DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date SEP 18 2000

0546 '00 SEP 22 P2:50

From (Acting) Division Director, Division of Standards and Labeling Regulations,
Office of Nutritional Products, Labeling and Dietary Supplements, HFS-820

Subject 75-Day Premarket Notification of New Dietary Ingredients

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	*Lactobacillus reuteri*
Firm:	McNeil Consumer Healthcare, Inc.
Date Received by FDA:	July 6, 2000
90-Day Date:	October 3, 2000

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary Ingredien should be placed on public display in Docket No. 95S-0316 after ~~August 20~~ OCT 3, 2000.

Geraldine A. Jere
for Felicia B. Satchell

95S-0316

RPT 78

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC

SEP 18 2000 0547 '00 SEP 22 P2:51

John C. Young
Director, Regulatory Affairs -Nutritionals
McNeil Consumer Healthcare
7050 Camp Hill Road
Fort Washington, Philadelphia 19034-2299

Dear Mr. Young

This letter is in response to your letter to the Food and Drug Administration (FDA) dated June 30, 2000, making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act). Your letter notified FDA of your intent to market a product containing a new dietary ingredient named *Lactobacillus reuteri.* FDA received your submission on July 6, 2000.

21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor of a dietary supplement that contains a new dietary ingredient submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under 21 U.S.C 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully evaluated the information in your submission, including the data concerning the use of *Lactobacillus reuteri* by infants and young children. The agency has concerns about the adequacy of the evidence in your submission regarding whether a dietary supplement containing *Lactobacillus reuteri* will reasonably be expected to be safe for use by infants and young children. You provided two abstract reports of clinical studies on gastrointestinal effects of *Lactobacillus reuteri* in healthy children aged 12 to 36 months. However, one of these abstracts lacks information on the amount of *Lactobacillus reuteri* consumed by the children during the study. Both of the abstracts lack detailed information that is needed to fully evaluate the effect of *Lactobacillus reuteri* on clinical measurements (e.g., hematology, chemistry, immunology) that are generally needed to evaluate the chronic use of a substance. Your submission also includes two published studies and one unpublished

study of *Lactobacillus reuteri* used in the treatment of infants and young children aged 6 to 36 months hospitalized with diarrhea. The subjects of these studies were sick infants and children. These studies are of limited relevance to evaluating the use of *Lactobacillus reuteri* in healthy infants and children. Additionally, these studies lack physiological and biochemical analyses. Furthermore, the duration of treatment with *Lactobacillus reuteri* is unclear in these studies. For these reasons, the information in your submission does not provide a sufficient basis to establish that a dietary supplement containing *Lactobacillus reuteri*, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe for infants and young children. Therefore, a dietary supplement containing *Lactobacillus reuteri* that is intended for use by infants and young children may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

As you may know, FDA is convening its Food Advisory Committee on September 26 and 27, 2000, to discuss existing information and needs with respect to probiotics. The issues pending before the committee include the use of probiotics in foods, probiotics and the immune system, and probiotics and infants. This meeting is open to the public and we encourage you to participate. For your convenience, we are enclosing a notice published in the *Federal Register* on September 11, 2000, announcing the public meeting.

In anticipation of this meeting, FDA is not responding at this time on the adequacy of the information in your submission concerning the safety of *Lactobacillus reuteri* for use in dietary supplements for populations other than infants and young children. However, FDA may further respond to the information contained in your submission after the FDA Advisory Committee meeting on September 26 and 27, 2000. Please note that, under 21 CFR 190.6(f), failure of the agency to respond to a notification does not constitute a finding by the agency that a new dietary ingredient or the dietary supplement that contains it is safe or is not adulterated under 21 U.S.C. 342.

Your submission will be kept confidential for 90 days from the date of receipt, and after October 3, 2000, your submission will be placed on public display at Dockets

Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact me if you have questions concerning this matter.

Sincerely yours,

Felicia B. Satchell

Felicia B. Satchell
(Acting) Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

Food Advisory Committee; Notice of Meeting

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

This notice announces a forthcoming meeting of a public advisory committee of the Food and Drug Administration (FDA). The meeting will be open to the public.

Name of Committee: Food Advisory Committee.

General Function of the Committee: To provide advice and recommendations to the agency on FDA's regulatory issues.

Date and Time: The meeting will be held on September 26, 2000, 8:30 a.m. to 5 p.m. and September 27, 2000, 8:30 a.m. to 2 p.m.

Location: Hilton Towers (Ballston Metro Stop), Gallery I and II, 950 North Stafford St., Arlington, VA.

Contact Person: Catherine M. DeRoever, Center for Food Safety and Applied Nutrition (HFS–6), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202–205–4251, FAX 202–205–4970, or e-mail: cderoever@cfsan.fda.gov., or FDA Advisory Committee Information Line, 1–800–741–8138 (301–443–0572 in the Washington, DC area), code 10564. Please call the Information Line for up-to-date information on this meeting.

Agenda: On September 26 and 27, 2000, the committee will meet to discuss existing information and needs with respect to probiotics.

Procedure: Interested persons may present data, information, or views, orally or in writing, on issues pending before the committee (such as the use of probiotics in foods, probiotics and the immune system, probiotics and infants, etc). Written submissions may be made to the contact person by September 20, 2000. Oral presentations from the public will be scheduled between approximately 3:30 p.m. and 4:30 p.m. on September 26, 2000. Time allotted for each presentation may be limited. Those desiring to make formal oral presentations should notify the contact person before September 20, 2000, and submit a brief statement of the general nature of the evidence or arguments they wish to present, the names and addresses of proposed participants, and an indication of the approximate time requested to make their presentation.

