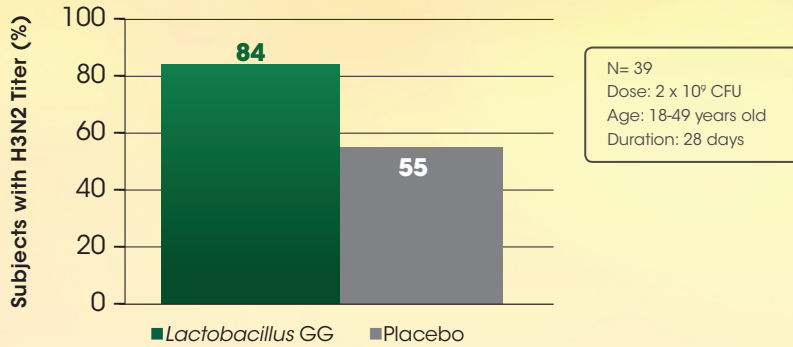
Live attenuated influenza vaccine (LAIV) protects against influenza by mucosal activation of the immune system. Initially, however, compared to inactivated influenza vaccine (IIV), LAIV was thought to be less effective for healthy adults (17-49 years old). Studies in animals and humans have demonstrated that probiotics improve the immune response to mucosally-delivered vaccines<sup>45</sup>.

As illustrated in Figure K, *Lactobacillus* GG was shown to improve adults' response to the influenza vaccine.<sup>46</sup> Vaccinated subjects were assigned to self-administer *Lactobacillus* GG or placebo for 28 days. Here, an objective measure of immunoglobulin in response to the vaccine was significantly higher in intervention group than in those who received placebo. *Lactobacillus* GG has the potential to improve the immune response to LAIV, enhancing the effectiveness of the vaccine.

## 4. *Lactobacillus* GG Clinical Support

Figure K

### *Lactobacillus* GG Improves Influenza Vaccine Response in Adults



Davidson, et al. *Eur. J. Clin. Nutr.*, 2011; 65(4):501-507

***Lactobacillus* GG behaves as an adjuvant to improve influenza vaccine immunogenicity. *Lactobacillus* GG increased seroprotection for H3N2.**

Further evidence in support of *Lactobacillus* GG supplementation as an immune adjuvant is described by de Vrese and colleagues.<sup>45</sup> They found that *Lactobacillus* GG administration increased titers of virus-specific neutralizing antibodies following a polio vaccination (oral booster). Titers of polio-specific IgA, IgG, and IgM were detected at twice that of vaccinated subjects administered placebo.

A third study of the effects of *Lactobacillus* GG as an immune adjuvant involved thirty healthy volunteers randomized to receive *Lactobacillus* GG, *Lactococcus lactis*, or placebo for seven days.<sup>47</sup> On days 1, 3, and 5 they also received an attenuated *Salmonella typhi* Ty21a oral vaccine to mimic an enteropathogenic infection. Although there was an increase of IgA-specific antibody secreting cells (sASC) against anti-*S. typhi* Ty21a in the *Lactobacillus* GG group compared to *L. lactis* and placebo groups, the increase wasn't significant. The mechanism of this adjuvant effect has not been clarified, but these findings indicate an

enhancement of systemic protection against infections with *Lactobacillus* GG supplementation. Additionally, studies administering *Lactobacillus* GG to children have also shown effects on all immunoglobulin classes and pathogen specific antibody responses.<sup>36,48-50</sup>

