

## Abstracts from Digestive Disease Week, May, 2006

### **Development of Strain-Specific Molecular Method for the Identification of Bifidobacteria infantis 35624.**

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**Background & Aim:** Evidence from controlled clinical trials indicates that the benefits of probiotic supplementation are strain specific, and limited to specific clinical conditions. Bifidobacterium infantis 35624 is the only probiotic strain clinically proven to provide multiple symptom relief in IBS. The literature also shows that many commercial probiotic preparations lack necessary quality control, as often the probiotic preparations are nonviable, incorrectly identified or worse contain microorganisms not recognized as probiotics. Thus, 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. **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. **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 the other Bifidobacteria 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.

## **Safety and tolerability of the probiotic organism *Bifidobacterium infantis* 35624: clinical experience and molecular basis.**

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**Introduction** Evidence for efficacy for probiotics in a variety of gastrointestinal disorders accumulates. While probiotics are generally regarded as safe, there have been few large-scale investigations of short-term tolerance or long-term safety and theoretical concerns have been raised regarding short-term tolerability in functional disorders and risk of systemic infections among those with impaired barrier function. **Aim** To evaluate the tolerability and safety of a probiotic organism *Bifidobacterium infantis* 35624. **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. **Results** In the IBS study, 270 subjects were randomised to one of the three doses of the organism and 92 to placebo; 330 completed the study, including 243 on active treatment. A total of 17 subjects withdrew due to adverse events (AE's), 9 from the placebo group and 8 from the three treatment groups combined. The majority were occasioned by worsening of IBS symptoms. The overall incidence of all AE's was similar in the four groups at 48%, 37%, 52% and 43% for placebo,  $10^6$ ,  $10^8$ , and  $10^{10}$ , respectively with the majority (29%, 37%, 28% and 24%) being IBS-type gastrointestinal symptoms. The incidence of severe AE's adjudged as treatment related was highest in the placebo group at 9%; rates for the three treatment groups were 0%, 1% and 2%, respectively. In the inflammatory bowel disease (IBD) study, 32 patients with Crohn's disease and 50 with ulcerative colitis completed the study. No instances of systemic or organ sepsis were recorded. 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.

## Abstracts from American College of Gastroenterology, October, 2005

### **Probiotic use results in normalization of bowel movement frequency in IBS. Results from a clinical trial with the novel probiotic *Bifidobacteria infantis* 35624.**

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**Purpose:** Benefits associated with a novel probiotic strain, *B. infantis* 35624 in IBS have previously been reported. The aim of this analysis was to determine the impact of probiotic therapy on bowel movement (BM) frequency.

**Methods:** Data from a randomized, double-blind, placebo-controlled clinical study in female IBS subjects were evaluated. BM frequency at baseline was calculated using data from the last 7 days of the run-in phase. BM frequency was then evaluated in a similar manner during each week of a 4-week treatment phase. To determine effect of probiotic on BM frequency, *B. infantis* 35624 1x10<sup>8</sup> CFU per capsule (n=85) and placebo (n=80) were compared for subjects with differing baseline bowel movement frequencies (i.e. ranging from constipation, at one end, to diarrhea, at the other) using analysis of covariance.

**Results:** At baseline, the median BM frequency was 1.43 BM/day, with an inter-quartile range of 1 BM/day to 2.29 BM/day. The distribution of BM frequencies over the entire range of percentiles is illustrated in the table. While there were no statistical differences between placebo and *bifidobacterium* at the mid-point of the distribution frequency (inter-quartile range), significant differences (p<0.05) were noted at both ends (i.e. below the 15<sup>th</sup> percentile: constipation and above the 81<sup>st</sup> percentile: diarrhea) of the frequency distribution with the *bifidobacterium* treated group experiencing a normalization of bowel habit in each instance.

**Conclusion:** The probiotic *B. infantis* 35624 normalised bowel habit among IBS patients with diarrhea or constipation at baseline by increasing BM frequency in constipated subjects and reducing BM frequency in those with diarrhea at baseline. These results suggest that supplementation with the novel probiotic *B. infantis* 35624 results in normalization of BM frequency for IBS sufferers at both ends of the spectrum.