Notice of this meeting is given under the Federal Advisory Committee Act (5 U.S.C. app. 2).

Dated: August 29, 2000.

Linda A. Suydam,

Senior Associate Commissioner.

[FR Doc. 00–23163 Filed 9–8–00; 8:45 am]

BILLING CODE 4160–01–F

McNeil Consumer Healthcare, 7050 Camp Hill Road, Fort Washington, PA 19034-2299 (215) 273-7000

JUN 30 2000

Office of Nutritional Products, Labeling and
Dietary Supplements (HFS-800)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

RE: New Dietary Ingredient Notification

Dear Sir or Madam:

McNeil Consumer Healthcare ("McNeil") submits the attached information to the Food and Drug Administration pursuant to Section 413(a) of the Federal Food, Drug and Cosmetic Act in anticipation of its marketing of a dietary supplement which contains the new dietary ingredient Lactobacillus reuteri. McNeil intends to incorporate the Lactobacillus reuteri ingredient into a dietary supplement in tablet form.

Since this submission is made under section 413 of the Federal Food, Drug and Cosmetic Act, we request that it be accorded the 90-day confidentiality provisions relating to public notice.

If you have any questions, please do not hesitate to call me at 215/273-7695.

Sincerely
MCNEIL CONSUMER HEALTHCARE

John C. Young
Director, Regulatory Affairs – Nutritionals

enc.

PREMARKET NOTIFICATION OF *LACTOBACCILLUS REUTERI* AS A NEW DIETARY INGREDIENT

Prepared for

McNeil Consumer Healthcare
Fort Washington, Pennsylvania

Prepared by

ENVIRON International Corporation
Arlington, Virginia

June 30, 2000

TABLE OF CONTENTS

CONTENTS

TABLES

SECTION I

I. NAME AND ADDRESS OF MANUFACTURER

McNeil Consumer Healthcare
7050 Camp Hill Road
Fort Washington, Pennsylvania 19034

Contact: John C. Young
Telephone: (215) 273-7695
Facsimile: (215) 273-4049

SECTION II

II. NAME OF NEW DIETARY INGREDIENT

The new dietary ingredient that is the subject of this submission is *Lactobacillus reuteri* ATCC 55730.

SECTION III

III. DESCRIPTION OF DIETARY SUPPLEMENT

A. Intended Level of New Dietary Ingredient

1. Dietary Supplement Containing *Lactobacillus reuteri*

Lactobacillus reuteri , together with binders, excipients, and flavors, will be formulated into a chewable tablet that will be sold in the U.S. as a dietary supplement. Each tablet will contain between 10^8 and 10^9 colony forming units (CFU) of *Lactobacillus reuteri.*

B. Conditions of Use

The label directions indicate a recommended daily intake of one tablet. The dietary supplement is intended to support digestive function.

SECTION IV

IV. EVIDENCE OF SAFETY

A. Summary of Evidence of Safety

The subject of this notification is a new dietary ingredient, *Lactobacillus reuteri* strain ATCC 55730, which is to be contained in a dietary supplement containing 10^8 to 10^9 colony forming units (CFU) of this bacterium. This strain of *L. reuteri* is commonly referred to as strain SD2112 in the scientific literature, and that designation is used in this notification.

The evidence of safety is determined from a review of the safety of *Lactobacillus* strains as well as historical uses and exposure to *L. reuteri*. Dietary ingestion of various *Lactobacillus* strains has a long history, and the safety record is excellent. Since exposure to ingested lactobacilli is limited to the gastrointestinal tract, traditional toxicological approaches that examine systemic effects in animal assays have limited value in assessing the safety of probiotic bacteria. Animal studies that have been conducted with *L. reuteri* were done to evaluate the efficacy for probiotic effects. They are reviewed as part of the safety evaluation because in addition to efficacy, relevant safety endpoints were included. This submission also reviews human studies that were performed to evaluate the safety and efficacy of *L. reuteri*. The assessment of efficacy studies is limited to a review of the results related to safety and tolerability.

Lactobacillus species have been used in numerous food fermentations for many years; several lactobacilli are either sources of GRAS substances or have received prior sanctions as optional ingredients in specified standardized foods. *Lactobacillus* strains, including *L. reuteri*, are nonpathogenic and nontoxigenic. Many other *Lactobacillus* strains are used commercially as probiotics in products marketed in the U.S. and abroad. *L. reuteri* occurs naturally in a range of food products, including sourdough, Romano cheese, fermented rice noodles, and fermented cane molasses.

Recently *L. reuteri* SD 2112 has been intentionally added to food products in the United States, Europe, and Japan. In the United States, Stonyfield Farm has added *L. reuteri* and two other live, active cultures (in addition to traditional yogurt organisms) to its line of refrigerated and frozen yogurts for approximately one year. ToniLait, a Swiss company, has marketed since 1995 SymBalance, a product that contains *L. reuteri* in addition to other *Lactobacillus* strains. Milk supplemented with *L. reuteri* SD2112 and two additional probiotic bacteria was introduced in Sweden in 1991 under the tradename BRA, and this product now accounts for about 4% of the milk sold in Sweden. In Finland, *L. reuteri* SD2112 has been marketed under the Rela name in yogurt, fermented milk, orange juice, pineapple juice, and cottage cheese. In Japan, dairy

products containing *L. reuteri* SD2112 are sold under the names SymBalance and Reuteri. The levels consumed in a serving of these foods is comparable to the number of *L. reuteri* that will be consumed through the daily use of the intended supplement.

