Structure/Function Claims for Soil-Based Organisms™ Manufactured by Life Science Products, Inc., Houston, Texas

Executive Summary

Introduction
Soil-Based Organisms™

Life Science Products, Inc., Houston, Texas (manufacturer) has notified the Food and Drug Administration in a letter to the Office of Special Nutritional Products, Labeling and Dietary Supplements that Life Science Products, Inc., Soil-Based Organism™ products were being sold in the marketplace as dietary supplements. In accordance with 21 CFR 101.93, the manufacturer and its various distributors have provided the agency a dossier, containing information which defines Soil-Based Organisms™ and substantiates the claims to be made on the products. Information pertinent to the safety of Soil-Based Organisms™ is also included. The following discussion presents a balanced review of the literature supporting structure/function claims for Soil-Based Organisms™.

Soil-Based Organisms™: Description and Components

Soil-Based Organism™ (SBOs™) products consist of a mixture of eight (8) species of naturally occurring probiotic organisms embedded in a matrix of fermentation end-products and fulvic acid. The strains of probiotic bacteria used in these products were originally isolated from healthy soil, and hence have not been genetically altered in any way by either exposure to a particular human diet, or by the intestinal environment of any given human donor. While the particular species of bacteria used in the products may commonly be isolated from the human gut, e.g., *Lactobacillus lactis*, the isolates delivered in the Soil-Based Organism™ products were collected from the environment.

The bacterial species listed below in Table 1 are grown in a culture containing fulvic acid, vitamins and minerals. Together, the bacteria form a stable microbial consortium, in which one type of bacteria lives off the “left-overs” or end-products of

a bacterial group higher up on the food chain. This method allows the bacteria to move through several different growth cycles before being harvested, and thus produce the maximum amount of end products.

Table 1 Life Science Products, Inc., Soil-Based Organisms™

Lactobacillus acidophilus *Lactobacillus plantarum* *Lactobacillus fermentum* *Lactobacillus lactis* *Lactobacillus casei*	*Bifidobacterium bifidum* *Bacillus subtilis* *Bacillus licheniformis* Members of the *Cyanobacteria,* such as *Dunaliella bardawil* may also be included

The Host Medium for growing the probiotics has also been tested for contaminants, such as heavy metals, pesticide residues, and pathogens. The results of these studies showed no contaminants present. The medium was also tested for the presence of psychomotor or neurogenic drugs, antibiotics and hormones. No existing variety was detected. Results of these contaminant studies were published by several authors conducting human clinical trials. The publications appeared in a supplement to *Progress in Nutrition* (2002) [Halpern, G., Editor, Supplement 1, 2002].

Soil-Based Probiotic Bacteria:

The probiotic (or lactic acid) bacteria are known to alter the gastrointestinal environment through their metabolites and behavior. They are known to participate in maintaining the homeostasis of the intestine, and to regulate immune responses to environmental antigens [Heyman, *et al*., 2002]. It was Elie Metchnikoff in 1907 who first stressed the importance of *Lactobacilli* in promoting health and longevity, and the term probiotic was first popularized by R. Fuller in 1989 [Heyman, *et al*., 2002].

Probiotic bacteria influence the metabolism and well being of the gut and of the whole body through a variety of mechanisms, which scientists are finding to be more complex than originally thought. The human colon contains more than 500 different bacterial species, and there are about 10 times more bacteria in the gut, than there are cells in the human body [Bengmark, 1998]. These bacteria communicate with the body and the immune system through the epithelial cells of the gut wall using a complicated system of messenger chemicals [Heyman, *et al*., 2002 and Husebye *et al*., 2001].

Probiotic Bacteria, Mechanisms of Action:

Probiotic organisms are thought to have four basic mechanisms of action.

1. Through fermentation, they secrete helpful compounds that either nourish other cells (such as those in the colon or liver), alter the colonic environment, or serve as signals to communicate with the immune system. These compounds may include vitamins, antioxidants, enzymes, bioactive peptides, organic acids, and polysaccharides [Bravo, 1998, Tieking, *et al*., 2003, Zvauya, *et al*., 1997, Seppo, *et al*., 2003, Calderon, *et al*., 2003, Mensah, *et al*., 1995, and Olsen, *et al*., 1995].

2. They inhibit the growth of organisms that are harmful to humans by either secreting antimicrobial substances, or by blocking the ability of the harmful organisms to adhere to or puncture the gut wall [Rolfe, 2002];

3. They prevent the build-up of waste materials and toxic compounds in the colon by either blocking their formation or by breaking toxins and waste materials down into harmless molecules that can be easily eliminated [Rolfe, 2002].

4. They exhibit strong antioxidant activities, which include the ability to scavenge reactive oxygen species, chelate metal ions, such as iron and

copper, inhibit the formation of the enzymes that create reactive oxygen species, and reduce oxidants [Lin, *et al.,* 1999].

Safety Assessment

To ensure the safety of its products, the manufacturer of SBOs™, Life Science Products, Inc. has included only species of probiotics with a long history of safe use in foods. Further, the company has relied on the results of both human and animal studies sponsored by Life Science Products, Inc. and by others who have included SBOs as major components of their commercial products.

Identity of the SBOs

The organisms contained in the SBO™ probiotic mixture include non-pathogenic strains of:

Lactobacillus acidophilus
Lactobacillus plantarum
Lactobacillus fermentum
Lactobacillus lactis
Lactobacillus casei
Bifidobacterium bifidum
Bacillus subtilis
Bacillus licheniformis

The bacteria listed have all been associated with human food products for thousands of years. Many appear on FDA's database, entitled "Everything Added to Food in the US" (EAFUS), or on FDA's list of food ingredients that are considered "Generally Recognized as Safe" (GRAS). Others, like the *Lactobacilli* were commonly used in US Food products or in food products in other countries prior to January 1, 1958, and have therefore "grandfathered" as GRAS food additives.

To confirm this assertion, both animal and human feeding studies have been conducted using the SBOs™ as the test article. An animal toxicological study to determine the lethal dose for fifty percent of a group (LD_{50}) of 50 test rats was conducted by the Sociedad Internacional de Gerontologia Valparaíso 424, Tlanepantla, Mexico. In this study 90-day old, laboratory-certified healthy, non-vaccinated Norwegian black rats were fed five times the dose recommended for humans (110 mg/kg) for 5 days. The researchers increased the dose every 5 days until at the 25th day, the animals were consuming 30 times the recommended daily dose for humans. The conclusion of the study was that the LD_{50} was greater than the highest level tested. No animals died during the study, and their weight gain was 2% higher than normal for the species despite the occurrence of diarrhea, due to force feeding [Rothschild, *et al*., 2002 and Rothschild, *et al*.,1992]. The test material was at that time labeled under the trade name Natur-Earth.

A human study was then carried out under the auspices of the state Academy of Medicine at Tamaulipas [Rothschild, 1993b Letter of Certification]. In this study adult humans between the ages of 40 and 65 consumed 12 Natur-Earth capsules daily for 90 days. Capsules were to be taken 1 hour before meals. Subjects were selected based on chronic hypercholesterolemia (>300 mg/dl). Thirty five test subjects and an equal number of control subjects were enrolled in the study. All subjects were tested for cholesterol levels at the conclusion of the study. Thirty-one out of thirty-five test patients reported increased energy levels, while five out of thirty-five placebo patients reported similar experiences. No adverse effects were reported.

In a third human study, 35 humans between the ages of 15 and 35 were recruited to test the Natur-Earth product for 90 days. Each subject was diagnosed with chronic lymphocytic leukemia, hence with a compromised immune system. Each subject was paired with a healthy control. Analyses were made on the numbers of white blood cells, red blood cells, segmented neutrophils, lymphocytes, body weight, and average blood pressure [Rothschild, *et al.,* 1993c]. Twenty-seven of the 34 test subjects completing the study reported increased energy levels and a higher level of general well being.

It is important to note that all the studies carried out in Mexico during 1992-1993 used the same batch of material: Batch 89216/92, which was analyzed by the American Academy of Naturopathic Medicine in Washington, D.C. [Rothschild, *et al.*, 1993c]. Thus continuity was established between animal safety studies, and studies with humans at various levels of health, Importantly, no adverse effects were reported during any of the studies.

During 2002 a human study was carried out with 35 compromised human male and female adults, aged 25 – 60, who were unresponsive to medications for Chronic Digestive Disorder and Malabsorption Syndrome. The study was conducted at the Apostolic School of Natural Medicine, Peoples University of the Americas, Ponce City, Puerto Rico. The subjects received 6-caplets containing the SBOs™ (1100 mg each, where 350 mg were the SBO probiotic blend) 3 times daily 30 minutes before meals for a 30-day period, followed by 4 caplets 3 times per day for a 60 day period. An equal group of control individuals received placebos. The total duration of the study was 90 days.