Change from baseline comparisons at different baseline values					
Baseline percentile	Average BM/day	Week 4			
		<i>B. infantis</i> 35624	Placebo	Difference	p-value
10th	.71	+.57	+.31	+.25	.037
15th	.80	+.51	+.27	+.23	.049
25th	1.00	+.36	+.18	+.18	.098
50th	1.43	+.04	-.03	+.07	.457
75th	2.29	-.58	-.44	-.15	.145
81st	2.57	-.79	-.58	-.22	.049
88th	3.00	-1.11	-.78	-.33	.010
90th	3.14	-1.21	-.85	-.36	.007

**Who is the responder to probiotic therapy in IBS? Data from a controlled clinical trial with *Bifidobacterium infantis* 35624.**

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**Purpose:** Benefits in IBS with a novel probiotic strain, *B. infantis* 35624, have previously been reported. The aim of this analysis was to identify baseline characteristics that have a significant effect on the treatment response.

**Methods:** Subjects from a randomized, double-blind, placebo-controlled clinical study, were considered treatment responders if they had at least 2 weeks with adequate relief of IBS symptoms during a 4-week treatment phase. Predictors included in the analysis were: 12 symptom variables; 6 variables used in the Rome II classification of IBS sub-types; 6 demographic variables; and treatment effect (n=87 on active, n=86 on placebo). The best baseline predictors were obtained by fitting a logistic regression model using a backward elimination technique.

**Results:** The baseline characteristics most closely associated with responders were the symptom *urgency* (mean=1.96, SD=0.93); the Rome II variables *hard stool* and *straining*; and the demographic variable *alcohol consumption* (mean=4.67, SD=5.46). A total of 115 subjects (66.5%) had hard stools and 127 (73.4%) had straining in the last 3 months (Rome II). At the end of the treatment phase there were 55 (63.2%) responders in the active group and 40 (46.5%) in the placebo group (unadjusted OR=2.08). The table gives the main results of the best logistic regression model. A one-unit score increase for urgency increased the chances of being a responder by a factor of 1.45. Hard stool increased the OR whereas as straining decreased it. A one-unit increase in alcohol consumption decreased the chance of response by a factor of 0.88. After adjusting for these baseline predictors, the odds of response in the *B. infantis* 35624 group were 2.15 times that in the placebo group.

**Conclusion:** The baseline variables urgency, hard stool, straining and alcohol consumption have a significant impact on the chances of being a treatment responder to this probiotic. In the presence of the best baseline predictors treatment efficacy increased, as measured by the OR, from 2.08 to 2.15.

Main Results of the Best Logistic Regression Model				
Variable	Beta Estimate	SE	P-value	OR
Urgency	.38	.19	.05	1.45
Hard Stool	1.04	.45	.02	2.84
Straining	-1.11	.49	.02	.33
Alcohol	-.13	.03	<.01	.88
Treatment Effect	.76	.33	.02	2.15

**Abstracts from World Congress of Gastroenterology, September 2005.**

**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. The Procter & Gamble Company, Personal Health Care Technology Division, Health Care Research Center, Mason

