BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624) PROFESSIONAL MONOGRAPH

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)

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BACTERIAL BALANCE: A PROMISING RESEARCH AREA

One of the most promising areas of research in medicine today is that of the interface between the indigenous microbiota of the gut and the host. This research has, in turn, engendered scientific interest in probiotic science and the utility of beneficial bacteria in the maintenance of digestive health. Although this area of medicine is considered new and revolutionary, the concept of healthy bacteria actually dates back to ancient Roman times.

Why all the interest in bacteria? Here are some interesting facts about the bacterial composition of the human body:

- Bacterial cells comprise more than 95% of the total cells in the human body
- 100 trillion bacteria reside in the gastrointestinal (GI) tract compared to 10 trillion human cells
- The surface of the GI tract represents the human body's largest contact area with the external environment. The total surface area of the GI tract is calculated to be 150 to 200 meters square compared to approximately 2 meters square surface of the skin
- There are over 500 different identified species of bacteria residing in the intestinal environment, and may include as many as 1500 different species!
- Bifidobacteria were first described in 1899 as the predominant gut microflora in breast-fed infants
- Bifidobacteria work in concert with the immune system and other GI bacteria to maintain health
- Bifidobacterial populations in the GI tract decrease in numbers as we age

The microflora of the human digestive tract has been called a "hidden organ." In fact, experts believe that the intestinal flora does not exist as an entity by itself but is instead constantly interacting at a number of levels within the human body. These enteric microflora have been demonstrated to be responsible for numerous functions that contribute to overall health, including the:

- Synthesis of vitamins including thiamine (B_1), folic acid (B_9), pyridoxine (B_6), and vitamin K
- Absorption of calcium, magnesium, and iron
- Production of epithelial nutrients such as short-chain fatty acids (SCFAs)
- Degradation of food components
- Stimulation of the immune system
- Production of digestive and protective enzymes
- Prevention of colonization by opportunistic or pathogenic microorganisms

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BACTERIAL BALANCE: A PROMISING RESEARCH AREA

The human infant begins life with a sterile GI tract, with rapid bacterial colonization occurring in the first few hours and days of life. By about age 2, the intestinal environment is fairly well established. The fecal flora of breast fed infants is dominated by bifidobacteria, which are thought to provide protection against infection. In fact, the concentration and composition of bifidobacteria are considered more important than other lactic acid bacteria for the health of the newborn. While the composition of intestinal flora can fluctuate under some circumstances, it is relatively stable for most of the adult life, especially in healthy individuals. The composition of the intestinal microflora differs widely between individuals, and is considered as unique as a person's fingerprints.

The composition of the microflora is dominated by four types of bacteria: *Bacteroides, Bifidobacterium, Eubacterium* and *Peptostreptoccocus*. Bifidobacteria are widely thought to be the most important gram positive organism in the digestive environment. Bifidobacterial species remain primary residents of the digestive microflora throughout most of an individual's life, with the size and diversity of the bifidobacterial population influenced by age, stress, antibiotic use and other common activities of everyday life. Currently, more than 29 species of bifidobacteria have been described and commensal strains have been isolated from numerous sources including breast milk, human feces, and the human vagina.

Under normal conditions, the intestinal bacteria exist in equilibrium (symbiosis) with the human host that fosters a stable, balanced environment. One of the primary functions of the beneficial bacterial species is to protect against dysbiosis, a state in which both transient and resident strains of pathogenic bacteria can cause digestive upsets. Common factors that can disrupt the normal microbial balance include changes in diet, climate, stress, illness, aging, and the use of some medications, primarily antibiotics.

"There is a growing body of evidence that the complex and vast microbial world inside our gastro-intestinal tract, also termed the intestinal microbiota, contributes to health and disease."

Saxelin M et al, 2005

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BACTERIAL BALANCE: A PROMISING RESEARCH AREA

Abnormal shifts in the microfloral balance have been documented in individuals who suffer GI upsets ranging from mild and occasional constipation or diarrhea to doctor-diagnosed conditions like Irritable Bowel Syndrome (IBS). These shifts were observed by comparing the fecal bacterial composition and fermentation patterns of IBS patients to healthy controls. Importantly, these studies have found reduced levels of bifidobacteria species in individuals meeting the diagnostic criteria for IBS.

When bifidobacteria are ingested by humans, the profile of the fecal microflora—and by association, the digestive microflora—changes in positive ways. As the proportion of bifidobacteria increases, the relative proportion of enteric pathogens decreases. Bifidobacteria produce both acetic acid and lactic acid, short chain fatty acids that are bacteriostatic and inhibit the growth of enteric pathogens.

The word probiotic means "for life". The goal of probiotic supplementation is to build and maintain an optimal bacterial balance, providing a natural defense against occasional digestive upset. The highly specific nature of each person's microflora means that no commercial probiotic can permanently reside in the human intestine, no matter how effectively it is able to attach to the mucosa. Probiotic trials show that probiotic strains are eliminated relatively quickly from the intestinal ecosystem once regular consumption of the probiotic has ceased, and no probiotic strains have been shown to colonize the GI tract permanently. Therefore, continued supplementation with probiotic products is necessary for continued health benefits.

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PORTRAIT OF A PROBIOTIC

Not all probiotics are alike. The benefits of probiotics are widely accepted to be species, and even strain specific. Different strains of the same species differ in their stability, expression of enzymes and production of inhibitory substances, the ability to colonize the GI tract and, perhaps most importantly, clinical efficacy. Therefore, the recommendation is that all probiotics be independently tested and evaluated in clinical trials. It is especially important that probiotics that make claims of health benefits be studied in well-controlled clinical trials in humans. Many health claims rely on extrapolation of data from non-clinical laboratory (*in vitro*) methods, which may not be predictive of the actual clinical benefits (*in vivo*). While these laboratory experiments can offer important insights into the way probiotics work, they are considered insufficient on their own to support claims of health benefits.