## **LACTOBACILLUS GG MODULATES INFLAMMATORY & ALLERGIC RESPONSES**

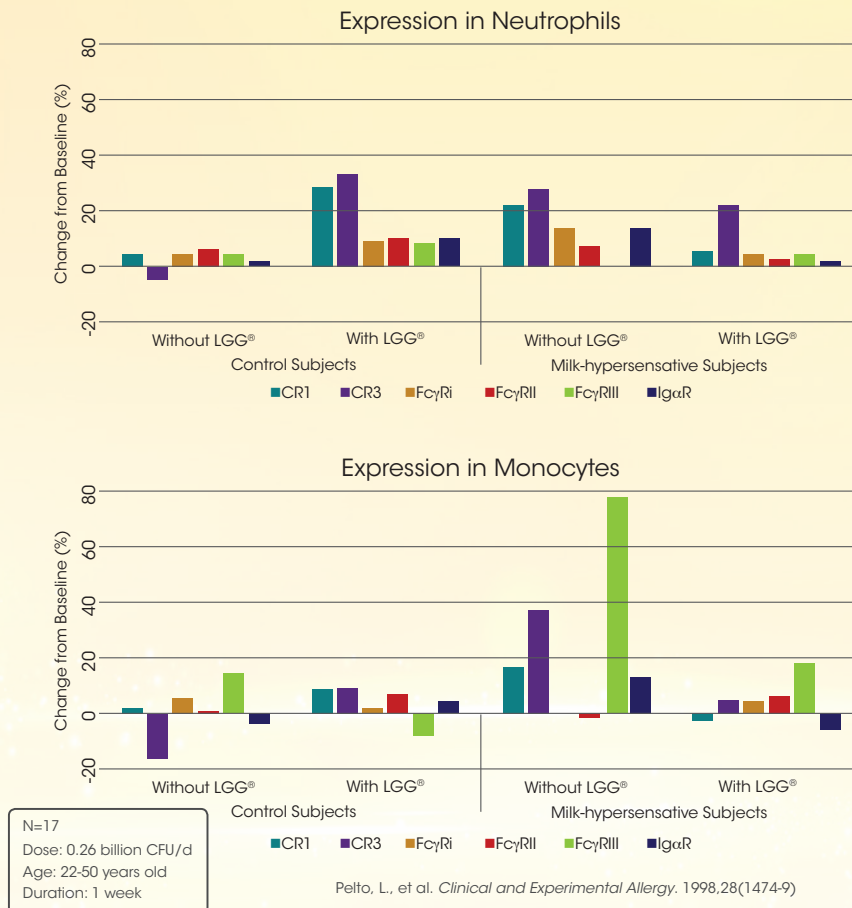
Hypersensitivity reactions, or allergies, occur within minutes of exposure to a challenging antigen. Following the antibody reaction, mast cells and basophils release histamine, causing smooth muscle contraction as well as increased blood flow and vascular permeability. Airborne allergens are common in developed countries and typically manifest allergic symptoms locally in the nasal passages, eyes, lower airway, and lungs. Additionally, certain foods can lead to an allergic reaction which can also manifest locally in the gastrointestinal tract or may spread to distant sites in the body via blood circulation, triggering a systemic response. Serious systemic reactions, or anaphylaxis, can be life threatening. Here we highlight two clinical trials that investigated the effects of *Lactobacillus* GG on adults with specific food allergies.

The first study, reported by Peltó and colleagues, determined that *Lactobacillus* GG can modulate nonspecific immune responses differently in milk tolerant and milk hypersensitive adults.<sup>51</sup> They compared the expression of phagocytic receptors on neutrophils and monocytes prior to and after a milk challenge between healthy and milk hypersensitive adults with or without *Lactobacillus* GG (0.26 billion CFU/d).<sup>51</sup> Phagocytosis, mediated by these receptors, is important to the early activation of the inflammatory response, even before antibody production. The consumption of milk significantly increased the expression of phagocytosis receptors CR1, CR3, FcγRIII, and FcαR in milk-hypersensitive individuals, while the addition of *Lactobacillus* GG supplementation attenuated this increase, and ultimately the immunoinflammatory response as shown in

## 4. *Lactobacillus* GG Clinical Support

Figure L. However, milk with *Lactobacillus* GG had the opposite effect in healthy participants where the phagocytic process increased and receptor expression was up-regulated. Consequently, *Lactobacillus* GG appears to be able to positively modulate an immune response depending on the participant's sensitivity to milk and is possibly the mechanism by which *Lactobacillus* GG improves gastrointestinal distress in these sensitive individuals.

**Figure L** *Lactobacillus* GG Supplementation Down-Regulates Milk-Induced Phagocytic Receptor Expression in Milk Hypersensitive Subjects



***Lactobacillus* GG can modulate the non-specific immune response differently in healthy and milk-hypersensitive subjects.**



Another form of food allergy, oral allergy syndrome (OAS) is a clinically recognized allergic condition characterized by itching or swelling of the mouth and tongue following the contact of specific foods (usually fruits and vegetables) with the oral mucosa. OAS is an IgE-mediated allergy known to be associated with cross-reactivity to certain pollens, such as birch pollen.<sup>52,53</sup> Cross-reactive IgE can be directed against the birch pollen allergen Bet v 1, which is structurally related to allergens found in fruits such as apples, and nuts such as hazelnuts. Individuals known to be allergic to birch pollen can be more susceptible to oral allergy syndrome after exposure to these foods.<sup>53</sup> To study the effects of probiotics on the oral immune response in adults, Piirainen and associates administered *Lactobacillus* GG (20 billion CFU/d) or placebo to adults with birch pollen allergy induced OAS for 5.5 months prior to the birch pollen season.<sup>52</sup> The authors conducted an oral apple challenge before, during, and after the pollen season and collected saliva and serum samples before each challenge. They found that *Lactobacillus* GG administration increased the levels of IgA and IgG specific to birch pollen and apple compared to placebo indicating that *Lactobacillus* GG stimulated the oral mucosal immune system. While not statistically significant, symptom scores decreased in the *Lactobacillus* GG group. **Overall this study indicates that supplementation with *Lactobacillus* GG has an immunostimulating effect on the oral mucosa.**

## IMMUNE BENEFITS: ADDITIONAL RESOURCES

### Role of probiotics in human health and disease: An update

Faujdar, S., et al. *International Journal of Current Microbiology and Applied Sciences*. 2016 5(3):328-44.

This review discusses the history of probiotics, the most common probiotic species today, and provides a plethora of examples of how probiotics may help us establish and maintain good health.

## 4. ***Lactobacillus* GG Clinical Support**

### **Immune system stimulation by probiotic microorganisms**

Ashraf, R. & Shah, N. *Critical Reviews in Food Science and Nutrition*. 2014 54:938-56.