**Background & Aim:** Clinical benefits for irritable bowel syndrome (IBS) with the unique probiotic strain *Bifidobacterium infantis* 35624 delivered from milk have previously been reported. The impact of *Bifidobacterium infantis* 35624 feeding on fecal floral composition in healthy (n=10) and IBS (n=13) subjects was examined. Additionally a study examined the levels of *Bifidobacterium* in stools of healthy (n=34) and IBS (n=47) subjects. **Methods:** In the feeding study subjects were fed a placebo milk preparation 2 weeks baseline period, followed by a 3 week study phase in which subjects were fed *B. infantis* 35624 ( $10^{10}$  CFU/day) in milk. Fecal samples were collected at baseline, and weeks 2 and 3 of feeding. Fecal samples were analyzed by microbiological plating using selective media for Coliforms, Lactobacilli, MRSA medium, Bacteroides, total anaerobes and enteric pathogens. *Bifidobacteria* was 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 and categorical data analysis evaluated the differences between groups. Comparisons of *Bifidobacterium* between IBS and healthy subjects were determined using quantitative PCR analysis. **Results:** At baseline IBS and healthy subjects had similar fecal counts of total anaerobes, Bacteroides, Coliforms and Lactobacilli. IBS subjects had directionally lower levels of bacteria on MRSA. IBS subjects had a higher level of enteric pathogens in their stool versus healthy subjects. After 2 weeks of feeding, the difference in enteric pathogen level diminished. In healthy subjects Lactobacilli counts increased during the feeding period. FISH demonstrated that as feeding progressed, *Bifidobacteria* levels increased in both groups. T-RFLP profiles demonstrated differences between healthy and IBS subjects. MspI generated terminal restriction fragments measuring 476, 551, and 553-554 base pairs were more common in healthy than IBS subjects. Fragments measuring 88, 91 and 552 appeared exclusively in IBS subjects. IBS subjects were found to have significantly fewer *Bifidobacterium* counts than healthy subjects. **Conclusions:** There are differences in the composition of fecal microflora between IBS and healthy subjects. Daily consumption of *B. infantis* 35624 impacted fecal flora with a reduction in enteric pathogens in IBS subjects and an enhancement in Lactobacilli in healthy subjects. The shift in microflora provides a possible explanation for the observed clinical benefits associated with this novel probiotic.

This research was funded by The Procter and Gamble Co., Cincinnati, USA

**Probiotic supplementation with a *Bifidobacteria infantis* 35624 capsule provides symptom relief in subjects with Irritable Bowel Syndrome: results of a multi-center, placebo-controlled clinical trial.**

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**Background & Aim:** Milk-based delivery of a novel probiotic strain, *Bifidobacteria infantis* 35624, has been reported in the literature (*Gastroenterology* 2005; 128:541-551) to have benefits for patients with irritable bowel syndrome (IBS). The aim of this study was to evaluate 3 encapsulated formulations of *B. infantis* 35624 in a large, multi-center, randomized, double-blind, placebo-controlled clinical study among IBS patients. **Methods:** After a 2 week baseline phase, 362 female subjects with Rome II-positive IBS were randomized to 1 of 3 formulations of *B. infantis* 35624, namely, 1x10<sup>10</sup> (n=90), 1x10<sup>8</sup> (n=90), or 1x10<sup>6</sup> (n=90) CFU/capsule, or to a Placebo (n=92). Capsules were given once daily for 4 weeks. Patients reported IBS symptoms daily, by telephone, using an interactive voice response system and a 6-point Likert scale. Stool frequency and form (Bristol Stool Scale) were also reported daily. The primary efficacy variable was the abdominal pain; secondary efficacy variables included other IBS symptoms, a composite symptom score (sum of the abdominal pain/discomfort, bloating/distension, and bowel habit satisfaction), subject’s global assessment (SGA) of IBS symptom relief, and quality of life. 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 (1x10<sup>10</sup> vs. 1x10<sup>8</sup> vs. 1x10<sup>6</sup> vs. Placebo) were based on Least-square Means. The efficacy variable SGA for symptom relief was analyzed via a logistic model with “centers” as a random effect. The estimated percents were not adjusted by any covariate. **Results:** At Week 4, 10<sup>8</sup> was significantly superior to Placebo for the primary efficacy variable, abdominal pain/discomfort, and the SGA (P-values = 0.0226 and 0.0074). In addition, the table below depicts other secondary efficacy variables for which 10<sup>8</sup> was significantly superior (P-value < 0.05) to placebo. There were no significant differences observed for either the 10<sup>10</sup> or 10<sup>6</sup> formulation compared to placebo. All probiotic formulations were well tolerated and no clinically significant adverse events were reported. **Conclusion.** *B. infantis* 35624 delivered in a capsule formulation at 10<sup>8</sup> CFU/day is effective in relieving all of the cardinal symptoms of irritable bowel syndrome. This study confirms benefits observed previously in a milk-based delivery while demonstrating the complexity of achieving stable and effective probiotic capsule formulations.