At the conclusion of the study 21 of 35 subjects in the control group had dropped out of the study, whereas, only 4 of the treatment group left the study. No adverse results due to the consumption of the SBOs™ were noted. Of the subjects in the treatment group, 86% returned to normal white blood cell counts, and 83% returned to normal red blood cell levels [Rothschild, *et al*., 2002]. Significantly, 75% of the test subjects reported via a visual analog scale, that they felt better while taking the SBO™ caplets, while the fourteen remaining control subjects reported no change in their condition (8), or that their condition had worsened (6) [Rothschild, *et al*., 2002].

A second study in humans was conducted by Dr. Paul A. Goldberg M.P.H., D.C. at the Goldberg Clinic, Marietta, Georgia [Goldberg, 2002]. In this study 17 compromised subjects, who had remained unresponsive to medical intervention for 3 or more years were given eighteen SBO™-containing caplets for 120 days (19.8 grams per day). At the conclusion of the study, no toxicological condition had been observed, and no subject reported any worsening of their condition after consuming the soil based probiotics. Sixteen of seventeen subjects reported an improvement in

their overall well being. Significantly, 8 of 9 subjects with elevated yeast counts showed a reduction in yeast population, and no subject experienced an increase in stool yeast population. Three subjects experienced a significant improvement in respiratory function, and eleven of twelve subjects experienced improved bowel function. None of the subjects reported any negative effects on bowel function. Fifteen of the seventeen subjects reported a moderate to significant increase in energy levels [Goldberg, 2002].

In summary the Soil-Based Organisms™ studied showed no toxicity in test animals or in humans with compromised health conditions. The safety results discussed above for the Soil-Based Organisms™ are consistent with or better than the safety profile reported for probiotics in general. Saarela, *et al*., 2000 reviewed the safety, functional and technological properties of probiotics. The authors concluded that probiotic products have been safely consumed in large quantities throughout Europe and Japan, but cautioned that immunocompromised persons may constitute a risk group when taking commercial probiotic preparations. The authors provided several lists of criteria for the assessment of probiotic safety. First, there must be studies on the intrinsic properties of the probiotic to be used, second, there must be studies on the pharmacokinetics of the probiotics, and thirdly, there must be studies on the interactions between the probiotic and the host [Saarela, *et al*., 2000]. Life Science Products, Inc. submits that both published literature as well as company supported studies support the safe use of the Soil-Based Organisms™.

Life Science Products, Inc. submits that both published literature as well as company supported studies support the safe use of the Soil-Based Organisms™.

Studies investigating the effects of the individual microorganisms found in Soil-Based Organisms™ in human and animal models have been published in several languages worldwide. Other studies sponsored by various companies using SBOs™ in their products support the safety and efficacy of the mixture of probiotic organisms, termed Soil-Based-Organisms™.

Supporting Statements

Documentation and Review

SBOs™ Support Gut Regeneration and Balance Gut Microbiology

Mangell, *et al*., 2002, investigated whether supplementation with *Lactobacillus plantarum* 299v would inhibit *Escherichia coli*-induced intestinal permeability. Intestinal permeability refers to a situation in which a person's gut wall becomes permeable to potentially harmful bacteria and other noxious substances. When unacceptable amounts of these bacteria or toxins enter the blood stream, they cause the immune system to react on a continual basis, using energy, and leading to immune stress. The purpose of the Mangell, *et al*., study was to investigate whether a probiotic bacterium, *Lactobacillus plantarum* 299v, could affect *Escherichia coli*-induced passage of mannitol (a sugar alcohol) across the intestinal wall. Sprague-Dawley rats were pretreated for one week by either tube feeding with *L. plantarum* 299v twice daily, or by having free access to *L. plantarum* 299v in their drinking water, or by receiving no bacteria in their regular feed (negative control). Intestinal segments were mounted in Ussing chambers and the mucosa was exposed to control medium, *E. coli*, and *L. plantarum* 299v (alone or together). $^{[14C]}$Mannitol was added as a marker of intestinal permeability and samples were taken from the side against the body cavity. *E. coli* exposure induced a 53% increase in mannitol passage across the intestinal wall ($P < 0.05$). One week of pretreatment with *L. plantarum* 299v in the drinking water abolished the *E. coli*-induced increase in permeability. Tube feeding for one week or short-term addition of *L. plantarum* 299v in the Ussing chambers had no effect on the permeability provoked by *E. coli* challenge. Notably, a single application of *L. plantarum* 299v itself did not change the intestinal passage of mannitol. These data demonstrate that pretreatment with *L. plantarum* 299v, a probiotic bacterium, protected against an *E. coli*-induced increase in intestinal permeability, provided that sufficient bacteria were consumed over the test period as in the free choice group [Mangell, *et al*., 2002].

SBOs™ Prevent Harmful Bacteria and Fungi from Colonizing the Gut

Lactic acid bacteria (LAB) produce a wide range of antagonistic factors that reduce the load of harmful organisms in the body. These include metabolic products, antibiotic-like substances, hydrogen peroxide, and bactericidal proteins. Collectively, these are termed bacteriocins [Kim, *et al*., 2003]. The organic acids, such as lactic and acetic acid, produced by the LAB also play important roles in the antagonism of microflora in the environment, the body, and in fermented foods. The lowering of colonic pH due to lactic and acetic acids, which account for 90% of the acids produced also has bactericidal or bacteriostatic effects.

Eight out of nine human subjects who began a study with elevated yeast counts as verified by stool and/or blood testing showed a significant reduction in yeast growth in their stools after having consumed 18 SBO-containing tablets for 120 days. In addition, four of the subjects, (50%) reported a 75% improvement in their health. All the subjects reported at least a 25% improvement in their well being. Based on these results, the study supervisor concluded that SBOs™ appeared to be effective at reducing yeast growth in these individuals (See Table below) [Goldberg, 2002].

Table: Effect of SBO™ Capsules on Yeast Growth in Humans

Subject/Subjective Outcome	Initial Yeast Overgrowth by species (Bowel)*	Yeast Assay Final	Initial Elevated Yeast (Blood)**	Blood Yeast Assay Final
C	*albicans* 3+	No Yeast		
C	*tropicalis* 3+	No Yeast		
A			900 U/ml	438 U/ml
A	*albicans* 4+, *zeylanoides* 2+	1+,0		
C	*albicans* 2+	1+		
B	*albicans* 4+, *lusitaniae* 3+	No Yeast		
A	*albicans* 1+, *krusei* 1+	No Yeast	1,688 U/ml	183 U/ml
A	*albicans* 3+	No Yeast		

A=75% or greater subjective improvement
B=50% or greater subjective improvement
C=25% or greater subjective improvement
D=No improvement
E=Worsening of symptoms

*Bowel Mycology Testing was performed at Doctor's Data Diagnostic Laboratories, Illinois, USA (Scale 1 – 4)
**Yeast Serum Antibody Tests was performed at Great Smokies Diagnostic Laboratories, South Carolina, USA

Another group of scientists studied whether *Lactobacillus fermentum* RC-14 could inhibit *Staphylococcus aureus* invasions of surgical implants in rats [Gan, *et al.,* 2001]. *Staphylococcus aureus* is a bacterium, which commonly invades hospitalized persons (particularly those who have undergone some type of surgery). The authors stated that the clinical impact of *S. aureus* is on the rise because of the global increase in the incidence of multidrug-resistant strains. As a result, there is a pressing need to identify new anti-staphylococcal agents that will help in building resistance to the effects of this organism. In this study the probiotic, *Lactobacillus fermentum* RC-14, and its secreted biosurfactant were successfully used to inhibit the growth of *S. aureus* in surgical implants in rats [Gan, *et al.,* 2001].

Cats, *et al.,* 2003, studied the effect of frequent consumption of a *Lactobacillus casei*-containing milk drink in *Helicobacter pylori*-colonized human subjects in the Netherlands. The research group tested whether a drink containing *Lactobacillus casei* strain Shirota inhibited *Helicobacter pylori* growth. In this

intervention study, 14-H. *pylori*-positive subjects were given a Yakult® drink containing 10^8 colony-forming units/mL of *L. casei*. The drink was given three times daily with meals for 3 weeks. Six untreated *H. pylori*-positive subjects served as controls. *H. pylori* bacterial loads were determined using the ^{13}Curea-breath test, which was performed before and 3 weeks after the start of *L. casei* supplementation. Urease activity decreased in nine of the 14 (64%) subjects with *L. casei* supplementation and in two of the six (33%) controls (P = 0.22). The authors concluded that viable *L. casei* were required for *H. pylori* growth inhibition, and that the inhibition did not result from changes in lactic acid concentration [Cats, *et al.,* 2003].

Lin, *et al.,* 1999, investigated nineteen strains of lactic acid bacteria for antioxidant activity. Antioxidant mechanisms including metal ion chelating ability, scavenge of reactive oxygen species, enzyme inhibition, and reducing activity were studied. All strains demonstrated reactive oxygen species scavenging ability. *B. longum* B6 showed the highest reducing activity among the 19 strains tested [Lin, *et al.,* 1999].