An adequate dose and duration of probiotic intake is necessary for the bacteria to colonize and exert an effect. What characteristics are important for probiotic effectiveness?

1. First, a probiotic must be alive in the product in which it is provided.

2. The probiotic must then be able to survive transit through the stomach, where the secretion of gastric acid is a primary defense against ingested micro-organisms.

3. The probiotic bacteria must have the ability to adhere to the human mucosa to allow it to reside within the gastrointestinal system long enough to elicit beneficial effects. The effectiveness of the probiotic and the dose needed to provide benefits are dependent on its adhesion affinity, with probiotics that have a high amount of adhesion able to achieve the desired probiotic effects at a lower dosage.

Isolation of bacterial species directly from the mucosa of healthy humans, rather than from the feces, is a preferred means of identifying strains of probiotic bacteria, since this isolates the bacteria from the environment where it needs to be able to function to be effective. At University College Cork, it was recognized that while lactobacilli isolated from human feces have been studied for some time, there is a scarcity of information regarding bacterial strains isolated directly from a healthy GI environment. One of the strains identified in this pivotal work, *Bifidobacterium infantis* 35624 (Bifantis®) is the first and only probiotic ingredient that was isolated directly from the epithelium of a healthy adult. Bifantis is well characterized and has been demonstrated to be bile tolerant and acid resistant, and to survive passage through the GI tract. Bifantis has been extensively researched by some of the most recognized and respected authorities in the fields of probiotic science, immunology, and GI health. The findings of the studies with this strain have been published in some of the world's leading peer-reviewed journals, including *Gastroenterology*, *The American Journal of Gastroenterology* and *Gut.*

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PORTRAIT OF A PROBIOTIC

Accepted criteria for the selection and assessment of probiotic lactic acid bacteria (LAB)*	
Requirement	**Bifantis**
Strain should be of human origin	Bifantis was isolated directly from the epithelium of the terminal ileum of a healthy human subject
Demonstrate nonpathogenic behavior	The genome has been fully sequenced, with no known regions of pathogenicity found. Bifantis is susceptible to all antibiotics with gram positive coverage.
Exhibit resistance to technologic processes (viability and activity in delivery vehicles)	Rigorous stability standard and testing of encapsulated product demonstrate ability to survive processing and maintain an effective level of viable organisms for at least 24 months at room temperature.
Prove resistant to gastric acid and bile	The Bifantis strain is readily recovered from the feces following supplementation, demonstrating the ability to survive the gastric environment.
Adhere to gut epithelial tissue	The Bifantis strain was isolated directly from intestinal epithelial tissue sample.
Be able to persist for short periods in the GI tract	The Bifantis strain continues to persist in fecal samples for at least 2 weeks following end of supplementation, with a steady decline in bacterial counts over this time.
Produce antimicrobial substances	*In vitro* studies have shown that the strain has both antibacterial and antiviral activity.
Modulate immune responses	Positive shifts in ratios of cytokines IL-10/IL-12 have been documented in both healthy individuals and IBS subjects.
Have the ability to influence metabolic activities	Bifantis strain has been shown to modulate metabolism and restore metabolic balance of GI flora.
Each strain well documented and tested independently on its own merit	Bifantis is a single strain probiotic with a well-documented genome sequence.
Extrapolation of data from closely related strains not acceptable	All testing has been conducted using the specific strain. Accordingly, no other probiotic strain can use Bifantis data in support of claims of benefit.
Probiotic strains, well-defined study preparations	Recommended daily level and form consistent with that tested in clinical studies.
Randomized human studies	All studies of Bifantis have been placebo controlled and randomized.
Results confirmed by several independent research groups	Study designed with input from a number of the world's thought leaders. Bifantis is the only probiotic strain to meet the primary endpoint in multiple clinical trials.
Publication in peer-reviewed journals	Data have been published in *Gastroenterology*, *The American Journal of Gastroenterology* and *Gut*, among others.
BIFANTIS SATISFIES ALL OF THESE CRITERIA.	

* Guarner & Shaafsma, 1998.

"The effect of a bacterium is strain specific and cannot be extrapolated even to other strains of the same species. For demonstration of probiotic activity, well-designed clinical trials are needed, which should be controlled, randomized, and double-blind."

Gaurner F and Malegelada J-R, 2003

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
CLINICAL TRIALS

Objective
To compare the response (symptoms and cytokine ratios) to ingestion of milk-based probiotic preparations containing well-characterized strains of *Lactobacillus* or *Bifidobacterium* in patients with IBS

Publication
O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan G, Kiely B, Collins JK, Shanahan F, Quigley EMM. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology*. 2005;128:541-551

Study Design
Randomized, multiple-dosage, parallel, placebo-controlled, double-blind clinical trial

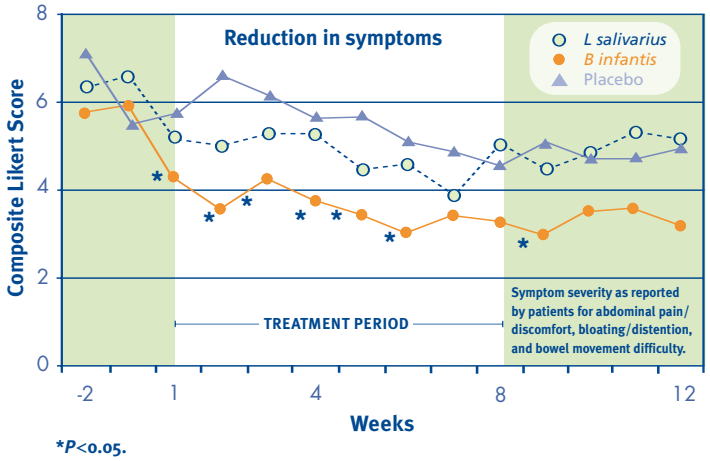
Patient Population
Seventy-seven male and female subjects aged 18 to 73 years and diagnosed with IBS according to Rome II criteria. Of these 77 subjects, 75 were considered evaluable (64% women and 36% men). Classification by IBS subtype found that 45% were alternators, 28% were diarrhea predominant, and 26% were constipation predominant.