Efficacy Variable (change from baseline)	Treatment Group (CFU/day)		
	Placebo	1x10 <sup>8</sup>	P-value
Abdominal Pain/Discomfort	-0.58±0.10	-0.89±0.10	0.0226
Bloating/Distension	-0.44±0.10	-0.71±0.10	0.0458
Incomplete Evacuation	-0.25±0.10	-0.54±0.10	0.0335
Straining	-0.07±0.09	-0.38±0.09	0.0152
Passage of Gas	-0.30±0.09	-0.54±0.09	0.0383
Overall IBS Symptom	-0.42±0.09	-0.76±0.09	0.0074
Bowel Habit Satisfaction	-0.26±0.09	-0.55±0.09	0.0151
Composite Score	-1.27±0.26	-2.12±0.25	0.0126
SGA Symptom Relief	42.0%	62.3%	0.0118

This research was funded by The Procter and Gamble Co., Cincinnati, USA

**Oral administration with a unique probiotic strain, *Bifidobacterium infantis* 35624, to IBS subjects normalizes their systemic immunological imbalance in cytokine profile toward healthy subjects.**

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**Background & Aim:** Investigate effects of a novel probiotic, *Bifidobacterium infantis* 35624, on cytokine production by peripheral blood mononuclear cells (PBMC) after oral administrations of a probiotic in healthy and irritable bowel syndrome (IBS) subjects. **Methods:** Thirteen self-reported IBS subjects (male and female) and 10 healthy subjects were included in this pilot study. Each individual was fed daily with  $10^{10}$  colony forming units of *Bifidobacterium infantis* 35624 in 4 oz (112 ml) milk for 3 weeks. Venous blood was collected from each study subject before and after 3-week oral feedings with the probiotic preparation. Isolated PBMC were cultured *in vitro*, either alone with medium or with a stimulant (LPS, *Bifidobacteria*), for 3 days. An array of human cytokines (IL1- $\beta$ , IL10, IL12, TNF- $\alpha$ , IFN- $\gamma$ ) present in the culture supernatant was analyzed with LINCoplex kit assay (Linco) in a Bioplex bead flow cytometer<sup>TM</sup> (Bio Rad). The differences in cytokine level between IBS and healthy subjects at baseline and post-feeding with *Bifidobacteria* were analyzed statistically by ANOVA. **Results:** At baseline without probiotic feeding, spontaneous production level of cytokines by PBMC of the IBS was not statistically significantly different from that of the healthy. However, *in vitro* LPS stimulation of PBMC from IBS subjects produced a significantly ( $p < 0.1$ ) higher level of pro-inflammatory cytokines (IL12, IFN- $\gamma$ , and TNF- $\alpha$ ) and a lower ratio of anti-inflammatory/pro-inflammatory cytokine (IL10/IFN- $\gamma$ ) than the healthy. Daily oral administration with *Bifidobacteria* for 3 weeks did not significantly affect the spontaneous production level of cytokines between 2 study populations. *In vitro* LPS stimulation of PBMC from *Bifidobacteria*-fed IBS population produced a lower level of IL12, TNF- $\alpha$ , and IFN- $\gamma$  and a significantly higher ratios of IL10/IL12 and IL10/IFN- $\gamma$ . When the same PBMC were stimulated *in vitro* with *Bifidobacteria*, it not only demonstrated an identical change pattern as described for the LPS-stimulated response but also elevated IL10. **Conclusions:** Systemic immune response by cytokine production in IBS subjects' PBMC is altered when compared to the healthy population. Immunological differences in cytokine production in IBS becomes apparent, following LPS stimulation of PBMC, that: (i) produces an elevated level of pro-inflammatory cytokines (IL12, TNF- $\alpha$ , IFN- $\gamma$ ) and (ii) shows a reduced ratio of anti-inflammatory/pro-inflammatory cytokine (IL10/IFN- $\gamma$ ). Therefore, systemic cytokine production of the IBS showed a bias toward a greater Th1 activity in the Th1-Th2 cytokine balance. Oral administration with *Bifidobacteria* normalizes immune response of IBS by producing: (i) a higher level of anti-inflammatory cytokine (IL10) when stimulated *in vitro* with *Bifidobacteria*; (ii) an elevated ratio of anti-inflammatory/pro-inflammatory cytokines (IL10/IL12, IL10/IFN- $\gamma$ ) when stimulated *in vitro* with LPS or *Bifidobacteria*.