Efficacy studies of *L. reuteri* administration in animals examined effects on the colonization of the gut, cholesterol levels, prevention of acetic acid induced colitis, decreases in carriage of pathogenic microorganisms and protection from *C. parvum* and salmonella infection. The studies did not demonstrate any adverse health effects or adverse effects on body weight. There was a beneficial effect of *L. reuteri* administration on colonization of the gut.

Use of *L. reuteri* at levels comparable to those which will result from use as discussed in this submission has been studied in infants, children, healthy adults and individuals with HIV infection. Two randomized double-blinded placebo controlled trials, one of healthy adult males and one of HIV infected subjects, examined appropriate physical, clinical chemistry, hematology, urinalysis and gastrointestinal tolerance parameters. *L. reuteri* SD2112 administration for 21 days of up to 1×10^{11} CFU/day did not result in any clinically significant adverse effects on safety or tolerance. Two other clinical studies in adults using *L. reuteri* SD2112 administration reported no adverse health effects. These studies demonstrated that *L. reuteri* has the ability to colonize the human intestinal mucosa. Five studies evaluating primarily efficacy in infants and children demonstrated no adverse effects on gastrointestinal tolerance, stool consistency, weight or length gain.

B. Overview of Lactic Acid Bacteria

The commercial significance in the dairy industry of lactic acid bacteria (LAB) arises from their ability to convert milk sugar (lactose) and other sugars to lactic acid. Lactic acid bacteria include certain species in the genera *Lactobacillus, Streptococcus, Pediococcus,* and *Leuconostoc. L. reuteri* is a species of *Lactobacillus* and is therefore a lactic acid producing organism. Streptococci and pediococci are homofermentative, the leuconostocs are heterofermentative, and lactobacilli include both homofermentative and heterofermentative types. Homofermenters convert carbohydrates primarily to lactic acid through the glycolytic (hexose diphosphate) pathway. Heterofermenters produce lactic acid and substances such as acetic acid, ethyl alcohol, and carbon dioxide using phosphoketolase as well as alternative pathways. Some homofermenters have the ability to be heterofermentative; these are referred to as facultative heterofermenters (Axelsson, 1989).

LAB have been used in food for many years and are generally considered to be harmless, thus they are afforded "generally recognized as safe, or GRAS" status (Lee and Salminen 1995). They have been used in numerous food fermentations and they play an important ecological role in food preservation (Stiles 1996). LAB that grow as adventitious microflora or that are added to foods as cultures do not pose a health risk to mankind (Holzapfel et al. 1995). If fact, there are

no food poisoning organisms associated with LAB, and no food allergies have been reported (Hammes et al. 1995).

To insure the safety of LAB in food, many strains that were selected for such use have been previously associated with man and animals. Species of human origin that are relatively common in the human intestinal tract are listed in Table 1. Strains such as *L. reuteri, L. plantarum*, and *L. casei* subsp. *rhamnosus* have been isolated from humans and administered to human subjects without reported adverse effects (Lee and Salminen 1995). This excellent tolerance and lack of adverse effects is a general property of *Lactobacillus* organisms.

TABLE 1
Species of Lactic Acid Bacteria Isolated from the Human Gastrointestinal Tract[a]

Lactobacillus reuteri	*Bifidobacterium adolescentis*
L. acidophilus	*B. angulatum*
L. animalis	*B. bifidum*
L. brevis	*B. breve*
L. buchneri	*B. catenulation*
L. casei	*B. infantis*
L. delbruecki	*B. longum*
L. gasseri	*B. pseudocatenulatum*
L. lactis	*B. dentium*
L. plantarum	*Enterococcus faecalis*
L. ruminis	*E. faecium*
L. salivarius	

[a]Adapted from Hammes and Tichaczek (1994)

There is evidence that certain LAB species contribute to the well being of man. Thus including them in the diet may have a beneficial effect on human health (Gorbach 1990; Rafter 1995; Scheinbach 1998). These organisms are typically referred to as probiotics, which is defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Fuller 1991). Several of these species are listed in Table 2.

TABLE 2
Lactic Acid Bacteria Used in Probiotic Products as Reported by Hammes and Tichaczek[a]

Lactobacillus acidophilus	*Streptococcus thermophilus*
L. bulgaricus	*Bifidobacterium adolescentis*
L. casei	*B. bifidum*
L. helveticus	*B. breve*
L. lactis	*B. infantis*
L. plantarum	*B. longum*
L. salivarius	*Enterococcus faecium*

[a]Adapted from Hammes and Tichaczek (1994)

Donohue and Salminen (1996) compiled a comprehensive review that outlines safety issues concerning the use of bacteria as probiotics. An example of a properly designed safety assessment is presented in Salminen et al. (1996a). In general, lactic acid bacteria have an excellent safety profile, and no major problems have arisen during widespread use. Safety studies have been well documented on several dairy strains (Saxelin et al. 1996a; Saxelin et al. 1996b) with no report of adverse effects. New species and more specific strains of probiotic LAB are constantly being developed and incorporated into our food supply, further demonstrating that LAB are safe for human consumption.

C. Taxonomy and Identification of *Lactobacilli*

There are approximately 64 identified species in the *Lactobacillus* genus. These organisms are straight to curved rods occurring singly or in chains. The rods vary from long and slender to short coccobacillary forms. Generally, they are nonmotile. Lactobacilli are typically considered to be gram positive, but as the culture ages, cells may become gram negative (Kandler and Weiss 1986).