Methods
Subjects were randomized to receive either *Lactobacillus salivarius* UCC4331 or *Bifidobacterium infantis* 35624 in a dose of $1x10^{10}$ live bacterial cells in a malted milk drink, or the malted milk drink alone as placebo, for a period lasting 8 weeks. The cardinal symptoms of IBS were recorded on a daily basis and assessed each week. Quality of life (QoL) assessment, stool microbiologic studies, and blood sampling for estimation of peripheral blood mononuclear cell release of the cytokines IL-10 and IL-12 were performed at the beginning and at the end of the treatment phase. Cytokine levels were compared to those of healthy volunteers (n=20) who were matched to the IBS subjects for age and gender.

Key Results
Subjects receiving *B infantis* 35624 had lower composite scores (abdominal pain/discomfort, bloating/distention, and bowel movement difficulty) than those receiving placebo for all weeks in the treatment phase and the entire washout period, with 10 of these 12 scores significantly lower ($P<0.05$). The 2 weeks that were not significantly lower were the washout weeks 11 and 12. In contrast, *L salivarius* UCC4331 was significantly different from placebo during the second week only.

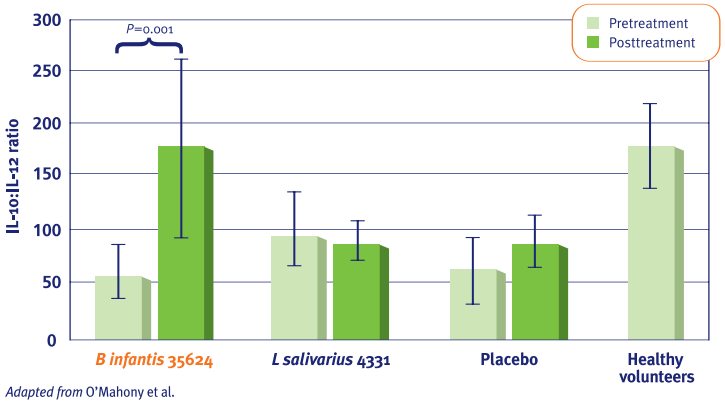
BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
CLINICAL TRIALS

For the individual symptom scores, *B infantis* 35624 was associated with a significant reduction (P<0.05) for all measures except bowel movement frequency and consistency during the treatment period of the study. These individual assessments included pain/discomfort, bloating/distention, and bowel movement difficulty.



*P<0.05.

Levels of the cytokines IL-10 and IL-12 were noted to be different at baseline between the IBS and healthy groups, with IL-10 levels lower in the IBS group and IL-12 levels increased. The ratio of IL-10/IL-12 was significantly different (P=0.003) between the groups. Following *B infantis* 35624 use, cytokine levels in the IBS subjects were similar to the levels in the healthy volunteers, while the levels were not significantly changed in either of the other treatment groups.



Adapted from O'Mahony et al.

Conclusions

B infantis 35624 alleviated symptoms in IBS, while the *Lactobacillus* strain did not. This symptomatic response was associated with a normalization of the ratio of an anti-inflammatory to a proinflammatory cytokine, suggesting an immune-modulating role for this organism in this disorder. The Bifantis strain was superior to both a lactobacillus strain and placebo for each of the cardinal symptoms of IBS and for normalization of the cytokine ratio.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
CLINICAL TRIALS

Objective
To confirm the efficacy of the probiotic *B infantis* 35624 (Bifantis) in a large scale, multicenter, clinical trial of women with IBS, and to determine the optimal dosage for administration in an encapsulated formulation.

Publications
Whorwell P, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, Kiely B, Shanahan F, and Quigley EMM. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *American Journal of Gastroenterology* 2006: 101: 1581-1590.

Study design
Multicenter, randomized, multiple-dosage, parallel, placebo-controlled, double-blind clinical trial

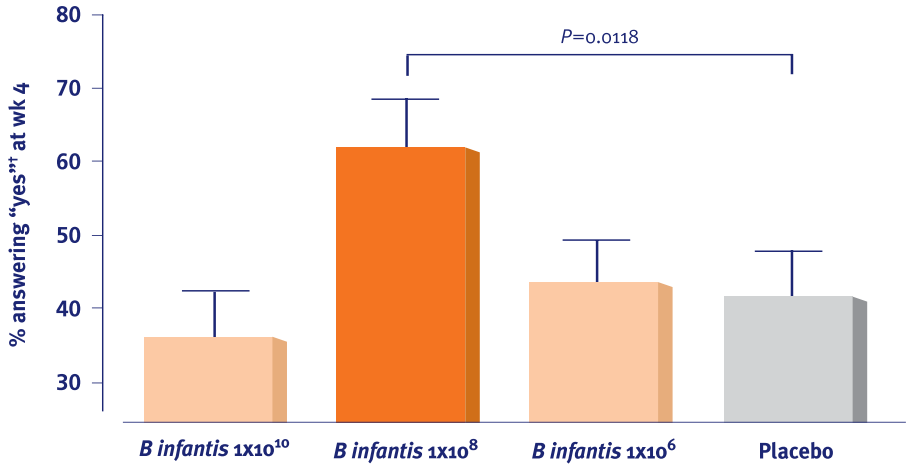
Patient population
Three hundred sixty-two female subjects aged 18 to 65 years and diagnosed with IBS according to Rome II criteria were recruited for the study and randomized to therapy. Of these 362 subjects, 330 completed the study, 293 considered evaluable per protocol and 362 comprising the ITT population. Classification by IBS subtype found that approximately 23.8% were alternators, 55.5% were diarrhea predominant, and 20.7% were constipation predominant.