This research was funded by The Procter and Gamble Co., Cincinnati, USA

## **Abstracts from Digestive Disease Week, May 2005**

### **Modulation of cytokine profiles in healthy and IBS subjects following supplementation with the unique probiotic strain, *Bifidobacterium infantis* 35624**

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**Background:** Clinical benefits in irritable bowel syndrome (IBS) have been observed with a unique strain of *Bifidobacterium infantis*. **Aim:** To evaluate *B. infantis* 35624 effect on cytokine production by peripheral blood mononuclear cells (PBMC) in healthy (n=10) and IBS (n=13) subjects. **Methods:** For 3 weeks, subjects were fed a preparation of *B. infantis* 35624 ( $10^{10}$  CFU/day). Venous blood was 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 stimulant (LPS, or *Bifidobacteria*). The presence of human cytokines (IL1- $\beta$ , IL10, IL12, TNF- $\alpha$ , IFN- $\gamma$ , TGF- $\beta$ ) in the supernatant was analyzed with LINCOpex kit assay (Linco) in a Bioplex bead flow cytometer<sup>TM</sup> (Bio Rad). Differences in cytokine levels were analyzed using analysis of variance. **Results:** Pre-feeding: no differences in cytokine levels were found in unstimulated PBMC in IBS and healthy subjects; while *in vitro* LPS stimulation of PBMC from IBS subjects produced a significantly ( $p < 0.1$ ) higher level of pro-inflammatory cytokines (IL12, TNF- $\alpha$ ) and a lower ratio of anti-inflammatory/pro-inflammatory cytokines (IL10/IL12, TGF- $\beta$ /IL12) than the healthy. Post-feeding: probiotic-feeding did not significantly affect the spontaneous production level of cytokines between the study populations. However, *in vitro* LPS stimulation of PBMC from *Bifidobacteria*-fed IBS subjects produced a significantly lower level ( $p < 0.1$ ) of IL12 and a higher ratio of IL10/IL12. 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 IL10 and IL10/IFN- $\gamma$  ratio, decreased IFN- $\gamma$ ). **Conclusions:** PBMC in IBS subjects exhibit altered function that becomes apparent following LPS stimulation by: (i) producing an elevated level of pro-inflammatory cytokines (IL12, TNF- $\alpha$ ) and (ii) a reduced ratio of anti-inflammatory/pro-inflammatory cytokines (IL10/IFN- $\gamma$  and TGF- $\beta$ /IL12). PBMC of the IBS skewed a bias toward greater Th-1 activity in the Th1-Th2 cytokine balance. Feeding with *B. infantis* 35624 improved the function of IBS PBMC by producing: (i) a higher level of anti-inflammatory cytokine (IL10) when stimulated *in vitro* with *Bifidobacteria*; (ii) an elevated ratio of anti-inflammatory/pro-inflammatory cytokines (IL10/IL12, IL10/IFN- $\gamma$ , TGF- $\beta$ /IL12) when stimulated *in vitro* with LPS or *B. infantis* 35624.

### **Impact of *Bifidobacteria infantis* 35624 on Fecal Flora from Healthy and IBS Subjects in a Chemostat model.**

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**Background & Aim:** Clinical benefits in irritable bowel syndrome (IBS) and impact on fecal flora by the novel probiotic strain *Bifidobacterium infantis* 35624 have previously been reported. These studies investigated this probiotic impact on fecal flora derived from healthy (n=5) and IBS (n=5) subjects in a chemostat model. **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<sub>2</sub> (20 psi), pH stated to 7.0, impeller rate 50 rpm, 37°C and nutrient feed of 60ml/hr. Baseline (4 consecutive days) was followed by additions of *B. infantis* 35624 (10<sup>10</sup> CFU/day) for 4 consecutive days. Chemostat samples were evaluated for bacterial content using selective media for total anaerobes, Bifidobacterium, Fusobacterium, 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 NMR spectroscopy. Multivariate analysis was used to extract spectra of components whose concentrations changed during baseline and treatment phase. **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, with probiotic addition resulted in an increase 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 the healthy flora. **Conclusions:** In the chemostat model compositional differences in flora derived from healthy versus IBS subjects were noted. Overall *B. 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.