The genus is divided into three groups based on sugar metabolism (Table 3). Group 1 species are obligate (restricted to) homofermenters of hexose sugars to lactate and do not ferment pentose sugars. Group 2 species are facultative (i.e. taking place only under certain conditions) heterofermenters of hexoses (they ferment hexoses by glycolysis to lactate or, under glucose limitation, to lactate, acetate, ethanol, and formate), whereas pentoses are fermented by the phosphoketolase pathway to lactate and acetate. Group 3 species are obligate heterofermenters of sugars (Cogan 1996).

TABLE 3 Grouping of *Lactobacillus* Species		
Group	**Organisms**	**Comments**
1	*Lactobacillus delbruecki* ssp. *bulgaricus*	[a]
	L. delbruecki ssp. *lactis*	[a]
	L. helveticus	[a]
	L. delbruecki	Does not ferment lactose[a]
	L. delbruecki ssp. *delbruecki*	Does not ferment lactose[a]
	L. acidophilus	[a]
2	*L. casei* ssp. *casei*	11-89% of strains ferment lactose[a]
	L. casei ssp. *pseudoplantarum*	[a]
	L. casei ssp. *rhamnosus*	[a]
	L. casei ssp. *tolerans*	[a]
	L. plantarum	[a]
	L. curvatus	[a]
3	*L. fermentum*	[a]
	L. reuteri	[b]
[a]Cogan (1996); [b] Kandler and Weiss (1986)		

Lactobacillus reuteri is a heterofermentative species that resides in the gastrointestinal tract of humans and animals (Kandler and Weiss 1986). It is comprised of slightly irregular, bent rods with rounded ends. It generally measures 0.7-1.0 x 2.0-5.0 μm in size and occurs singly, in pairs and in small clusters. Optimum growth for this organism occurs at 45°C.

D. Historical Uses of *Lactobacilli*

1. Use in Production of Foods

Lactobacillus strains have a long tradition of use in food production, and large levels of viable bacteria are present in many foods, especially uncooked foods such as yogurt, fermented milk, and cheeses. *L. reuteri* shares many important characteristics with lactobacilli with extensive histories of food use. These properties include the lack of pathogenicity and toxic metabolites and the ability to convert common food sugars into simple acids that improve the taste and stability of foods. Traditional *Lactobacillus* strains have an excellent history of safe use in the fermentation of dairy products and other foods (Table 3). Most strains are considered commensal microorganisms (i.e. they live in harmony with a host organism) with no pathogenic potential. Some *Lactobacillus* strains have "generally recognized as safe" (GRAS) status (Donohue and Salminen 1996).

Prior sanctions have been granted for the use of lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (Code of Federal Regulations 21, §131.12), sour cream (§131.160), cottage cheese (§133.128), and yogurt ((§131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophillus* are also used in yogurt. A urease enzyme preparation from *Lactobacillus fermentum* for use in the production of wine is affirmed as GRAS in 21 CFR part 184 (U.S. FDA, March 1998).

In the manufacture of Swiss-type cheeses and yogurts, lactobacilli such as *L. helveticus* and *L. delbrueckii* subsp. *bulgaricus* are added as part of the starter culture. *Lactobacillus casei* plays an important role in the ripening of hard cheeses such as provolone, parmesan and manchego (Stiles 1996). A cheddar cheese that contains human-derived strains of *L. paracasei* was developed in Ireland (Gardiner et al. 1998).

Lactobacillus acidophilus, L. kefir and L. kefiranofaciens are typically used in the production of fermented milks. Kefir is a fermented drink that has been consumed for thousands of years. Although *L. kefir* is the predominant organism in the beverage, *L. paracasei* subsp. *paracasei* and other homofermentative lactobacilli are the predominant organisms in the grain. Kefir has been popular in the former Soviet Union, Hungary, and Poland for many years. According to a trade journal report, kefir has been available in the United States for the past few years and is gaining popularity in Japan (Saloff-Costa, 1996).

Microbial fermentation with lactobacilli has been used successfully to extend the shelf life of several meat products. *Lactobacillus sake* and other homofermentative lactobacilli that grow under refrigerated conditions are the dominant lactobacilli in meat products (Hammes et al. 1995). Serine proteinase from *L. paracasei* subsp. *paracasei* NCDO 151 has been used to accelerate production of northern-type dry fermented sausages (Blom et al. 1996).

Fermentation with lactobacilli, including *L. bavaricus, L. brevis, L. sake,* and *L. plantarum,* is often used to preserve vegetables such as cucumbers and cabbage and are typically associated with pickles and sauerkraut. *L. pentosus* is used in olive production. Lactobacilli are also associated with the production of baked goods. For example *L. sanfrancisco* is used to make wheat and rye sourdough bread (Stiles 1996). The table below summarizes food uses.