SBOs™ Improve Overall Health and Well Being

A large German study assessed the hypocholesterolemic effect of yogurt supplemented with *Lactobacillus acidophilus* 145 and *Bifidobacterium longum* 913 in women [Kiessling, *et al.,* 2002]. The cross-over study consisted of three periods (7 weeks each). During the first period, all 29 women consumed control yogurt. During the second period, 18 women consumed probiotic yogurt and 11 women consumed control yogurt. During the third period, the groups were reversed. The twenty-nine healthy women were aged 19-56 years. Fifteen of these were normocholesterolemic and 14 women were hypercholesterolemic. The yogurt was dosed at 300 g daily and contained 3.5% fat and starter cultures of *Streptococcus thermophilus* and *L. lactis*. The probiotic yogurt was enriched with *L. acidophilus* 145, *B. longum* 913 and 1%

oligofructose, making a synbiotic mixture. The mean serum concentration of total cholesterol and the LDL cholesterol was not influenced by the synbiotic (P>0.05). However, the HDL concentration increased significantly by 0.3 mmol/1 (P=0.002). The ratio of LDL/HDL cholesterol decreased from 3.24 to 2.48 (P=0.001). The authors concluded that the long-term daily consumption of 300 g of yogurt over a period of 21 weeks increased the serum concentration of HDL cholesterol and lead to the desired improvement of the LDL/HDL cholesterol ratio [[Kiessling, *et al.,* 2002].

Test subjects consuming SBOs™ for a 90 day period experienced a marked improvement in markers of anemia (iron deficiency). The investigator concluded that the product seemed to have a "blood building" effect (See Table below) [Rothschild, *et al*., 2002].

Marker	Before SBOs™	After SBOs™	Change	Reference Range
Red Blood Cells	3.5	4.4	+26%	4.5-5.6 Millions/cm^3
Hemoglobin	9.3	12.9	+39%	12-16 g/dL

Kano, *et al*., 2001 conducting research in Japan examined the effects of *Lactobacillus sp*. on the development of autoimmune deficiency in mice with collagen-induced arthritis (CIA). CIA can be used as a model of some types of rheumatoid arthritis (RA) and autoimmune deficiencies. Autoimmune deficiencies can be induced in DBA/1J mice by immunizing them with bovine type II collagen (bCII). Oral intake of skimmed milk (SM) fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1 (SM/OLL1073R-1) was found to markedly inhibit the development of CIA in these mice, compared with a control group fed a control feed. The inhibitory effect of SM fermented with *L. delbrueckii subsp. bulgaricus* OLLI 102 (SM/OLL1102) or fresh SM was weaker than that of SM/ OLL1073R-1. A deMan Rogosa Sharpe (MRS) broth culture of OLL1073R-1 without any major components of SM had the same inhibitory effect as SM/OLL1073R-1, suggesting that the inhibitory effect of SM/OLL1073R-1 was attributable not only to SM components but also to OLL1073R-1 cells, their metabolites, or both. The researchers found that

SM/OLL1073R-1 and SM caused reduced secretion of the cytokine IFN-gamma by lymph node cells (LNCs) in response to bCII. A polysaccharide fraction secreted by OLL1073R-1 also exhibited the inhibitory effects on both development of CIA and secretion of IFN-gamma. The results of this model suggest that supplementation with probiotic species modulates the immune system, inhibiting over-reactivity [Kano, *et al*., 2001].

SBOs™ Increase Intestinal Absorption of Nutrients

Subjects consuming SBOs™ in a controlled study for 90 days experienced a marked improvement in blood levels of essential minerals, such as iron, calcium, phosphorous, and potassium (See Table below) [Rothschild, *et al*., 2002]. The test material consisted of 18 caplets (1100 mg each) (see attached assay) taken daily. The oral doses were divided into 6 caplets taken 3 times daily 30 minutes before meals. The caplets were taken with 8 ounces of pure water, for a 30-day period. This protocol was followed by taking 12 caplets 4 caplets 3 times per day 30 minutes before meals for a 60 day period. Each caplet included 350 mg of SBOs in a probiotic blend. Inclusion criteria for the study are listed below.

INCLUSION CRITERIA:

- Individuals of both sexes suffering from clinically confirmed Chronic Digestive Disorder and Malabsorption Syndrome who were resistant to conventional forms of medications. All selected patients were suffering from chronic and painful spastic intestinal contractions, persistent alternating diarrhea and constipation and consequent non-hematopoiesis-contingent anemia for a minimum of nine months. All subjects were shown to have elevated white blood counts, immune system imbalances, mineral deficiencies and enzyme deficiencies.
- Both sexes of ages between 25 and 60 years
- Average duration of symptoms 3 years
- Individuals who had not been treated with tranquilizers, anti-depressants, steroids and/or chemotherapeutic drugs for at least 3 months prior to the beginning of the study.
- Individuals who had not received any prescription medicines for at least 3 months.

Marker	Before SBOs	After SBOs	Change	Reference Range
Iron	25.2	43.5	+73%	(40-80) ug/dL
Calcium	6.4	9.2	+44%	(8.5-10.6) mg/dL
Phosphorus	1.5	2.9	+93%	(2.5-4.6) mg/dL
Potassium	2.7	3.9	+44%	(3.5-5.3) mg/L

Twenty-five of the same subjects, who tested low for blood protein levels at the beginning of the study, showed an increase in blood protein levels after consuming SBOs™. The study director concluded that these results signified improved absorption and utilization of protein from the diet [Rothschild, *et al*., 2002]. Figure 2 below shows normal, damaged and healing absorptive surfaces on the intestinal villi [Playford, 1996].

Marker	Before SBOs™	After SBOs	Change	Reference Range
Total Protein (25)	3.86	7.03	+82%	6.4-8.4 g/dL

SBOs™ Enhance Gut Metabolism and Promote Normal Bowel Function

Individuals consuming SBOs™ in the controlled study described above demonstrated a marked increase (from 100 to more than 300%) in their ability to produce digestive enzymes for starches, fats and proteins. The improvements in enzyme production were assayed through their blood levels (See Table below) [Rothschild, *et al*., 2002].

Enzyme	Before SBOs™		After SBOs™	Change	Reference Range
Amylase	16.8	DU/mm^3	40.2	+139%	20-110 U/mm^3
Lipase	4.6	FCCLU/mm^3	20.0	+335%	7-60 U/mm^3
Protease 6.0	18.2	HUT/mm^3	38.2	+110%	30-90 HUT/ mm^3
Protease 4.5	6.9	HUT/mm^3	15.8	+129%	12-40 HUT/ mm^3
Protease 3.0	4.6	HUT/mm^3	10.9	+137%	8-16 HUT/ mm^3

Marteau, *et al*., 1990, studied nine healthy French volunteers before, during, and after ingesting a fermented dairy product containing *Lactobacillus acidophilus, Bifidobacterium bifidum*, and mesophilic cultures (*Streptococcus lactis* and *S. cremoris*) for 3 weeks. Hydrogen and methane productions and fecal beta-galactosidase and beta-glucosidase activities were measured as indicators of fermentation capacity of the colonic flora. Fecal concentrations of nitroreductase, azoreductase, and beta-glucuronidase, which may be implicated in producing toxins in the colon were also assessed. Hydrogen and methane productions, fecal beta-galactosidase, beta-glucuronidase, and azoreductase activities did not change over three 3-week periods, whereas fecal beta-glucosidase activity increased (42 +/- 6, 91 +/- 12, and 40 +/- 6 IU/g N, [$P < 0.01$]) and nitroreductase decreased (0.87 +/- 0.13, 0.54 +/- 0.11, and 0.57 +/- 0.08 IU/g N, [$P < 0.05$]) [Marteau, *et al*., 1990]. These results indicate that probiotic bacteria promote the production of digestive enzymes and inhibit the production of certain enzymes associated with toxin production.

Marteau, *et al*., 1990, studied nine healthy French volunteers before, during, and after ingesting a fermented dairy product containing *Lactobacillus acidophilus, Bifidobacterium bifidum*, and mesophilic cultures (*Streptococcus lactis* and *S. cremoris*) for 3 weeks. Hydrogen and methane productions and fecal beta-galactosidase and beta-glucosidase activities were measured as indicators of fermentation capacity of the colonic flora. Fecal concentrations of nitroreductase, azoreductase, and beta-glucuronidase, which may be implicated in producing toxins in the colon were also assessed. Hydrogen and methane productions, fecal beta-galactosidase, beta-glucuronidase, and azoreductase activities did not change over three 3-week periods, whereas fecal beta-glucosidase activity increased (42 +/- 6, 91 +/- 12, and 40 +/- 6 IU/g N, [P < 0.01]) and nitroreductase decreased (0.87 +/- 0.13, 0.54 +/- 0.11, and 0.57 +/- 0.08 IU/g N, [P < 0.05]) [Marteau, *et al*., 1990]. These results indicate that probiotic bacteria promote the production of digestive enzymes and inhibit the production of certain enzymes associated with toxin production.