Methods
After a 2 week run-in phase, subjects were randomized to placebo (n=92), or one of three dosages of *B infantis* 35624: $1x10^6$ (n=90), $1x10^8$ (n=90), or $1x10^{10}$ (n=90) CFU/capsule, given once daily for 4 weeks. IBS symptoms were monitored daily by telephone using an interactive voice response system (IVRS) and scored according to a 6-point Likert scale; stool frequency and form (using the Bristol Stool Scale) were also monitored daily. The primary efficacy variable was the abdominal pain score; secondary efficacy variables included other IBS symptoms, a composite symptom score, and subjects' global assessment (SGA) of IBS symptom relief and QoL. In all IBS symptom efficacy analyses, "centers" and "subjects within centers" were treated as random factors. All results were adjusted by baseline so dosage comparisons (placebo vs 10^6 CFU/day vs 10^8 CFU/day vs 10^{10} CFU/day) were based on least square (LS) means.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
CLINICAL TRIALS

Key results

Subjects receiving *B infantis* 35624 at the $1x10^8$ dosage had significantly ($P<0.05$) lower symptom scores at week 4 of the treatment phase for abdominal pain/discomfort, bloating/distention, sense of incomplete evacuation, passage of gas, straining, and bowel habit satisfaction than those receiving placebo. The global response score for the probiotic was 62.3% versus 42.0% for placebo, a therapeutic gain of more than 20% and a therapeutic index of 48%.



† Responses to the question "Compared to the way you felt before beginning the medication, have you had adequate relief of your IBS symptoms?"

An assessment by IBS subtype demonstrated a normalization of bowel habit, with a significant difference ($P < 0.05$) in BM frequency noted for all subjects outside the median baseline percentile (1.00-2.29 BMs/day) when the probiotic group was compared to placebo.

Conclusions

This study provided additional evidence of the benefits of the probiotic strain *B infantis* 35624 delivered in a capsule formulation at $1X10^8$ CFU/day for relief of all of the cardinal symptoms of IBS. A normalization effect on stool frequency was observed, demonstrating efficacy among all subjects, no matter the Rome II IBS-subtype designation at baseline. The findings of this study highlight the need for rigorous quality control and clinical trials of probiotic in the final dosage form.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
MECHANISTIC STUDIES

Probiotic supplementation impacts metabolism and immune response in healthy and IBS subjects

Objective
To investigate the effects of a dietary supplement, *B infantis* 35624, on the intestinal microflora of subjects with and without symptoms of IBS. Secondarily, to understand the differences in fecal microflora metabolism between IBS subjects and healthy subjects

Publication
Fecal flora effects following oral supplementation with *Bifidobacteria infantis* 35624 in healthy and IBS subjects. Charbonneau DL, Altringer LA, Carryl OR, Chen KS, Kidd KJ, Darcy T, Fawcett DH, Trowbridge MM, Jang C, Luo F, Poehner RD, Meller ST. World Congress of Gastroenterology; September 2005; Montreal, Canada.

Study design
Multiple-dose, open-label clinical trial

Patient population
Male and female volunteer subjects at least 18 years of age who met the Rome II criteria were age-matched to healthy subjects (in generally good health and IBS-symptom free). A total of 24 subjects completed all phases of the study, with 23 subjects in the intent-to-treat (ITT) population for statistical analysis: 18 (78%) females; 5 (22%) males. Subjects averaged 41.7 years of age (range of 30–54 years). Eighteen (78.3%) were Caucasian, 3 were African American, and 2 were Asian. No statistically significant difference was found between the IBS and healthy groups for age, gender, or race. Among the 13 subjects with IBS, 9 (69.2%) were diarrhea predominant, 3 were constipation predominant, and 1 was alternating.

Methods
Subjects consumed a placebo milk preparation during a 2-week baseline period, followed by a 3-week study phase in which they consumed *B infantis* 35624 ($1x10^{10}$ colony-forming units (CFU)/day in milk. Fecal samples were collected at baseline and during weeks 2 and 3 of probiotic consumption. Fecal samples were analyzed by microbiologic plating using selective media for coliforms, lactobacilli, methicillin-resistant *S aureus* (MRSA) medium, bacteroides, total anaerobes, and enteric pathogens. Bifidobacteria were monitored by fluorescent in situ hybridization (FISH) using a selective probe. DNA was extracted from fecal samples and bacterial community analysis was performed by terminal restriction fragment length polymorphism analysis (T-RFLP). Analysis of variance (ANOVA) and categorical data analysis evaluated the differences between groups. Comparisons of bifidobacteria between IBS and healthy subjects were determined using quantitative polymerase chain reaction (PCR) analysis. An analysis of SCFAs was also conducted.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
MECHANISTIC STUDIES

Probiotic supplementation impacts metabolism and immune response in healthy and IBS subjects

Venous blood samples were drawn before and after the feeding period, and systemic cytokines analyzed. Isolated PBMC were cultured *in vitro* for 3 days, either alone with medium or with stimulant (LPS, or bifidobacteria). The presence of human cytokines (IL-1-β, IL-10, IL-12, TNF-α, IFN-γ, TGF-β) in the supernatant was analyzed with LINCOplex kit assay (Linco) in a Bio-Plex® bead flow cytometer (Bio-Rad). Differences in cytokine levels were analyzed using ANOVA.