TABLE 4 Foods and Their Associated *Lactobacillus* Species	
Food	**Culture**
Swiss cheese	*Lactobacillus delbrueckii* ssp. *bulgaricus; L. helveticus*
Dairy products in general	*L. brevis; L. buchneri; L. casei; L. paracasei; L. fermentum; L. plantarum*
Fermented milks - yogurt - acidophilus milk - kefir	 *L. delbrueckii* ssp. *bulgaricus* *L. acidophilus* *L. kefir; L. kefiranofaciens*
Meats - raw - semi-preserved - fermented meat	 *L. sake: L. curvatus* *L. viridescens* (spoilage) *L. sake; L. curvatus; L. farciminis*
Fish - marinated fish products	 *L. alimentarius*
Fermented vegetables - cucumbers, sauerkraut - olives	*L. plantarum; L. sake; L. buchneri; L. fermentum* *L. bavaricus; L. brevis; L. sake; L. plantarum* *L. pentosus*
Baked goods - sourdough bread	 *L. sanfrancisco* (wheat and rye sourdough) *L. farciminis; L. fermentum; L. brevis; L. plantarum;* *L. amylovorus; L. reuteri; L. pantis* (rye sourdough)
[a]Adapted from Stiles (1996)	

2. *Lactobacillus* Supplementation of Foods

There has been an increasing commercial interest in *Lactobacillus* supplementation of foods, and several U.S. and European companies are now marketing products that contain various *Lactobacillus* strains (Table 5). Gefilus® (Valio Dairy, Helsinki, Finland) is a fruit flavored whey drink fermented with *L. casei* subsp. *rhamnosum* strain GG. The physiological and clinical properties of the GG strain are well known and results from a number of clinical studies have indicated that the strain is safe for human consumption (Salminen 1996a). Biogaia Biologics (Stockholm, Sweden) introduced *L. reuteri* SD2112 into Scandanavian dairy products in the early 1990's, and this organism was subsequently introduced in foods in the US and Japan.

TABLE 5 Commercially Used Probiotic *Lactobacillus* And *Bifidobacterium* Species[a]	
Company	**Strain**
BioGaia, Stockholm, Sweden	*Lactobacillus reuteri* SD2112[a]
Valio Dairy, Helsinki, Finland	*Lactobacillus rhamnosus* GG[b]
Nestle, Lausanne, Switzerland	*Lactobacillus johnsonii* LA1 (Lj1)[b]
Yakult, Tokyo, Japan	*Lactobacillus casei* *Bifidobacterium breve* strain Yakult[b]
Morinaga Milk Industry Co. Ltd., Zama City, Japan	*Bifidobacterium longum* BB536[b]
Rhodia, Madison, Wisconsin, USA	*Lactobacillus acidophilus* NCFM®[b]
Chr. Hansens, Milwaukee, USA	*Lactobacillus casei* CRL 431 Gilliland (La-Mo)[b]
Probi, Lund, Sweden	*Lactobacillus plantarum* 299V *Lactobacillus rhamnosus* 271[b]
Urex Biotech Inc., London, Ontario, Canada	*Lactobacillus fermentum* *Lactobacillus rhamnosus*[b]
Danone, Le Plessis-Robinson, France	*Lactobacillus casei* DN014 001 (*Immunitas*)[b]
Biocodex, Inc. Seattle, Washington, USA	*Saccharomyces boulardii*[b]
Meiji Milk Products, Tokyo, Japan	*Lactobacillus delbruekii* subsp. *bulgaricus* 2038[b]
Bona, Inc., Whitehouse, New Jersey	*Lactobacillus casei*[b]
Snow Brand Milk Products, Tokyo, Japan	*Lactobacillus acidophilus* SMT-2062 *Bifidobacterium longum* SBT-2928[b]
[a]Adapted from Casas and Dorbrogosz (submitted for publication); [b]Adapted from Sanders and Huis in't Veld (1999)	

Yakult® (Yakult Honsha Co., Japan) has become a well established and popular drink in Japan since it was first introduced in 1935. The recent demand for low-sugar and low-calorie products has led to the introduction of a light type of Yakult®. The light version contains a minimum of 15 billion live *L. casei* subsp *shirota*, whereas the regular product contains 6.5 billion bacteria in a 65-ml bottle of Yakult®.

Yakult® is now sold in several countries including Japan, Singapore, Hong Kong, Korea, Brazil, Mexico, Argentina, Netherlands, France, Germany, Belgium, and the UK. Although the Yakult Honsha Company does not distribute the product directly to the United States, Yakult® can be purchased in US branches of Japanese supermarkets.

Bona, Inc. (Whitehouse, New Jersey) introduced a European style drinkable yogurt in the U.S. in May of 1998. This product, which contains five different dairy cultures including *L. casei,* has been formulated and marketed in Poland since 1993 (Durling 1998).

Actimel (Danone, Le Plessis-Robinson, France) is a fermented milk drink that contains *L. casei* DN114 001 (*Immunitas*) in addition to traditional yogurt cultures. This product is currently being test marketed in dairy cases across the US.

E. Foods Containing *Lactobacillus reuteri*

Sourdough bread is widely consumed in Europe and the US. A starter, called sourdough, is used to initiate the acidification of rye flour-containing doughs and in the flavoring of wheat doughs. A sponge of sourdough is made from wheat or rye flour plus water. The sourdough sponge is prepared daily by mixing fresh ingredients with mature sourdough in order to maintain the activity of the microorganisms. *Lactobacillus reuteri* has been isolated from the microflora of sourdoughs (Vogel et al. 1994) and sourdough sponges (Okada et al. 1992).

Lactobacillus reuteri is also used in dairy foods. In addition to traditional yogurt cultures, Stonyfield Farm (Londonderry, NH) adds *L. reuteri* SD2112 and two other live, active cultures to their refrigerated and frozen yogurts (Gorski 1998b).

A Swiss company (Toni Lait) manufactures SymBalance, a fermented milk product which contains *Lactobacillus reuteri* SD2112, *L. casei* 01, *L. acidophilus* La5 and *Bifidobacterium* (Sanders and Huis in't Veld 1999). The product has been on the market in Switzerland since October 1995.