SBOs™ Help Overcome Bloating and Gas

Nobaek, *et al*., 2000, studied the influence of the gastrointestinal (GI) microflora in persons with abnormal bowel function to see if those persons had an imbalance in their normal colonic flora. The authors stated that some bacterial taxa were more prone to gas production than others. They also wanted to study whether the flora could be altered by supplementation. The study comprised 60 subjects with normal colonoscopy or barium enema. None of the subjects had a history of malabsorption. The subjects were divided into two groups, one received 400 ml per day of a rose-hip drink containing 5×10^7 cfu/ml of *Lactobacillus plantarum* (DSM 9843) and 0.009 g/ml oat flour, and the other group received a plain rose-hip drink, comparable in color, texture, and taste. During the four-week study, the subjects recorded their own GI function, starting 2 weeks before the study and continuing throughout the study period. Twelve months after the end of the study all patients were asked to complete the same questionnaire regarding their symptomatology. The subjects receiving *Lactobacillus plantarum* maintained populations of these

bacteria in their colons. There were no major changes of Enterobacteriaceae in either group, before or after the study, but the *Enterococci* increased in the placebo group and remained unchanged in the test group. The investigators observed that flatulence was rapidly and significantly reduced in the test group between weeks 1 and 2 as compared to weeks 5 and 6 for the controls. Forty four percent of the test group reported a reduction in flatulence of at least 50%, while the placebo group reported a reduction of 18%. There was also a clear trend toward a more rapid and more pronounced reduction in abdominal pain over the same period on test group (36%) over the placebo group (18%), although these results were not statistically significant. At a 12-month follow-up, patients in the test group maintained a better overall GI function than control patients. The results of the study indicated that the administration of *Lactobacillus plantarum* as a supplement decreased pain and flatulence in subjects with abnormal bowel function [Nobaek, *et al.,* 2000].

SBOs™ Normalize Bowel Function

Five individuals consuming SBOs™ (18 caplets per day) in a 120-day controlled pilot study demonstrated a marked improvement in their gastrointestinal function following chronic (mean 5.5 years) gastrointestinal dysfunction [Goldberg, 2002]. All five subjects with either constipation or diarrhea reported a 75% or greater improvement (See Table below). Each of the caplets contained 350 mg of a blend of Soil Based Organisms™ along with a fulvic acid matrix and fermented plant material [Goldberg, 2002].

Effect of SBOs™ on Chronic Bowel Complaints

Complaint	Perceived Benefit
Chronic Constipation	A
Chronic Constipation	A
Chronic Constipation	A
Chronic Diarrhea	A
Chronic Diarrhea	A
A=75% or greater subjective improvement	
B=50% or greater subjective improvement	
C=25% or greater subjective improvement	
D=No improvement	

SBOs™ Improve Immune Function

Thirty-five individuals with abnormal immune markers, who consumed SBOs™ in a controlled study showed a trend towards a modulation of the immune system as evidenced by a significant decrease in white blood cells, an increase in neutrophils, a normalization of lymphocytes, and a decrease in elevated monocytes, eosinophils and basophils (See Table below) [Rothschild, *et al.*, 2002]. The subjects consumed 18 caplets (1100 mg) per day for 30 days followed by 12 caplets per day for 60 days. Each caplet contained 350 mg of a blend of SBOs™.

Marker (Subjects)	Before SBOs™	After SBOs™	Change	Reference Range
White Blood Cells (31)	11.2	8.6	-30%	(4.5-9.5) Th/cm^3
Low Neutrophils (18)	45.3	55.3	+22%	(50-72) %
High Lymphocytes (4)	44.75	33	-26%	(26-40) %
Low Lymphocytes (16)	20	31	+55%	(26-40) %
High Monocytes (27)	13.4	6.9	-49%	(0-8) %
High Eosinophils (6)	6.7	5.7	-15%	(0-4) %
High Basophils (21)	10.3	2	-81%	(0-5) %

Marker (Subjects)	Before SBOs™	After SBOs™	Change	Reference Range
White Blood Cells (31)	11.2	8.6	-30%	(4.5-9.5) Th/cm^3
High Lymphocytes (4)	44.75	33	-26%	(26-40) %
High Monocytes (27)	13.4	6.9	-49%	(0-8) %
High Eosinophils (6)	6.7	5.7	-15%	(0-4) %
High Basophils (21)	10.3	2	-81%	(0-5) %

Kishi, *et al.,* 2001 designed a study to determine whether the oral administration of *Lactobacillus brevis subsp. coagulans* modulates immunological responses in human subjects, and whether there were differences in response between live and heat-treated preparations. As a marker for immune modulation, they observed the effect of the oral administration of *Lactobacillus brevis subsp. coagulans* (Labre) on the interferon-alpha (IFN-alpha) producing capacity of apparently healthy subjects for 4 weeks. Sixty volunteers were divided into five groups for the determination of virus-induced IFN-alpha production in response to various doses of live and to heat-killed Labre. 2-5A synthetase activity was measured to detect trace amounts of IFN production. Routine blood tests were also performed to determine the state of health of the subjects involved in this study and to test any side effects of the Labre treatment. The oral administration of live Labre showed a statistically significant increase in IFN-alpha production at 2 weeks ($p < 0.05$) and at 4 weeks ($p < 0.05$) in the group receiving 600 million bacteria/day and at 4 weeks ($p < 0.05$) in the group receiving 300 million bacteria/day. In particular, IFN-alpha production in those with initially low levels rose significantly when either 300 million or 600 million bacteria/day were ingested. Consumption of heat-killed Labre 300 million bacteria/day did not result in a statistically significant change in IFN-alpha production. The level of 2-5A synthetase activity remained the same in the control and experimental groups [Kishi, *et al.,* 2001].

A research team from New Zealand stated that many elderly subjects are at increased risk of infectious and noninfectious diseases due to an age-related decline in lymphoid cell activity (immunosenescence) [Gill, *et al.,* 2001]. Noninvasive means of enhancing cellular immunity were therefore thought desirable in the elderly. Previous reports had suggested that dietary supplementation could represent an effective means of enhancing the activity of circulating natural killer (NK) cells in elderly populations. In the Gill, *et al.,* study, the authors conducted a pre-post intervention trial to determine the

impact of dietary supplementation with probiotic lactic acid bacteria (LAB) on peripheral blood NK cell activity in healthy elderly subjects. Twenty-seven volunteers consumed low-fat/low-lactose milk supplemented with known immunostimulatory LAB strains (*Lactobacillus rhamnosus* HN001 or *Bifidobacterium lactis* HN019) for a period of 3 weeks. A dietary run-in of milk alone was shown to have no significant effect on NK cells. In contrast, the proportion of CD56-positive lymphocytes in peripheral circulation was higher following consumption of either LAB strain, and *ex vivo* peripheral blood mononuclear cells (PBMC) tumoricidal activity against K562 cells was also increased. Supplementation with *Lactobacillus rhamnosus* or *Bifidobacterium lactis* increased tumoricidal activity by an average of 101 and 62%, respectively; these increases were significantly correlated with age, with subjects older than 70 years experiencing significantly greater improvements than those under 70 years. These results demonstrated that dietary consumption of probiotic LAB might offer benefit to elderly consumers to reverse some of the deleterious effects of immunosenescence on cellular immunity [Gill, *et al.,* 2001].

A Finnish study used thirty healthy volunteers randomized into three different treatment groups who consumed *Lactobacillus GG, Lactococcus lactis* or placebo (ethyl cellulose) for 7 days [Fang, *et al.,* 2000]. On days 1, 3 and 5, an attenuated *Salmonella typhi* Ty21a oral vaccine was given to all subjects to mimic an enteropathogenic invasion. All subjects responded well to the vaccine, but no significant differences were observed in numbers of IgA-, IgG- and IgM-secreting cells among the different groups. There was a trend towards a greater increase in specific IgA among the subjects receiving the vaccine in combination with Lactobacillus GG. Those receiving *L. lactis* with their vaccine evinced significantly higher CR3 receptor expression on neutrophils than those receiving either the placebo or *Lactobacillus GG*. These results indicated that probiotic species might influence the humoral immune response to oral *S. typhi* vaccine differently, when this vaccine was being used as a model antigen [Fang, *et al.,* 2000].

A protein from SBOs™ titled SRL 172 has been reported in a limited number of case studies to shift cytokine function from the pro-inflammatory T Helper 2 (TH2) pathway to the more effective anti-inflammatory T Helper 1 (TH1) profile. Improvements were also observed in cell mediated immunity and antibody production [No authors listed, 1998].

SBOs™ Enhance the Cleansing Function of the Gut

The normal intestinal microbiota has a diverse composition; a conservative estimate is that it consists of at least 400 species. Because a large part of the intestinal microbiota can not be cultured with current techniques, it has been suggested that the number of microbial species in the human intestine may, in fact, exceed 1000 [Ouwehand, 2007].