Key results

Differences were noted in the microfloral composition and metabolism at baseline when samples from the IBS subjects were compared to samples from the healthy subjects:

- At baseline, the IBS group had significantly (P<0.10) higher levels of enteric pathogens than the healthy group
- At baseline, the IBS group had a directionally higher level of acetate than the healthy group

Consumption of *B infantis* 35624 resulted in positive benefits for both the IBS and healthy subjects:

- After only 2 weeks of probiotic use, the IBS and healthy groups had significant increases in both the total and percent bifidobacteria counts (P<0.10) when compared to baseline levels
- After 3 weeks of probiotic use, both the IBS and healthy groups had reduced levels of total microbial cell counts, with a statistically significant reduction in the IBS group (P<0.10)
- After 2 weeks of probiotic consumption, the IBS group had a significant (P<0.10) reduction in total anaerobe and bacteroide counts in comparison to baseline levels
- After 3 weeks of probiotic consumption, the healthy group had significantly higher lactobacilli counts than at baseline (P<0.10)

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
MECHANISTIC STUDIES

Probiotic supplementation impacts metabolism and immune response in healthy and IBS subjects

Key results (cont'd)

- Reductions in the levels of the SCFA acetate and propionate were observed in the IBS group from baseline to week 2 and week 3 of the probiotic use period
- The changes in the SCFA profile among the IBS subjects was a significantly higher (P<0.10) magnitude than that of the healthy subjects

Analysis of cytokines:

- At baseline, there were no differences in cytokine levels of unstimulated PBMC in IBS and healthy subjects; however, *in vitro* LPS stimulation of PBMC from IBS subjects produced a significantly higher (P<0.10) level of proinflammatory cytokines (IL-12, TNF-α) and a lower ratio of anti-inflammatory/proinflammatory cytokines (IL-10/IL-12, TGF-β/IL-12) than that of healthy subjects
- Probiotic consumption did not significantly affect the spontaneous production level of cytokines between the study populations. However, *in vitro* LPS stimulation of PBMC from IBS subjects produced a significantly lower level (P<0.1) of IL-12 and a higher ratio of IL-10/IL-12. When PBMC from IBS subjects were stimulated *in vitro* with *B infantis* 35624, the same change pattern in the aforementioned cytokines was observed, along with changed levels in other cytokines (elevated IL-10 and IL-10/IFN-γ ratio, decreased IFN-γ)

The probiotic was well tolerated in this study, with no serious adverse events reported.

Conclusions

Differences in the composition of fecal microflora between IBS and healthy subjects were demonstrated in this study. These observed differences were associated with altered production of SCFAs. Daily consumption of the probiotic *B infantis* 35624 resulted in positive changes to the fecal floral composition in both healthy and IBS subjects, and also resulted in a normalization of the SCFA profile in the IBS subjects. Systemic immune response by cytokine production in IBS subjects' PBMC is altered when compared to the healthy population.

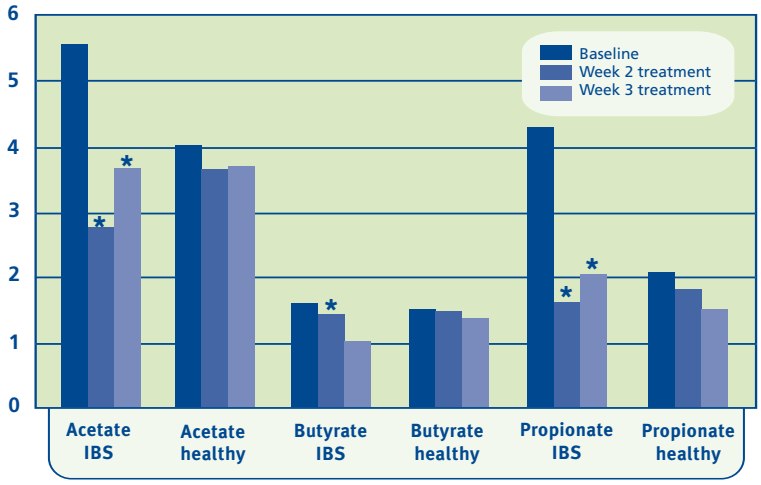
BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)

MECHANISTIC STUDIES

Probiotic supplementation impacts metabolism and immune response in healthy and IBS subjects

Bifidobacteria normalized immune response of IBS by producing: (i) a higher level of anti-inflammatory cytokine (IL-10) when stimulated *in vitro* with bifidobacteria; (ii) an elevated ratio of anti-inflammatory/proinflammatory cytokines (IL-10/IL-12, IL-10/IFN-γ) when stimulated *in vitro* with LPS or bifidobacteria.

Changes in SCFA production in healthy and IBS subjects with probiotic consumption



*$P<0.10$.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
MECHANISTIC STUDIES

Metabolic effects of probiotic supplementation

Objective
To investigate the impact of *B infantis* 35624 on fecal flora derived from healthy (n=5) and IBS (n=5) subjects in a chemostat model

Investigators
Charbonneau DL, Baria M, Poehner RD, Mccauleymyers D, Eads C, Furnish C, Donovanbrand R

Study design
In vitro evaluation of fecal samples from IBS and healthy subjects in a chemostat model

Patient population
Male and female volunteer subjects at least 18 years of age who met the Rome II criteria were age-matched to healthy subjects (in generally good health and IBS symptom free). A total of 5 IBS subjects and 5 healthy subjects provided stool samples for experimentation in a chemostat.