This review discusses how probiotics stimulate the immune system and *Lactobacillus* GG is highlighted throughout, although other probiotics are also discussed. The authors also discuss the benefits of probiotic supplementation during pregnancy and infancy for short and long term benefits.

### **Mucosal immunology and probiotics**

Dongarra, M., et al. *Curr Allergy Asthma Rep*. 2013 13:19-26.

This review discusses some of the recent findings related to the effect of probiotics, including *Lactobacillus* GG, on mucosal immunology with a focus on innate immunity. The authors highlight their own research within innate immunity, modulating dendritic cells using probiotics.

## 4.3 **EMERGING SCIENCE**

### **EMERGING SCIENCE: THE GUT MICROBIOTA AND IRRITABLE BOWEL SYNDROME**

Irritable bowel syndrome (IBS) is the most common diagnosis in gastroenterology.<sup>54</sup> While the benefits of *Lactobacillus* GG have been well established in adults for immune health, antibiotic associated diarrhea, and Traveler's diarrhea, positive research regarding the use of *Lactobacillus* GG to treat IBS has only been published within the past few years. A clinical trial by Pederson and colleagues investigated the effect of a low FODMAP (fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols) diet versus *Lactobacillus* GG (6 billion CFU/d) on IBS and found that participants in both groups experienced a significant reduction in self-reported severity symptom scores (SSS) compared to the control group on a western diet.<sup>55</sup> There was no difference in the self-reported quality of life between either of these groups.

This finding indicates that *Lactobacillus* GG supplementation can match the symptom reduction of a FODMAP diet- the standard of care for IBS patients today. Another study by O’Sullivan and associates administered *Lactobacillus* GG, at a dose of 10 billion CFU/d, to a small group of people (n=25) and saw no difference in SSS scores for intervention group participants compared to those in the placebo group.<sup>56</sup> They did find, however, a trend in the reduction of unformed bowel movements in patients with diarrhea receiving the *Lactobacillus* GG intervention.

The battery of outcomes measured in the clinical use of *Lactobacillus* GG ranges throughout the body. Scientists continue to substantiate the known benefits of *Lactobacillus* GG, as well as those benefits that require further support, such as disease management of cystic fibrosis, diabetes, and psychiatric disorders. Clinicians continue to study the benefits of *Lactobacillus* GG in more vulnerable populations such as cancer patients, the elderly, and premature infants to establish additional therapeutic options for these groups.

## EMERGING SCIENCE: ADDITIONAL RESOURCES

### **Functional dynamics of the gut microbiome in elderly people during probiotic consumption.**

Eloe-Fadrosh, E. et al., *MBio*. 2015 March/April 6(2):e00231-15.

This study used 16S rRNA and RNA-seq metagenomic and metatranscriptomic analysis of fecal samples of 12 elderly participants before, during, and after supplementation with *Lactobacillus* GG and found significant transcriptional changes in the commensal gut microbiota post supplementation with *Lactobacillus* GG. Although more research is needed to explore the mechanism by which *Lactobacillus* GG affects gene expression, this study found that supplementation with *Lactobacillus* GG transiently affects the expression of genes that potentially promote anti-inflammatory pathways in the commensal gut microbiota.

## 4. ***Lactobacillus* GG Clinical Support**

### **Transcriptomic profile of whole blood cells from elderly subjects fed probiotic bacteria *Lactobacillus rhamnosus* GG ATCC 53103 (LGG) in a phase I open label study.**

Solano-Aguilar, G., et al. *PLOS ONE*. 2016 Feb 9 11(2):e0147426..

This study examined the immunological responses by gene expression in whole blood cells from elderly participants before and after supplementation with *Lactobacillus* GG. While more research is needed to explore the mechanism by which *Lactobacillus* GG down-regulates the genes involved in these processes, this study indicates that *Lactobacillus* GG supplementation may have an anti-inflammatory effect in the elderly.

### **Stress and the microbiota-gut-brain axis in visceral pain: Relevance to irritable bowel syndrome**

Molony, r., et al. *CNS Neuroscience & Therapeutics*. 2016 22(2):102-117

Many patients with function gastrointestinal disorders (FGIDs), such as irritable bowel syndrome (IBS), also experience comorbid behavioral disorders, including anxiety and depression, making IBS a gut-brain disorder. This review discusses and highlights evidence for the connection between visceral pain, stress, and the gut microbiota.

### **Collective unconscious: How gut microbes shape human behavior**

Dinan, T., et al. *Journal of Psychiatric Research*. 2015 63:1-9

This review discusses the role of the gut microbiota in unconscious human behavior. It highlights recent investigations that indicate our gut microbiota has the capacity to produce neuroactive compounds that regulate cognitive function, behavior patterns, social interaction, and stress management. The authors propose that the development of the gut microbiota is critical in brain development as demonstrated in animal studies focusing on cognitive function and behavior.



## Lactobacillus GG Clinical Support (pgs.22-36)

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## 4. **Lactobacillus GG Clinical Support**

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