## **Benefits associated with supplementation with an encapsulated probiotic preparation in subjects with Irritable Bowel Syndrome.**

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**Background.** We have previously demonstrated benefits in irritable bowel syndrome (IBS) with a milk-based probiotic preparation of a novel probiotic strain, *Bifidobacteria infantis* 35624. **Aim.** To evaluate benefits in IBS with an encapsulated preparation of the same probiotic strain. **Methods.** After a 2 week run-in phase, 291 female subjects with Rome II-positive IBS were randomized to placebo (n=73), or one of three doses of *B. infantis* 35624:  $10^6$  (n=74),  $10^8$  (n=74), or  $10^{10}$  (n=70) 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, subject's global assessment (SGA) of IBS symptom relief and quality of life. 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.  $10^6$  vs.  $10^8$  vs.  $10^{10}$ ) were based on Least-square Means. **Results.** For the primary efficacy variable, abdominal pain/discomfort ( $-0.58 \pm 0.10$  vs.  $-0.41 \pm 0.10$  vs.  $-0.89 \pm 0.10$  vs.  $-0.46 \pm 0.10$ ) as well as for all secondary variables of composite score ( $-1.16 \pm 0.26$  vs.  $-1.11 \pm 0.26$  vs.  $-2.13 \pm 0.26$  vs.  $-1.07 \pm 0.26$ ), bloating/distension ( $-0.41 \pm 0.10$  vs.  $-0.37 \pm 0.10$  vs.  $-0.71 \pm 0.10$  vs.  $-0.39 \pm 0.10$ ), incomplete evacuation ( $-0.22 \pm 0.10$  vs.  $-0.26 \pm 0.10$  vs.  $-0.52 \pm 0.10$  vs.  $-0.21 \pm 0.10$ ), passage of gas ( $-0.26 \pm 0.09$  vs.  $-0.21 \pm 0.09$  vs.  $-0.51 \pm 0.09$  vs.  $-0.27 \pm 0.09$ ) and SGA for symptom relief ( $-0.20 \pm 0.26$  vs.  $-0.20 \pm 0.25$  vs.  $0.74 \pm 0.27$  vs.  $-0.74 \pm 0.28$ ), bifidobacterium in a dose of  $10^8$  was significantly superior (P-value < 0.05) to placebo and all other bifidobacterium doses. The efficacy variable SGA for symptom relief was analyzed using a logistic model so its associated results are given on the logit scale. The corresponding success rates are 45% vs. 45% vs. 68% vs. 32%. No significant adverse events were recorded. **Conclusion.** *B. infantis* 35624, in a dose of  $10^8$  bacteria/day is effective in relieving all of the cardinal symptoms of irritable bowel syndrome. This study confirms benefits observed in previous studies, at a lower daily dose of probiotic in a capsule form, while demonstrating the complexity of achieving stable probiotic formulations.

## **Gender differences in colonic mucosal gene expression – an important consideration in relation to male/female variations in disease prevalence**

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**Background & Aims:** Molecular approaches to the understanding of several gastrointestinal diseases increasingly involves gene expression profiling using microarray technology. This requires rigorous controls on technical and biologic variables. One of the factors that has received limited attention is gender difference, which might be particularly relevant in disorders such as irritable bowel syndrome which predominantly occurs in women. Therefore we assessed gender as a determinant of individual differences in colonic gene expression. **Methods:** Rectosigmoid colonic biopsies (20 cm from anal verge) were taken from male (n=12) and female (n=8) healthy volunteers after informed consent and ethics board approval. Biopsies were taken at approximately the same time of day in each case to exclude diurnal variation. Biopsies were immediately placed in a preservative (RNAlater). Following RNA extraction, a genome-wide assessment of gene expression levels was performed using the Affymetrix system (U-133 Plus 2.0 chips). Alterations in gene expression were considered significant at  $p < 0.05$  for male versus female comparison. In addition, the data was subjected to bioinformatic assessment using software such as PathBinder, GeneNarrator and GOClusterer. **Results:** Of the 38,500 genes examined, we noted gender-dependent expression levels for 634 genes. Only a subset of these changes was related to Y-linked genes but the majority of changes were observed in non-Y linked genes. The differential gene expression between males and females related primarily to genes involved in immune function (e.g. chemokines and T cell activation) and metabolism (including drug metabolism). **Conclusions:** Molecular studies of disease mechanisms should control for gender differences in gene expression within the mucosa. This may be particularly relevant to disorders such as irritable bowel syndrome where there are marked male/female differences in disease prevalence.