Methods
Fecal samples were homogenized into slurries and filtered through cheesecloth to eliminate large particulates, then inoculated into a Braun Model M2 fermentor operated as a chemostat. Chemostat conditions were: anaerobic via a continuous flow of N_2 (20 psi), pH stated to 7.0, impeller rate 50 rpm, 37^{O}C, and nutrient feed of 60mL/hr. Baseline (4 consecutive days) was followed by additions of *B infantis* 35624 ($1x10^{10}$ CFU/day) for 4 consecutive days. Chemostat samples were evaluated for bacterial content using selective media for total anaerobes, bifidobacteria, fusobacteria, clostridia, enteric pathogens, and bacteroides. Random bacterial colonies from selective media were further classified by 500 base-pair sequence analysis of the 16s rRNA gene. Quantitative analysis of short-chain volatile fatty acids was conducted and changes in the chemical composition of the growth medium studied using nuclear magnetic resonance (NMR) spectroscopy. Multivariate analysis was used to extract spectra of components whose concentrations changed during baseline and treatment phase.

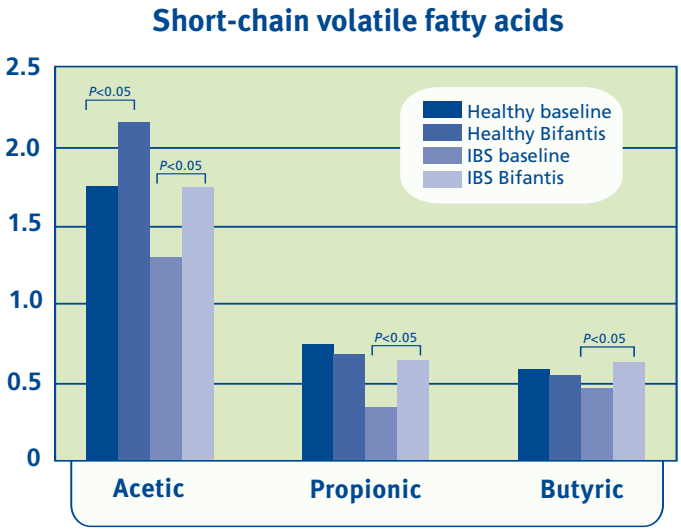
BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
MECHANISTIC STUDIES

Metabolic effects of probiotic supplementation

Key results
B infantis 35624 reduced enteric pathogens from healthy subjects and black pigmented bacteroides populations from IBS subjects, but had little to no effect on butyrate formation from either type of flora. *B infantis* 35624 increased acetic acid production and reduced propionic formation in healthy flora while it stimulated both acetic and propionic formation in IBS flora. NMR analysis found metabolites produced in higher concentrations in healthy versus IBS flora. Probiotic addition resulted in increased production of these metabolites in IBS flora to levels similar to healthy flora. Probiotic addition also reduced the levels of metabolites elevated in IBS flora to levels comparable to those in healthy flora.

Conclusions
In the chemostat model, compositional differences in flora derived from healthy versus IBS subjects were noted. Overall, *B infantis* 35624 addition resulted in a change in the IBS profile (flora and metabolites) to mimic the healthy condition. These shifts provide a possible explanation for the observed clinical benefits associated with this novel probiotic.

Publication
Charbonneau D, Baria M, Poehner R, Mccauleymyers D, Eads C, Furnish C, Donovanbrand R. Impact of *Bifidobacterium infantis* 35624 on fecal flora from healthy and IBS subjects in a chemostat model. *Gastroenterology.* 2005;128:A-661.



BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
TOLERABILITY

Objective
To evaluate the tolerability and safety of the probiotic organism *Bifidobacterium infantis* 35624 and, specifically, to address:

- Short-term tolerability in a functional disorder (IBS).
- Risk of systemic infections among those with impaired barrier function (IBD)

Publication
Safety and tolerability of the probiotic organism *Bifidobacterium infantis* 35624: clinical experience and molecular basis. Quigley EMM, Whorwell PJ, Shanahan F, Van Sinderen D, Xu J, Altringer L, O'Mahony L, Guarner F and the PROGID investigators. *Gastroenterology* 2006:130(4)S2:493

Methods
Safety data from two randomised, placebo-controlled, double blind trials, a four-week dose-ranging study, (*B infantis* 35624 10^6 vs 10^8 vs 10^{10}) in subjects with irritable bowel syndrome (IBS) and a one-year study among subjects with active Crohn's disease and ulcerative colitis, were reviewed for evidence of short-term tolerability and long-term safety, respectively. The genome of the organism was also evaluated for evidence of genetic features of pathogenicity.

Key Results
IBS Study
17 subjects withdrew due to adverse events (AE's):

- 9 (10%) from the placebo group vs. 8 (3%) from the 3 treatment groups combined. The majority were occasioned by worsening of IBS symptoms.
- The percentages of subjects reporting at least one AE were similar across groups. Subjects on placebo were the most likely to report multiple AEs.
- The severity of AEs was similar between the placebo and active legs, with 5% judged as severe in the placebo leg and 6% across the active legs.
- AEs were judged to be treatment related slightly more often in the placebo leg (9%) than for the active legs (3%).