This microbiota has a metabolic activity that equals that of the liver, our metabolically most active organ. The microbiota contributes to the digestion of exogenous and endogenous substrates, such as fibers and mucins. This provides the host with additional energy in the form of fatty acids. It may, however, also expose the host to detrimental metabolic end products such as amines, sulfides, ammonia, etc. These toxins are removed from the gut by the SBOs™ as part of the metabolism of the probiotic cells [Rolfe, 2002].

de Waard, *et al.,* 2001 examined the effects of orally administered viable *Lactobacillus casei* Shirota strain YIT9029 on the immunity parameters of Wistar and Brown Norway rats. For this purpose, they used the *Trichinella spiralis* (a parasite found in certain raw pork) host resistance model. Two weeks before and during *T. spiralis* challenge, rats were fed 10^9 viable *L. casei* bacteria 5 days per week. The *T. spiralis*-specific delayed-type hypersensitivity (DTH) response was significantly enhanced in both Wistar and Brown Norway rats given *L. casei*. In both rat strains fed *L. casei*, serum *T. spiralis*-

specific immunoglobulin G2b (IgG2b) concentrations (antibodies) were also significantly increased. In the model, no significant effects of *L. casei* on larval counts or inflammatory reactions in the tongue musculature, body weights, or lymphoid organ weights were observed. Serum specific antibody responses, other than IgG2b, were not changed by feeding of *L. casei*. In contrast to *L. casei*, it was shown that orally administered *Bifidobacterium breve* or *Bifidobacterium bifidum* had no influence on antibody production against *T. spiralis* [de Waard, *et al.,* 2001].

SBOs™ Are a Source of Probiotics for Children

Dairy products containing live bacteria that possess lactase activity are used for dietary management of lactose maldigestion [Montes, *et al.,* 1995]. However, the efficacy of acidophilus milk and the effect of consuming unfermented milk that had been inoculated with yogurt bacteria had not been examined in children until the publication of a study by Montes, *et al.,* 1995. That research group compared scores for breath H_2 excretion and the symptoms of 20 lactose-maldigesting children after consumption of 250 ml of uninoculated milk with two identical milks inoculated with 10^{10} cells of *Lactobacillus acidophilus* or with a commercial yogurt starter culture containing 10^8 cells of *Lactobacillus lactis* and 10^{10} cells of *Streptococcus thermophilus*. Nine of 10 subjects who were symptomatic following ingestion of uninoculated milk experienced a reduction in symptoms following ingestion of milk inoculated with *L. acidophilus*, without a decline in H_2 excretion (breath hydrogen [H_2] excretion is a measure of the amount of fermentation taking place in the colon). Five of 6 subjects who were symptomatic following uninoculated milk had decreased symptoms and a significant reduction in H_2 excretion following milk inoculated with the yogurt culture. The researchers concluded that for lactose-maldigesting children, milks inoculated with *L. acidophilus* or with a yogurt culture were associated with decreased symptoms compared with uninoculated milk maldigestion [Montes, *et al.,* 1995].

SBOs™ Increase Resistance to Harmful Bacteria and Fungi

As a result of their bile resistance, *L. acidophilus* Ki and *Bifidobacterium lactis* Bo have been shown to establish themselves in the intestine and become important in the microbial ecology of the intestinal environment. These bacterial species have also been shown to control serum cholesterol concentrations and to increase colonization resistance against pathogenic bacteria such as *Salmonella enteritidis* and *Clostridium perfringens* [Weerkamp, *et al*., 1996].

Viable spores of several species of the spore-forming *Bacilli*, including *subtilis* and *licheniformis* have been found to enhance the growth of various species of *Lactobacillus*, suppress the growth of pathogenic organisms, such as *E. coli* 078:K80 and *Helicobacter pylori*, and produce antibiotic substances, which retard the growth of harmful organisms, and help the body restore its natural flora. [Hong, *et al*., 2005 and Hoa, *et al*., 2001]. For example, both *Bacillus subtilis* and *Bacillus licheniformis* are widely used in probiotic formulas in the livestock and poultry industries, especially in Europe, where the feeding of antibiotic growth promoters has been banned. In this instance, the spores of these organisms act as competitive exclusion agents. One study showed that 1-day old chicks, who consumed 2.5 X 10^8 (250,000,000) *Bacillus subtilis* spores, were able to completely resist infection by the pathogenic *E. coli* strain mentioned above. Other studies have shown that antimicrobials secreted by *Bacillus sp.* were able to inhibit and eliminate resistant *Staphylococcus aureus* from the contents of the large intestine in human patients with ulcerative colitis [Tropko, 2000].

SBOs™ Inhibit Overactive Immune Systems in Adults and Children

Atopic dermatitis, allergic rhinitis, and bronchial asthma in childhood represent a significantly large segment of chronic disease in the Western world. According to Kopp, *et al*., (2007), improved hygiene, the increased use of antimicrobial medication, the consumption of nearly sterile food, and reduced family size all resulting in lower rates of infection during childhood have reduced early contact to microbes. Researchers have found that this may interfere with the development of a child's immune system, which tends to be directed toward a T-helper (Th)2 phenotype in infants, whereas maturation of the immune system is associated with gradual inhibition of Th2 and an increasing use of the anti-inflammatory Th1 pathway [Kopp, *et al* 2007]. It has been shown that it is the cytokine, IL-10 which stimulates the maintenance of the "allergic" or Th2 phenotype, whereas IL-12 stimulates a shift toward the "tolerant" or Th1 phenotype. Th3 cells, through the production of transforming growth factor-b, further stimulate a shift toward tolerance. IgE may activate mast cells and cause allergic symptoms; IgA on the other hand may provide allergen exclusion [Ouwehand, 2007].

Experimental and epidemiologic data show strong evidence for the hypothesis that early microbial exposure is a critical feature for Th1-skewed immune response in healthy children during the postnatal period. Less exposure to microbial agents at an early age results in reduced activation of the immune system and subsequent polarization toward a Th2 phenotype, which is less efficient and more inflammatory [Kopp, *et al* 2007].

This concept is supported by epidemiologic data, which shows that children with allergy have a different intestinal flora with higher levels of *Clostridia* and lower levels of *Bifidobacteria* in comparison to healthy children. Based on these data, "harmless" microbial agents, that is, probiotics, have been test administered for their efficacy in the treatment and prevention of allergy in infants.

SBOs™ Help to Mature the Immune System in Young Children

The intestine is the body's largest immune organ; most of the antibody-producing cells reside in the intestine. A relatively recently recognized function of the intestinal microbiota is to provide stimulation of the immune system. According to Ouwehand (2007), consumption of probiotics (and prebiotics) is, in most cases, aimed at modulating the composition and/or activity of the intestinal microbiota. This modulation can be expected to influence the immune system. Several probiotic strains have been observed to modulate some immune parameters after sufficient (time and amount) consumption [Ouwehand, 2007].

Ouwehand (2007) states that at birth, the immune system of an infant is not fully developed and tends to be directed toward a T-helper (Th)2 phenotype to prevent rejection *in utero*. The Th2 phenotype leads, however, to the stimulated production of IgE by B cells and thus increases the risk for allergic reactions through activation of mast cells. Microbial stimulation early in life will reverse this Th2 bias and will stimulate the development of a Th1 phenotype as well as the activity of Th3 cells. The combined action of the Th1 phenotype and the Th3 cells will lead to the production of IgA by B cells. IgA contributes to allergen exclusion and will thereby reduce exposure of the

immune system to antigens. Cytokines produced by the Th1 phenotype will also reduce inflammation and stimulate tolerance toward common antigens [Ouwehand, 2007].

In the case of allergy, the rationale for modulating the intestinal microbiota is supported by observations that allergic children have a different microbiota composition than healthy infants. Children with allergy were found to have an aberrant microbiota even before the onset of allergy; they had higher levels of *Clostridia* and lower levels of *Bifidobacteria*. In addition to these quantitative differences in the *Bifidobacterium* microbiota, qualitative differences have also been observed. Infants with atopic dermatitis have been found to have a more adult type *Bifidobacterium* microbiota with high prevalence of *B. adolescentis*

SBOs™ Help to Promote Normal Immune Function in Children and Adults

Ouwehand (2007), reports that not only the composition of intestinal microbiota but also the metabolic activity of the microbiota may be different between healthy children and children with allergies. Swedish children, who are at high risk to develop allergy, were found to have significantly higher levels of fecal volatile fatty acids butyrate, isovalerate, and caproate then Estonian children, who historically have a low risk for developing allergies [Ouwehand, 2007].

Allergy may manifest in infants even when they are exclusively breast-fed. Standard treatment involves the feeding of extensively hydrolyzed formula. Supplementation of this type of formula with *Bifidobacterium lactis* Bb-12 or *Lactobacillus rhamnosus* GG has been found to lead to an earlier recovery than standard treatment alone, 2 mo vs. 6 mo. A combination of 2 *Lactobacillus*

strains, *L. rhamnosus* 19070–2 and *L. reuteri* DSM 122460, was found to significantly reduce the clinical scoring of atopic dermatitis (SCORAD) in 1- to 13-y-old children with a positive skin prick test. But the SCORAD of children with no positive skin prick test remained unchanged. Interestingly, more than half of the subjects reported an improvement in their eczema, whereas only 15% in the placebo group reported improvement. The 2 studies discussed above used different probiotic preparations, which may explain the observed differences in outcome. Ouwehand states that the differences may also relate to the differences in age of the patients studied. In young infants, the immune system is still developing. There is still a possibility to direct it toward tolerance. In older children, the allergic phenotype is already established, and here one may only be able to relieve the symptoms [Ouwehand, 2007]. Similarly, probiotics have not been very successful in alleviating symptoms of respiratory allergy. *L. rhamnosus* GG was not able to reduce the symptoms of birch pollen allergy in adults despite its effectiveness in children. Similarly, *L. acidophilus* L-92 was reported only to relieve the subjective symptoms of cedar pollen allergy in adults [Ouwehand, 2007].