Milk supplemented with *L. reuteri* SD2112 and two additional probiotic organisms was introduced in Sweden in 1991 under the BRA brandname. In Finland, products containing *L. reuteri* SD2112 are sold under the Rela brandname; these products include yogurt, orange juice, pineapple juice, and cottage cheese. In Japan, dairy products containing *L. reuteri* SD2112 are sold under the SymBalance and Reuteri names.

F. Lack of Pathogenicity and Toxicogenicity of *Lactobacillus reuteri*

A critical review of pertinent studies and literature identified in searches conducted through online bibliographic retrieval systems, including Medline and Dialog, found no evidence of pathogenicity or toxicogenic effects of *Lactobacillus reuteri.* The search criteria used to identify the studies that relate to this issue were: *L. reuteri*, pathogen, mycotoxin, toxin, toxigenic, infection or disease. These terms were truncated to include different forms of the word. The databases searched included Medline, Toxline, Cancerlit, HealthStar, Cab Health, Agricola, Cab Abstracts, Food Sci.&Tech. Abs, Foodline, Biosis Previews, SciSearch, Elsevier Biobase, Embase, Life Sciences Collection, TGG Health & Wellness DB and Embase Alert.

G. Metabolites of *Lactobacillus reuteri*

Axelsson et al. (1989) reported that *Lactobacillus reuteri* converted glycerol into a potent, broad-spectrum antimicrobial that was termed "reuterin." Reuterin has been isolated, purified and identified using nuclear magnetic resonance, mass spectrometry and infrared analysis (Talarico and Dobrogosz 1989). It was shown to be a mixture of monomeric, hydrated monomeric acetal and cyclic dimeric forms of β-hydroxypropionaldehyde. This low molecular weight, neutral, water soluble compound is capable of inhibiting growth of species representing several bacterial genera including *Escherichia*, *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Clostridium* and *Staphylococcus* (Axelsson et al. 1989). Lactic acid bacteria belonging to the genera *Streptococcus*, *Pediococcus*, *Leuconostoc* and *Lactobacillus* are also affected by reuterin but to a lesser degree. Chen et al. (1999) determined that the minimum concentration of reuterin required to inhibit growth of various bacterial strains ranged from 0.6 to 4.0% MIC (v/v).

There are no reports of toxicity of reuterin to organisms other than microbes. The β-hydroxy moiety of reuterin renders its aldehyde function reactive, capable of spontaneous reaction with available amino and sulfhydryl functional groups. Thus, it is not systemically absorbed.

Chung et al. (1989) showed that reuterin was synthesized under environmental conditions similar to those which exist in the regions of the GI tract where *L. reuteri* has been isolated, although *in vivo* production of reuterin has not been demonstrated. Reuterin synthesis was stimulated by contact with other bacteria such as *E. coli*, *Salmonella typhimurium*, *Shigella*, *Proteus*, *Pseudomonas fluorescens*, *Staphylococcus epidermidis*, *Bacillus megaterium*, *Clostridium sporogenes*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides* and *Streptococcus cremoris*.

Production by *L. reuteri* of other metabolites, aside from the organic acids resulting from sugar fermentation, has not been reported.

H. Production of *Lactobacillus Reuteri*

1. Origin of *L. reuteri* and Maintenance of the Organism

L. reuteri strain SD2112, the subject of this notification, was originally isolated by Dr. Ivan Casas from a sample of breast milk obtained from a mother in Peru. The original identification and characterization of this strain was performed by Drs. Ivan Casas and Walter Dobrogosz at North Carolina State University. The current stock strain is maintained at the R&D facility of BioGaia Biologics in Lund, Sweden. This strain has also been deposited with the American Type Culture Collection as accession number ATCC 55730. This strain is frequently denoted in the literature as SD2112. This strain and its uses are

covered by United States Patents 5,439,678; 5,800,813; 5,837,238; 5849,289; and additional pending applications.

2. Preparation of Lyophilized Bacteria

Lyophilized (freeze-dried) *L. reuteri* SD2112 is used in the manufacture of the intended dietary supplement.

The production of a lot of *L. reuteri* begins with the aseptic production of a broth culture in the laboratory. After the broth culture reaches stationary phase, it is used to inoculate a starter fermenter.

After the starter fermenter culture reaches early stationary phase, the culture is aseptically transferred to a large production fermenter. The media for the fermenter is batch sterilized, except for heat sensitive components that are sterilized separately. Sterilization consists of heating the media under pressure to 121°C, and holding it at that temperature for 30 minutes. The media is cooled by circulating water through the jacket of the fermentation tank.

Lactobacillus reuteri produces lactic acid during growth, and sodium hydroxide solution is added to maintain the pH at close to neutrality. The progress of bacterial growth is monitored by the total base consumption. Base consumption diminishes markedly when the culture nears stationary phase. Stationary phase is attained about 8-12 hours after the inoculation of the large fermentation vessel. Harvesting of the bacteria begins 30 minutes after the bacteria reach stationary phase.

Harvesting consists of chilling the broth culture in a plate and frame heat exchanger, and then passing the broth culture into a Sharples-style continuous centrifuge. All contact surfaces are steam sterilized prior to use. After the bacteria are deposited in the centrifuge, the cells are washed two times.