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)

TOLERABILITY

IBD Study

- 115 subjects discontinued the study due to relapse, with the rates similar across all treatment legs and across both study arms. There were no discontinuations due to AEs.
- The percentages of subjects reporting at least one AE were the lowest in the *B infantis* legs for both study populations.
- The severity of AEs was also lower in the *B infantis* legs in both study populations.
- AEs were more likely to be judged as treatment related in the placebo leg in both study populations. AEs in the *B infantis* leg were the least likely to be judged as treatment related in both arms.

Genome Analysis

From genome analysis it was apparent that *B. infantis* 35624 did not contain DNA that was homologous to known pathogenicity islands or transferable antibiotic resistance markers.

Conclusions

B. infantis 35624 is well tolerated in the short term by patients with IBS and is not associated, in long-term therapy, in a susceptible population (IBD), with any evidence of risk for systemic sepsis. These clinical findings are supported by genome analysis.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)

SAFETY OF PROBIOTICS

While a number of species and strains of bifidobacteria have been identified as normal inhabitants of the GI system, expert opinion is that the benefits conferred by these organisms when used as probiotics are strain specific, and therefore, each individual probiotic strain should be independently tested and evaluated.

The safety of probiotics has been the focus of a number of reviews in the medical literature. In these reviews, the safety of the most common probiotic species (Lactobacillus and Bifidobacteria) have been supported, while concerns have been raised about other species:

- *Lactobacillus* spp and *Bifidobacterium* spp have a long history of safe use as probiotics without any significant established risk to humans
- No pathogenic or virulence properties have been identified for bifidobacteria
- No cases of infections from bifidobacteria in probiotics have been reported, and bifidobacteria are infrequently encountered in clinical tissues. The isolated species *B dentium, B denticolens,* and *B inopinatum* have been associated with human dental caries. B dentium has been isolated from various clinical materials such as lower respiratory tract specimens. This further highlights the importance of using only strain-specific products that have rigorous quality control standards from reputable manufacturers.
- Enterococcus species have emerged as an important cause of nosocomial infections and isolates of this species have been noted to be increasingly resistant to antibiotics, in particular vancomycin.
- *Saccharomyces boulardi* is used widely as a probiotic, however, this yeast has been associated with episodes of systemic fungal infections (fengicemia).
- *Lactobacillus sporogenes*, a spore-forming bacterial species, is not recognized as a probiotic. Even so, this nomenclature is used by a number of companies to describe the organisms in their products.

The safety of Bifantis has been affirmed by the US Food and Drug Administration through its New Dietary Ingredient notification process. Since the enactment of the Dietary Supplement Health and Education Act of 1994, more than 350 applications for new dietary ingredients have been submitted for FDA review, with only about 30% considered adequate to support market introduction of the dietary ingredient.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)

SAFETY OF PROBIOTICS

A set of criteria was recommended by a joint FAO/WHO Working Group in what is widely considered to be the most authoritative report on establishing the identity, benefit, and safety of the use of probiotics for human consumption. *B infantis* 35624 satisfies these requirements as outlined in the table below. Clearly, the quality control advantages of using a specific strain in a marketed product far outweigh any disadvantages.

A joint FAO/WHO Expert consultation has recently published several criteria and standards for assurance of quality and reliability in the use of probiotics in humans	
Criteria	***Bifantis***
Genus and strain identification by acceptable DNA sequencing	Bifantis genome has been fully sequenced, and strain specific criteria are used in manufacturing to insure product quality and control.
Proper product labeling stating the exact genus, species, strain, and quantity	Product label states all required information
Safety tests, including antibiotic resistance	Bifantis is susceptible to antibiotics that would be expected for a gram-positive bacteria, including ampicillin, ciprofloxacin, erythromycin, gentamycin, penicillin, tobramycin and vancomycin.
Evidence-based measurements of health benefits from well-controlled randomized trials of sufficient power	Benefits have been demonstrated in the 2 largest, properly controlled clinical trials conducted to date with a probiotic strain. Both studies were placebo controlled, double blind, randomized clinical trials. The first study included over 70 patients, and the second study included over 360 patients.
***Bifidobacterium infantis* 35624 (Bifantis) meets all of these criteria.**	

Reference: Joint FAO/WHO Working Group. 2002. Guidelines for the Evaluation of Probiotics in Food, FAO/WHO.

"The safety record of probiotics, and lactobacilli and bifidobacteria in particular, is good. However, in order to maintain this good record, it is of major importance that all strains be correctly identified."

O' Brien J et al, 1999

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
APPROACH TO QUALITY CONTROL

Objective
The literature shows that many commercial probiotic preparations are nonviable, incorrectly identified or worse contain microorganisms not recognized as probiotics or specific on the product label. Given that the benefits of probiotic bacteria are strain specific, there is a need for strain-specific methodologies to assure consumers of product quality. We examined a method for the strain-specific identification of B infantis 35624.

Publication
Development of Strain-Specific Molecular Method for the Identification of Bifidobacteria infantis 35624. Charbonneau D, Poehner R, Donovan-Brand R, Xu J and Fawcett D. *Gastroenterology* 2006:130(4)S2:314

Methods
A library of 32 strains of Bifidobacteria were obtained from ATCC and used as references. Where appropriate, library strain identity was confirmed using species-specific PCR reactions. Library strain identity was further confirmed via 16S gene sequencing. B. infantis 35624 was grown in pure culture, and examined in a freeze-dried powder preparation. The method of rep-PCR was examined as a means for strain differentiation. DNA was isolated using the Ultra Clean Microbial DNA Isolation Kit as modified by Diversilabs. DNA extracts were processed using the Diversilab Bacterial Bar-Code system.