The precise mechanisms behind the favorable effects of probiotics on allergy are not entirely known. Several mechanisms have been observed *in vitro* and in animal studies. In addition to modulation of the intestinal microbiota, probiotics have been observed to improve the barrier function of the intestinal mucosa, reducing leakage of antigens through the mucosa and thereby exposure to them. Direct modulation of the immune system may be through the induction of anti-inflammatory cytokines or through increased production of secretory IgA. IgA will contribute to an exclusion of antigens from the intestinal mucosa. Further, enzymatic degradation of dietary antigens by

enzymes from probiotics will reduce the load of and exposure to antigens [Ouwehand, 2007].

Odamaki, *et al*., (2007) also cites the hygiene hypothesis, postulating that the decrease in opportunity for exposure to immunostimulating pathogens in early childhood causes an increased prevalence of allergic diseases, such as asthma and atopic dermatitis [Odamaki, *et al*., 2007]. Animal studies have demonstrated that if sufficient microbial stimuli are not available to the developing immune system during infancy, further maturation becomes inhibited, potentially resulting in the persistence of dysfunctional type 2 helper T cell (TH2) responses. Studies on the composition of intestinal microflora between allergic and non-allergic 2-year-old children have indicated that the prevalence of *Bifidobacteria* is lower in allergic infants, whereas counts of *Staphylococcus aureus* and *Enterobacteria* are higher. In comparison with healthy infants. Odamaki, *et al*., states that babies with allergies are less often colonized with *Enterococci* during the first month of life and with *Bifidobacteria* during the first year of life. In line with this hypothesis, oral probiotic bacteriotherapy with lactic acid bacteria may reduce the risk of atopic eczema, and improve the clinical symptoms of perennial allergic rhinitis and intestinal inflammation associated with food allergies [Odamaki, *et al*., 2007].

Bifidobacterium species are one of the major components of gut microbiota in humans, and are frequently associated with health-promoting effects. Studies on the gut microflora between allergic and non-allergic individuals have indicated distinct compositions, including different *Bifidobacterium*

colonization. Studies have also shown the immunostimulating and antitumor effects of *Bifidobacteria* upon oral administration. Specifically, many physiological effects such as stimulating immunity, reducing cancer risk, and preventing harmful bacterial infection have been reported for strain BB536, and this strain has been commercially applied to various aspects of the food industry in many countries. The *in vitro* and *in vivo* studies reported by Odamaki, *et al*., (2007) have also implied that strain BB536 has the potential for immune modulation (results not published). It is suggested that the yogurt supplemented with BB536 promoted a healthy intestinal environment by increasing the counts and the relative ratios of *Bifidobacteria*, in addition to increasing defecation frequency after consumption for 2 weeks.

To support this hypothesis, the authors measured the induction of major immunoregulatory cytokines. *In vitro* studies indicated that strains of the *B fragilis* group induced significantly more IL-6 which is one of the TH2-type cytokines, but significantly less TH1-type cytokines (IFN-γ and IL-12) compared with those of *Bifidobacterium* species in PBMC derived from JCPsis volunteers. Cytokine induction in PBMC from four healthy subjects was also assayed with each of 10 strains of *Bifidobacteria* and *B fragilis* group; the pattern was similar to JCPsis subjects (data not shown). The study indicated there were different capacities for the induction of cytokines by the *B fragilis* group and *Bifidobacteria* in PBMCs, and this potentially suggests the immunoregulatory ability of these bacteria in the intestinal tract of JCPsis subjects, during the pollen season. An alternative explanation is that allergic sensitization promotes the growth of *Bacteroides* by causing modifications in the gut microbiota. The results presented by Odamaki, *et al*., (2007) indicate a noticeable fluctuation in fecal microbiota during the pollen season in Japan.

However, supplementation with BB536 yogurt modulated the microbiota in a manner that could contribute to the alleviation of allergic symptoms. The authors added the caveat that since placebo yogurt containing ordinary lactic acid bacteria was used in the study, certain effects from placebo yogurt on microbiota could not be excluded [Odamaki, *et al*., 2007].

Scientists have also uncovered another aspect of the healthful benefits of the SBOs™. A group of scientists led by Dr. Georges Halpern tested 35 commercial preparations *in vitro* for their ability to effect the immune system. In this study the SBOs™ were extracted in DMSO and then filtered through a 0.45μ filter. The filtered solution was then diluted with PBS (pH 7.2) to yield a 200μg solution. This solution was then tested against the human hepatoma cell line, HEP G2, and the colon carcinoma cell line Caco-2. The SBOs™ were the most effective in inhibiting growth from these cell lines. Thus another indication that the SBOS™ help to maintain homeostasis in the gut [Halpern, *et al.,* 2002}.

SBOs™ Help to Promote Normal Weight in Children

Obesity is viewed as one of the important public health problems of our times, and the rate for becoming obese is highest in children. According to Kallioma, *et al*., (2008) children may find themselves in a vicious circle: obese children often become obese adults and maternal obesity overnourishes the fetus, thereby programming the child to have a heightened risk of obesity as an adult. Recent scientific advances point to systemic low-grade inflammation and local gut microbiota as contributing factors for overnutrition [Kallioma, *et al*., 2008].

The gut microbiota enables hydrolysis of indigestible polysaccharides to easily absorbable monosaccharides and activates lipoprotein lipase by direct action on the villous epithelium. Consequently, glucose is rapidly absorbed and fatty acids are excessively stored. Both processes boost weight gain. The authors explained that increased numbers of *Bacteroides* in the gut microbiota were shown in an experimental animal model to predispose the animals toward energy storage and obesity. Gut microbiota alterations in humans during the critical maturational period have been linked to the development of various inflammatory conditions such as allergy. An ambiguous relationship between obesity and asthma has also been suggested. Kallioma, *et al*., therefore, have evaluated the gut microbiota in infants at high risk for allergy in relation to controls.

In brief, subjects were examined by one of the authors (MK) at birth and at the ages of 3, 6, 12, 18, and 24 mo and at 4 and 7 y. Weight and height were assessed, and body mass index (BMI; in kg/m2) was calculated when the subjects reached 7 y of age. Instead of adult BMI criteria, which the investigators felt underestimated the extent of adiposity in childhood, they established weight status according to the International Obesity Task Force criteria for overweight and obesity. These criteria identify BMI values for each age associated with a predicted BMI of 25 or 30, respectively, at age 18 y [Kallioma, *et al*., 2008].

As a result of their previous research the group suggested that *Bifidobacteria* constitute an internal link between breastfeeding and weight development. First, human milk contains *Bifidobacteria* and also the oligosaccharides that

allow *Bifidobacteria* to thrive in the infant gut. Genomic studies have shown convincingly that *Bifidobacteria* present in the gut of breastfed infants, eg, *B. longum*, are specially equipped to use breast milk oligosaccharides as nutrients. *B. longum* is also adapted to the conditions in the lower human gut, where energy harvest from slowly absorbable carbohydrates takes place. These bacteria dominate the gut microbiota, representing 60–90% of the total microbiota in healthy infants during breastfeeding, and they influence the total metabolic activity of the gut microbiota by the mucosal cross-talk between microbes and the host. Moreover, breast milk also contains soluble pattern recognition receptors that recognize microbial constituents in the gut and thereby regulates activation of the innate immune system in such a way that prevents pathology of the immune system [Kallioma, *et al*., 2008]. The authors stated that breastmilk-derived soluble receptors are also vital in linking fatty acids with the innate immune system, further strengthening the beneficial host microbe cross-talk for inflammation control. In view of the recent demonstration that adiposity is characterized by low-grade inflammation, the authors hypothesized that control of inflammatory pathways might provide new potential approaches for future therapy [Kallioma, *et al*., 2008].

Infants receive their first microbial inoculation at the time of delivery. These inoculated bacteria reflect the microbiota of the mother's vagina and gastrointestinal tract. This inoculation is further reinforced during breastfeeding by breast-milk-derived galactooligosaccharides, bacteria in breast milk and the breast's skin. Thus, part of the maternal microbiota is transferred to the infant, influencing future intestinal microbiota development in the infant. Recent studies have suggested that especially skin-derived

bacteria may have an important contribution in early colonization of the gut [Kallioma, *et al*., 2008].