A slurry of washed bacteria is pumped into sterilized trays. These sterilized trays are placed in a batch lyophilizer. Filling of the trays and the lyophilization occurs in a clean room under positive pressure with HEPA filtered air (1,000-10,000 particle range). All personnel in this area wear sterile gowns, gloves, facemasks, and hair covers. Lyophilization is carried out at -50°C.

After lyophilization, the freeze-dried bacteria are milled and placed into polyethylene lined containers. Bacteria may subsequently be packed into lined aluminum foil pouches. All subsequent handling prior to manufacture of dietary supplements occurs at 4°C or colder temperature.

In-process microbiological samples are taken at each step. Also, the final freeze-dried bacteria are analyzed for strain identity (determined by biochemical reaction profile) and

lack of contamination by other microorganisms, including those of public health significance.

I. Animals Studies of *L. reuteri* Administration

Studies of *L. reuteri* administration in animals have examined effects on colonization of the gastrointestinal tract and support of digestive function. These studies are summarized in Table 6 (Alak et al. 1997; Piva and Morelli 1997; Edens and Parkhurst 1991, presentation; Fabia et al. 1993; De Smet et al. 1998; Molin et al. 1992; Ratcliffe et al. 1986). These studies have generally been directed toward identifying efficacy of *L. reuteri*. This notification reviews primarily the safety aspects of *L. reuteri* (i.e. tolerability, lack of toxicity and pathogenicity) that were reported in these studies.

In a study that was directed primarily toward safety assessment, mature sows (n=6) were supplemented with *L. reuteri* ($1.2x10^9$ CFU/day) and *L. acidophilus* ($8x10^8$ CFU/day) for 21 days. There were no adverse effects. They had lower yeast, coliform and clostridia counts, reduced ammonia levels in the jejunum and reduced acetate to propionate ratio in the cecum. Eighty percent of the lactic acid bacteria adhering to the intestinal epithelium were provided by the diet. *L reuteri* density tended to decrease from the stomach to the cecum and *L. acidophilus* levels increased. The results show that *L. reuteri* and *L. acidophilus* supplementation colonized the gut, and reduced the proliferation of pathogenic microorganisms and decreased ammonia production in the gut (Piva and Morelli 1997).

In two separate experiments, Edens and Parkhurst (1991) examined the effects of *L. reuteri* and whey on growth and mortality in animals challenged with *Salmonella typhimurium* ST-10 and *senftemberg*. In the first experiment, 90 turkey poults were assigned to eight groups. Respective groups were challenged with high doses of salmonella and treated with or without *L. reuteri* and whey, or they were not challenged and not treated with *L. reuteri* and whey, or they were not challenged but treated with whey. In the second experiment, 90 poults were again challenged with salmonella, and some groups also received *L. reuteri*. In both experiments the dose of *L. reuteri* was administered via the feed at a level of $5x10^5$ *L. reuteri* g^{-1} from hatching to 11 days of age. The results suggest that *L. reuteri* has protective effects on mortality and growth in animals challenged with salmonella. No adverse effects or tolerability problems were associated with the *L. reuteri* consumption.

Alak et al. (1997) also reported the protective effect of *L. reuteri* against *C. parvum* infection in immunosuppressed C57BL/6 mice. The mice developed immunodysfunction four months after intraperitoneal inoculation with 0.3 ml of LP-BM5. The mice were challenged with *C. parvum* parasite ($6.5x10^6$ organisms) after priming for 10 days with *L. reuteri*. The level of bacteria ingested and duration of treatment was not reported. The *L. reuteri* treatment was continued throughout the experimental period. Mice primed and treated with *L. reuteri* cleared

the infection and no *C. parvum* parasites were detected in the intestinal epithelium, whereas the control mice developed cryptosporidiosis and shed high levels of oocysts in the feces. No significant difference in body weight was observed between the groups, and there were no *L. reuteri* treatment-related adverse effects.

Fabia et al. (1993) examined the effects of *L. reuteri* in treating acetic acid induced colitis in rats. This study used a rat-derived *L. reuteri* strain (R2LC strain 156) and a human strain (HLC). Nine groups of rats were assigned either to the control normal group, the control colitis group, or to *L. reuteri* treatment groups. A single dose (5 ml of $7x10^7$ CFU/ml) or three doses (each dose was one ml of $7x10^7$ CFU/ml) of *L. reuteri* R2LC strain 156 was administered into the exteriorized colon as freeze-dried fermented oatmeal soup or as purified suspension. The single dose was administered immediately after inducing colitis with acetic acid or one day after induction. In the three dose treatment, one dose was administered immediately after inducing colitis and the other two doses were given on the following days. An additional group was administered a single dose of the human HLC strain (5 ml of $7x10^7$ CFU/ml in physiological saline). Finally, one further group was given unfermented oatmeal soup after acetic acid administration. The colonic mucosa was preserved and the development of colitis was prevented in rats treated with the rat-derived *L. reuteri.* No adverse effects were associated with exposure to either *L. reuteri* strain.

Ratcliffe et al. (1986) determined that no adverse consequences were associated with *L. reuteri* supplemented milk. They evaluated the effect of fermented milk with *L. reuteri* strain 14 on gastrointestinal flora in pigs weaned at two days of age in a series of five experiments. In three experiments the pigs were treated with either yogurt, acidified milk with lactic acid, base milk from which yogurt was made, base milk supplemented with vitamins or yogurt supplemented with vitamins for 12 days. In the remaining two experiments pigs were either fed fortified milk fermented with *L. reuteri* or fortified base milk for 12 days, or after 12 days they received their respective diets or fortified base milk for three days. The beneficial effects of yogurt, acidified milk and *L. reuteri* fermented milk on the intestinal tract were similar in that they decreased coliform counts and pH, and increased *Lactobacillus* counts (acidified milk decreased *Lactobacillus* counts). The *L. reuteri* containing treatments were well tolerated, and no adverse effects were associated with these treatments.