Key Results
The Diversilabs Bacterial Bar-code system was very reproducible and effective in strain-specific identification among Bifidobacteria. This assay distinguished *B infantis* 35624 from 32 other Bifidobacterial strains regardless of whether the DNA was isolated from pure cultures or the freeze-dried preparation.

Conclusions
Bacterial Bar-Code assay provided a molecular method for strain-specific identification of *B infantis* 35624. These results further confirm the uniqueness of this clinically effective probiotic *Bifidobacterium infantis* 35624.

The quality control advantages of using a specific strain like *B infantis* 35624 far outweigh any disadvantages.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
FREQUENTLY ASKED QUESTIONS

How can you be confident the results from your clinical studies with Bifantis were clinically meaningful?

In both studies, the primary endpoint was reduction in abdominal pain/discomfort, the cardinal symptom of IBS, with Bifantis significantly better than placebo in both studies. The larger of the 2 studies included a global response score, a widely recommended measure of efficacy used in numerous IBS clinical trials. The response to Bifantis (62.3%) and the therapeutic margin over placebo (42.0%, index of 48%) is the largest response seen to date in any IBS study.

What is the recommended daily supplement level for Bifantis, and how does it compare to what was studied in your clinical trials?

Bifantis has been formulated into capsules, each providing $1X10^9$ CFU per day. The recommended supplement level is one capsule daily. The probiotic level found to be effective in clinical trials has ranged from $1X10^8$ CFU/day to $1X10^{10}$ CFU/day. Importantly, capsules containing Bifantis have been formulated to stay within this range throughout the labeled "best used by" date on the bottle.

Can Bifantis be used with antibiotics?

Antibiotic use commonly results in disruption of the natural balance of bacteria in the GI tract. Probiotics help re-establish this lost balance by adhering to epithelial cells and displacing pathogens, competing for nutrients, modifying the pH, and even producing antimicrobial substances. Bifantis is sensitive to antibiotics as would be expected for a gram-positive organism, so the recommendation is to stop probiotic supplementation during use of one of these antibiotics, and begin again as soon as the antibiotic regimen is complete.

Does Bifantis help with lactose intolerance?

Bifantis is a lactic acid bacteria, and has the ability to digest lactose and convert it to lactic acid. We expect that the use of this probiotic would benefit individuals who are lactose intolerant; however, we have not conducted clinical trials to verify this.

How can I find information on what products contain Bifantis?

Bifantis.com contains information on dietary supplements that contain Bifantis

Who should not use Bifantis?

Bifantis should be avoided by individuals who are allergic to soy or milk protein, as these proteins are used in the process of growing and protecting the Bifantis bacteria, and trace amounts of these proteins remain in the finished product.

How quickly should they start to see results after they begin supplementing with Bifantis?

People respond individually to probiotics like Bifantis, and your individual patients should expect slightly different adjustments as they begin probiotic use. For example, during the first week of therapy, your patients may notice some adjustments, such as a temporary increase in the amount of bloating, as their system begins to rebalance itself. This is normal and a sign that Bifantis is starting to work with the digestive system— so you should encourage them to stick with it. Based on the results from Bifantis studies, your patients should experience a noticeable improvement by the third or fourth week of Bifantis use.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)
FREQUENTLY ASKED QUESTIONS

How long should I recommend that my patients continue to use Bifantis?

The goal of probiotic supplementation is to maintain an optimal bacterial balance, providing a natural defense against occasional digestive upset. Because of the relatively stable nature of the digestive environment, probiotic supplementation is unlikely to permanently change the composition of the flora, making continued supplementation necessary to sustain the health benefits that Bifantis provides.

What can my patients expect if they stop using Bifantis?

Taking Bifantis every day helps maintain the optimum level of friendly bacteria, which can work as a buffer against common triggers of occasional digestive upsets like stress, eating out, and travel. Some people have noticed that when they have missed taking Bifantis for several consecutive days, their digestive imbalance begins to return. Developing a regular habit of taking the probiotic each day will help your patients avoid the ups and downs that come from this imbalance, and stay on track for normal digestive health.

Why should I recommend Bifantis when there are other bifidobacteria strains on the market?

Many probiotic products claim to include bifidobacterial species, often labeling the product as simply bifidus without identification of the species of bacteria included in the product. Several species of bifidobacteria—specifically *B dentium, B denticolens, and B inopinatum*—have been associated with dental caries and are not recommended for use in human products. Bifantis contains only the specific strain *B infantis* 35624, with rigorous manufacturing standards to ensure product purity and quality.

Can Bifantis be used for children?

Bifantis is a probiotic strain that can be used by everyone, regardless of age, to help improve problems with digestive balance. However, all of the clinical trials with Bifantis have been conducted in adults, and as for any product, there are concerns about the extrapolation of these results to predict effectiveness in children.

Does Bifantis have an antibacterial effect? Can it be used in Small Intestine Bacterial Overgrowth?

When probiotic bacteria are present in the system at adequate levels, this fosters the development of an environment which is unfavorable for the pathogenic bacteria. There are a number of mechanisms by which probiotic bacteria accomplish this: most notably by competing for food and attachment sites, strengthening host defense mechanisms, and secreting inhibiting substances such as lactic acid – and in the case of bifidobacteria, both lactic acid and acetic acid. A recent editorial by Dr. Doug Drossman highlights that only a subset of patients with IBS have bacteria overgrowth, with the recommendation that only upon a confirmed diagnosis of SIBO based on positive breath gas measurement should antibiotics be recommended, followed immediately by probiotic supplementation.

BIFANTIS® (*BIFIDOBACTERIUM INFANTIS* 35624)

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