Coagulase-negative *Staphylococci* are now recognized as characteristic first colonizers of the newborn gut, regardless of the mode of delivery. Improved hygiene was suggested to be a cause for this early colonization of the gut by traditional skin bacteria. Moreover, a majority of the infants are colonized by *S. aureus* during the first months of life by parental skin. Such colonization is long term in nature, and many strains produce toxins that can act as superantigens, carrying proinflammatory potential [Kallioma, *et al*., 2008].
Indeed, a recent study found a strong specific association between early intestinal colonization with *S. aureus* and an increase in circulating soluble CD14, a marker of systemic inflammation. Hence, we speculate that *S. aureus* may indeed act as a trigger of low-grade inflammation, contributing to the development of obesity. The authors recommend that taken together, a physically active lifestyle and avoidance of excessive energy intake prvides the foundation for the prevention of obesity. That being said, their results indicate that changes in the gut microbiota may be linked not only to the development of allergy but also to other chronic inflammatory conditions common in the Western world, among them obesity, thus extending the concept of the hygiene hypothesis.

SBOs™ Produce Lactoferrin an Important Line of Defense

The SBOs produce an iron-binding compound in the gut, which has been described as one of the body's first lines of defense against pathogens. This compound functions to bind iron and hold it tightly, thereby stealing it from pathogenic organisms, parasites and viruses in the gut. Lactoferrin releases the

iron to either the blood stream, or organ targets only where specific binding sites are available. This mechanism allows very small amounts of iron to be nutritionally significant. In addition to being secreted by the SBOs, Lactoferrin can also be found in human milk, tears, and saliva [Bravo, 1998, Tieking, *et al*., 2003, Zvauya, *et al*., 1997, Seppo, *et al*., 2003, Calderon, *et al*., 2003, Mensah, *et al*., 1995, and Olsen, *et al*., 1995].

Summary

The discussion above has demonstrated that Soil-Based Organisms™ are capable of enhancing the health of individuals of all ages, including children and the elderly. Significantly, supplementation with these probiotic organisms has been shown to improve and restore the functioning of not only the gastrointestinal tract, but also of every system in the body, which is affected by the immune system.

What is especially noteworthy is that Soil-Based Organisms™ supply a mixture of eight species of probiotic organisms derived from pristine soils. Since each probiotic species has been shown to supply slightly different metabolites to the body, it is important to supply a variety of different types of organisms, in order to have an impact on total health. To date the majority of the probiotic products that have been studied have used between one and five species.

The point made throughout the published papers discussed above, is that the effect of probiotics is often dose dependent. In order to see the best results the consumer must take between 10^8 and 10^{10} organisms per unit over a significant period of time. This is between 100 million and ten trillion organisms. Human subjects taking Soil-Based Organisms™ saw similar or better results taking probiotic units with 10^7 (tens of millions) organisms, one to several orders of magnitude less. The manufacturer suggests that the difference in potency is due to the fermentation process, which transforms these organisms and their metabolites into a more bioactive state.

References

	Vol	Tab
Arnao MB, Cano A, Alcolea JF, and Acosta M., 2001. Estimation of free radical-quenching activity of leaf pigment extracts. *Phytochem Anal.* Mar-Apr;12(2):138-43.	2	1
Bengmark, S., 1998. Ecological Control of the gastrointestinal tract. The role of probiotic flora. *Gut*, 42:2-7.	2	2
Bravo, L, 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, 56(11):317-333.	2	3
Calderon, M, Loiseau, G, and Guyot, JP, 2003. Fermentation by *Lactobacillus fermentum* Ogi E1 of different combinations of carbohydrates occurring naturally in cereals: consequences on growth energetics and α amylase production. *International Journal of Food Microbiology,* 80:161-169.	2	4
Cannon, P., 1999. Microbial diversity and its relevance to European agriculture. Comment for CABI Bioscience.	2	5
Cats A, Kuipers, EJ, Bosschaert, MA, Pot, RG, Vandenbroucke-Grauls, CM, and Kusters, JG, 2003. Effect of frequent consumption of a *Lactobacillus casei*-containing milk drink in *Helicobacter pylori*-colonized subjects. *Aliment Pharmacol Ther* Feb;17(3):429-35.	2	6
Carr, FJ, Chill, D, and Maida, N., 2002. The lactic acid bacteria: a literature survey. *Crit Rev Microbiol*, 28(4):281-370.	2	7
Conserving Land: Population and Sustainable Food Production. The Toll of Intensification, Undated. Population Action International. Washington, DC.	2	8
Conway, P, 1995. Microbial Ecology of the Human Large Intestine. In: *Human Colonic Bacteria; Role in Nutrition, Physiology, and Pathology.* Eds. Gibson, G.R. and Macfarlane, G.T., CRC Press, Ann Arbor, MI, USA.	2	9
De Waard, DR, Garssen, J, Snel, J, *et al.*, 2001. Enhanced Antigen-Specific Delayed-Type Hypersensitivity and Immunoglobulin G2b Responses after Oral Administration of Viable *Lactobacillus casei* YIT9029 in Wister and Brown Norway Rats. *Clin Diag Lab Immuno,* Jul:762-767.	2	9
Duffy, CF, Killeen, GF, Connolly, CD, and Power, RF., 2001. Effects of dietary supplementation with *Yucca schidigera* Roezl ex Ortgies and its saponin and non-saponin fractions on rat metabolism. *J Agric Food Chem*. Jul;49(7):3408-13.	2	10

	Vol	Tab
Fang, H, Elina, T, Heikki, A, and Seppo, S., 2000. Modulation of humoral immune response through probiotic intake, *FEMS Immunol Med Microbiol*, Sep;29(1):47-52	2	11
Galvano, F, Piva, A, Ritieni, A, and Galvano, G., 2001. Dietary strategies to counteract the effects of mycotoxins: a review. *J Food Prot*, Jan;64(1):120-31.	2	12
Gan, BS, Kim, J, Reid, G, Cadieux, P, and Howard, JC., 2002. *Lactobacillus fermentum* RC-14 inhibits *Staphylococcus aureus* infection of surgical implants in rats. *J Infect Dis*, May 1;185(9):1369-72	2	13
Gill HS, Rutherfurd KJ, and Cross ML., 2001. Dietary probiotic supplementation enhances natural killer cell activity in the elderly: an investigation of age-related immunological changes, *J Clin Immunol*, Jul;21(4):264-71.	2	14
Goldberg, P, 2002. Primal Defense™ Soil-Based organisms as applied to medically unresponsive chronic disease conditions in adults, *Progress in Nutrition*, 4: 109-115, Suppl. 1..	2	15
Heyman, M, Ménard, S., 2002. Probioitc microorganisms: how they effect intestinal pathophysiology, *Cellular and Molecular Life Sciences*, 59:1151-1165.	2	16
Hoa TT, Baccigalupi L, Huxham A, Smertenko A, Van, PH, Ammendola S, Ricca, E, Cutting, SM. Characterization of *Bacillus* species used for oral bacteriotherapy and bacterioprophylaxis of gastrointestinal disorders. *Applied and Environmental Microbiology.* 2001 Sep; 67(9): 3819-23.	2	17
Hoa TT, Duc LH, Isticato R, Baccigalupi L, Ricca E, Van PH, Cutting SM. Fate and dissemination of *Bacillus subtilis* spores in a murine model. *Applied and Environmental Microbiology.* 2001 Sep; 67(9): 3819-23.	2	18
Hong HA, Duc le H, Cutting SM, 2005. The use of bacterial spore formers as probiotics. *FEMS* Microbiological Review Sep;29(4):813-35.	2	19
Husebye, E, Hellstrom, PM, Sundler, F, *et al*., 2001. Influence of microbial species on small intestinal myoelectric activity and transit in germ-free rats. *Am J Physiol Gastrointest Liver Physiol*, 280:G368-G390.	2	20
Kallioma¨ki, M., Maria Carmen Collado, Seppo Salminen, and Erika Isolauri, 2008. Early differences in fecal microbiota composition in children may predict overweight. *Am JClin Nutr* 2008;87:534–8.	2	21