## **Alterations in Immunological Gene Expression from Colonic Biopsies of Female IBS patients following probiotic consumption.**

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**Background & Aims:** Evidence is accumulating to support the role of prior infection and immune activation in Irritable Bowel Syndrome (IBS). We previously reported that oral administration of a unique probiotic strain, *Bifidobacterium infantis* 35624, provides symptom relief and improves quality of life in patients with IBS. To explore possible mechanisms of action in vivo, we examined gene expression in mucosal biopsies before and after feeding patients the probiotic (n=34) or placebo (n=17) in a blinded controlled manner. **Methods:** Sigmoid colonic biopsies were taken from all patients. Following RNA extraction, relative expression levels of IL1 $\beta$ , IL8, IL10, IL12, CCL5, CCL20 and TGF $\beta$  were analysed by Quantitative Real Time PCR using TaqMan Assays on the ABI 7000 Sequence Detection System. Relative quantification was carried out using the  $2^{-\Delta\Delta Ct}$  method. Analysis was blinded prior to statistical analysis using the Wilcoxon matched pairs test. **Results:** TGF $\beta$  exhibited the greatest increase in gene expression (p=0.0003) in the colonic tissue of IBS patients following probiotic treatment (n=34) in comparison to placebo treatment (n=17). Significant increases in the relative expression of IL1 $\beta$  (p=0.0022), and CCL5 (p=0.0010) were also observed. No significant alterations in the expression levels of IL8, IL10, IL12, and CCL20 were detected. **Conclusions:** Engagement of the immune system occurs following consumption of this probiotic strain. Modulation of cytokine gene expression (e.g. TGF $\beta$ ) may provide a useful biomarker of probiotic performance particularly in patients with IBS.

**Modulatory impact of commensal probiotics on intestinal epithelial cell responses to pro-inflammatory stimuli.**

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**Background and Aims:** The human intestinal mucosa response to the presence of pathogenic bacteria is well described while the response to commensal organisms is not well understood. We investigated the epithelial cell response to individual pathogenic and commensal micro-organisms as well as the modulatory effects of probiotic commensals on intestinal epithelial cell responses to pro-inflammatory stimuli. **Method:** HT-29 human intestinal epithelial cell gene expression following co-incubation with *Salmonella typhimurium*, *Lactobacillus salivarius* or *Bifidobacterium infantis* was examined by gene array analysis (847 genes associated with immune responses). In addition, HT-29 cells were pre-treated with or without commensal *L. salivarius* and *B. infantis* for 2 h. Subsequently, the cells were infected with *S. typhimurium*. NF- $\kappa$ B activation in epithelial nuclear extracts was determined using the TransAM assay while IL-8 protein was measured by ELISA. **Results:** In contrast to *S. typhimurium*, which increased expression of 36 genes associated with pro-inflammatory responses (e.g. TNF- $\alpha$ , IL-8, NF- $\kappa$ B), lactobacilli and bifidobacteria did not enhance expression of these genes. However, when co-cultured with *S. typhimurium*, the lactobacilli and bifidobacteria delayed NF- $\kappa$ B activation and reduced IL-8 production. These effects could not be attributed to interference with salmonella binding to the epithelium and occurred under both atmospheric and normoxia conditions. In contrast to their impact on salmonella-induced epithelial responses, probiotics did not offset TNF- $\alpha$ -induced epithelial activation. **Conclusion:** Commensal bacteria which are currently used as probiotics do not induce the well described epithelial cell response to pathogens. In contrast, they have the capacity to attenuate epithelial responses to *S. typhimurium*.