	Vol	Tab
Kano, H, Kaneko, T, and Kaminogawa, S., 2002. Oral intake of *Lactobacillus delbrueckii subsp. bulgaricus* OLL1073R-1 prevents collagen-induced arthritis in mice. *J Food Prot,* Jan;65(1):153-60.	2	22
Kiessling G, Schneider, J, and Jahreis, G. 2002. Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. *Eur J Clin Nutr* 2002 Sep;56(9):843-9.	2	23
Kim, TS, Hur, JW, Yu, MA, Cheigh, CI, Kim, KN, Hwang, JK, and Pyun YR, 2003. Antagonism of Helicobacter pylori by bacteriocins of lactic acid bacteria. J Food Prot, Jan;66(1):3-12.	2	24
Kishi, A, Uno K, Matsubara, Y, Okuda C, and Kishida T., 1996. Effect of the oral administration of *Lactobacillus brevis subsp. coagulans* on interferon-alpha producing capacity in humans. *J Am Coll Nutr* , Aug;15(4):408-12.	3	25
Kopp,M.V., Isabell Hennemuth, Andrea Heinzmann and Radvan Prevention: No Clinical Effects of Lactobacillus GG Supplementation Randomized, Double-Blind, Placebo-Controlled Trial of Probiotics for Primary *Pediatrics* 2008;121;e850-e856; originally published online Mar 10, 2008; DOI: 10.1542/peds.2007-1492	3	26
Lin, MY, Yen, CL., 1999. Antioxidative ability of lactic acid bacteria. *J Agric Food Chem,* Apr;47(4):1460-6.	3	27
Lin, C, Bolsen, KK, Brent BE, *et al.*, 1992. Epiphytic microflora on alfalfa and whole-plant corn. *J Dairy Sci*. Sep;75(9):2484-93.	3	28
Mangell, P, Nejdfors, P, Wang, M, Ahrne, S, Westrom, B, Thorlacius, H, and Jeppsson B, 2002. *Lactobacillus plantarum* 299v inhibits *Escherichia coli*-induced intestinal permeability. *Dig Dis Sci*, Mar;47(3):511-6.	3	29
Marteau, P, Pochart, P, Flourie, B, Pellier, P, Santos, L, Desjeux, JF, and Rambaud JC, 1990. Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. *Am J Clin Nutr,* Oct;52(4):685-8.	3	30
McAllister, TA, Annett, CB, Cockwill, CL, Olson, ME, Wang, Y, and Cheeke, PR. Studies on the use of *Yucca schidigera* to control giardiosis. *Vet Parasitol*, May 22;97(2):85-99.	3	31

	Vol	Tab
Mensah, P, Ndiokwelu, CI, Uwaegbute, A, Ablordey, A, van Boxtel, AM, Brinkman, C, Nout, M J, and Noddy, PO, 1995. Feeding of lactic acid-fermented high nutrient density weaning formula in paediatric settings in Ghana and Nigeria: acceptance by mother and infant and performance during recovery from acute diarrhea. Int J Food Sci Nutr, 46(4):353-62.	3	32
Miyakoshi, M, Tamura, Y, Masuda, H, Mizutani, K, *et al.*, 2000. Antiyeast steroidal saponins from *Yucca schidigera* (Mohave yucca), a new anti-food-deteriorating agent. *J Nat Prod*, Mar:63(3):332-8.	3	33
Molin, G, 2001. Probiotics in foods not containing milk or milk constituents, with special reference to *Lactobacillus plantarum* 299v. *Am J Clin Nutr*, Feb;73(2 Suppl):380S-385S.	3	34
Montes, RG, Bayless, TM, Saavedra, JM, Perman, JA, 1995. Effect of milks inoculated with *Lactobacillus acidophilus* or a yogurt starter culture in lactose-maldigesting children. *J Dairy Sci* , Aug;78(8):1657-64.	3	35
Morinobu, T, Tamai, H, Murata T, *et al*., 1994. Changes in beta-carotene levels by long-term administration of natural beta-carotene derived from *Dunaliella bardawil* in humans. *J Nutr Sci Vitaminol* (Tokyo), Oct::40(5):421-30.	3	36
Mundt, JO, and Hammer JL, 1968. *Lactobacilli* on plants. *Appl Microbiol*, Sep;16(9):1326-30.	3	37
Nobaek, S, Johansson, ML, Molin, G, Ahrne, S, and Jeppsson B, 2000. Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. *Am J Gastroenterol*, May;95(5):1231-8.	3	38
Naruszewicz, M, Johansson, ML, *et al.*, 2002. Effect of *Lactobacillus plantarum* 299v on cardiovascular disease risk factors in smokers, *Am J Clin Nutr*, 76: 1249-55.	3	39
Odamaki, T., J-Z Xiao, N Iwabuchi, M Sakamoto, N Takahashi, S Kondo, K Iwatsuki, S Kokubo, H Togashi, T Enomoto,4 Y Benno., 2007. Fluctuation of Fecal Microbiota in Individuals With Japanese Cedar Pollinosis During the Pollen Season and Influence of Probiotic Intake. *J Investig Allergol Clin Immunol* Vol. 17(2): 92-100	3	40
Olsen, A, Halm, M, and Jakobsen, M, 1995. The antimicrobial activity of lactic acid bacteria from fermented maize (kenkey) and their interactions during fermentation. *Journal of Applied Bacteriology*, 79:506-512.	3	41

	Vol	Tab
Ouwehand, A.C., 2007. Antiallergic Effects of Probiotics. from Presentations and discussions at the World Dairy Summit 2003 of the International Dairy Federation (IDF) in a joint IDF/FAO symposium entitled ''Effects of Probiotics and Prebiotics on Health Maintenance—Critical Evaluation of the Evidence,'' held in Bruges, Belgium. J. Nutr. 137: 794S–797S.	3	42
Rolfe, RD, 2000. The role of probiotic cultures in the control of gastrointestinal health, *J Nutr*,130:396S-402S.	3	43
Rothschild, PR, Huertas, JG, 2002. Ambulatory treatment of chronic digestive disorder with malabsorption syndrome with Primal Defense™. *Progess in Nutrition,* 4: 97-108, Suppl. 1.	3	44
Saarela, M, Mogensen, G, Fonden, R, Matto, J, *et al*., 2000. Probiotic bacteria: safety, functional and technological properties. *J Biotech,* 84:197-215.	3	45
Saran, S, Gopalan, S, and Krishna TP, 2002. Use of fermented foods to combat stunting and failure to thrive. *Nutrition, May;18(5):393-6.*	3	46
Seppo, L, Jauhiainen, T, Poussa, T, and Korpela, R, 2003. A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects*, Am J Clin Nutr*, 77:326-30.	3	47
Sharma A, Kapoor AC, 1996. Levels of anti-nutritional factors in pearl millet as affected by processing treatments and various types of fermentation. *Plant Food Hum Nutr.* Apr;49(3):241-52.	3	48
Singh, A, Singh, SP, and Bamczai, R, 1996. Modulatory influence of chlorophyllin on the mouse skin papillomagenesis and xenobiotic detoxication system. *Carcinogenesis,* Jul:17(7):1459-63.	3	49
Tieking, M, Lorakli, M, Ehrmann, MA, Gänzle, MG, and Vogel, RF, 2003. *In situ* production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. *Applied and Environmental Microbiology*, 69(2):945-952.	3	50
Tropko, LV. 2000. Comparative evaluation of effectiveness of the effect of antibiotics and bacterial substances on clinical strains of *Staphylococcus aureus* isolated from patients with nonspecific ulcerative colitis. *MikrobiolZ Jul-Aug;* 62(4):38-42.	3	51
Verbiscar, AJ, Bandgan, TF, Weber, CW, *et al.*, 1981. Detoxification of jojoba meal by lactobacilli. *J Agric Food Chem*, Mar-Apr;29(2):296-302.	3	52

	Vol	Tab
Vogel, RF, Knorr, R, Muller, MR, *et al*., 1999. Non-dairy lactic fermentations: the cereal world. *Antonie Van Leeuwenhoek*, Jul-Nov;76(1-4):403-11.	3	53
Xavier LC, Germida JJ, 1999. Impact of human activities on mycorrhizae. *Microbial Biosystems*: New Frontiers.	3	54
Yamamoto N, Maeno M, Takano T., 1999. Purification and characterization of an antihypertensive peptide from a yogurt-like product fermented by *Lactobacillus helveticus* CPN4. *J Dairy Sci*, Jul;82(7):1388-93	3	55
Yu, YM, Wu, CH, Tseng, YH, Tsai, CE, and Change, WC, 2002. Antioxidative and hypolipidemic effects of barley leaf essence in a rabbit model of atherosclerosis, *Jpn J Pharmacol* 89:142-148.	3	56
Zvauya, R, Mygochi, T, and Parawira, W, 1997. Microbial and biochemical changes occurring during production of masvusu and mangisi, traditional Zimbabwean beverages. *Plant Foods for Human Nutrition*, 51:43-51.	3	57
Weerkamp, A. H., I. Bovee-Oudenhoven, and R. van der Meer. 1996. Effect of probiotics on infections with pathogenic bacteria in human gut flora-associated rats. Page J36 *in* Proc. Fifth Symp. on Lactic Acid Bacteria—Genetics, Metabolism, and Applications, Cahen, France.	3	58
Weerkamp, A. H., F.A.M. Klaver, and R. van der Meer. 1993. "Assimilation" of cholesterol by lactobacilli is due to their bile salt deconjugating activity. FEMS Microbiol. Rev. 12:P185. .	3	59
Drossman, DA., 1993. Indigestion: gas, bloating, abdominal pain, etc. Digestive Disease Science. 38:1569-80.	3	60
Galland, L., 2007. Leaky Gut Syndromes: Breaking the Vicious Cycle. Foundation for Integrated Medicine.	3	61
Hawrelak, J., 2004. The causes of intestinal dysbiosis: a review. Alternative Medicine Review.	3	62
National Digestive Diseases Information Clearing House: Digestive Disease Statistics., 1999-2000	3	63
Salminen, E., et al., 1988. Preservation of intestinal; integrity during radiotherapy using live Lactobacillus acidophilus cultures. Clinical Radiology. 39:435-437	3	64