One study examined the effects of *L. reuteri* on lowering cholesterol levels in pigs (De Smet et al. 1998). Twenty pigs (10 males and 10 females) were fed a 'Western type' diet for four weeks (2 g/day cholesterol for first two weeks and 4 g/day cholesterol for last 2 weeks) and supplemented with or without *L. reuteri* (1.18×10^{11} *L. reuteri* cells per day) during this period. Body weights were not different between the groups and there were no adverse effects or illness related to the *L. reuteri* treatment. Total and LDL-cholesterol decreased significantly, but HDL-cholesterol and triglycerides levels were not affected by *L. reuteri* treatment. *Lactobacillus*

counts in the feces increased, but the streptococci and enterobacteriaceae levels decreased in the *L. reuteri* group. The *L. reuteri* treatment was well tolerated, and the results show that *L reuteri* treatment did not affect body weights, cause adverse effects, or increase the growth of pathogenic microorganisms in pigs.

In a study designed to examine cholesterol lowering effects in rats, Molin et al. (1992) reported no adverse effects from administration of six *Lactobacillus* strains, including three *L. reuteri* isolates (R21c, Hj108 and Hj108^{ery-}). Ninety rats (10/group) were assigned to either fermented oatmeal soup (FOS) treatment containing six *Lactobacillus* strains (total CFU/g was $1x10^7$) for 9 days or to unfermented oatmeal treatment for 10 days. No significant differences in weight gain or variation in clinical condition were observed among the groups. Serum cholesterol levels decreased with oatmeal feeding, but no further decrease was observed with *Lactobacillus* feeding.

Summary

Studies done in animals show that *L. reuteri* administration has no adverse health effects and does not adversely affect body weight. It has beneficial effects on promoting normal gastrointestinal function.

TABLE 6 Studies of *L. reuteri* Administration in Animals	
Piva and Morelli 1997 *Zool. Nutr. Anim.* 1997;23:147-155 Italy	
Study design	Randomized controlled experiment
Species and Strain	Six mature sows (Landrace x Large White) weighing 200 kg. The pigs were fed standard diet before and during the experimental period. The experimental group received an additional 40 g/day of commercial preparation containing $3x10^7$ *L. reuteri* and $2x10^7$ *L. acidophilus* cells per gram. At the end of the supplementation period all animals were fasted for 24 hours and then sacrificed.
Dose	$1.2x10^9$ CFU/day of *L. reuteri* and $8x10^8$ CFU/day of *L. acidophilus*
Duration	21 days (3 weeks)
Safety Results	No adverse effects were associated with *Lactobacillus* consumption. Yeast counts in the lumen of the stomach and on the mucosa of the stomach, jejunum, and colon were reduced in the experimental animals. Coliforms were reduced in the cecal and colon contents and on the gastric and cecal mucosa of the experimental animals. Clostridia were reduced in the ileum and colon contents and on the stomach, ileum and colon mucosa of the experimental animals. The pH in the cecum and jejunum was lower in animals treated with bacteria. Ammonia concentration in the jejunum was significantly reduced in the treated animals. Acetic acid to propionic acid ratio was reduced in the cecal samples of the treated pigs.
Safety Conclusion	Colonization of the gastrointestinal tract with *L. reuteri* and *L. acidophilus* was well-tolerated, and was not associated with adverse biochemical or physiological effects.

TABLE 6 (continued) Studies of *L. reuteri* Administration in Animals	
Edens and Parkhurst 1991 Research report presented at the Southern Poultry Science Society Annual Meeting. January 1991 Georgia	
Study design	Randomized controlled experiment
Species and Strain	Nicholas turkey poults
Dose	Experiment 1: 8 groups. All groups were challenged with *Salmonella senftenberg* and *typhimurium* 10^6 CFU/poult via gavage. The amount of *L. reuteri* administered was $5x10^5$ *L. reuteri* g^{-1} of feed. Both *L. reuteri* and whey were added in the feed. Each treatment group contained 90 poults. Group 1: control group challenged at hatch with no *L. reuteri* or whey treatment Group 2: challenged at hatch and treated with whey and *L. reuteri* Group 3: challenged at day 5 after the hatch with no whey or *L. reuteri* treatment Group 4: challenged at day 5 after the hatch and treated with whey and *L. reuteri* Group 5: continuously challenged with no whey or *L. reuteri* treatment. Poults challenged daily with salmonella 10^4 CFU/poult at hatch, then 10^6 CFU/poult from 5 days until 11 days post hatch. Group 6: continuously challenged and treated with whey and *L. reuteri*. Poults challenged daily with salmonella 10^4 CFU/poult at hatch, then 10^6 CFU/poult from 5 days until 11 days post hatch. Group 7: No challenge and no *L. reuteri* or whey treatment Group 8: No challenge and treated with whey Experiment 2: 6 groups. All groups were challenged with Salmonella $2x10^3$ CFU/poult via gavage. The amount of *L. reuteri* administered was $5x10^5$ *L. reuteri* g^{-1} of feed. Both *L. reuteri* and whey were added in the feed. Each treatment group contained 90 poults. Group 1: challenged at hatch with no *L. reuteri* or whey treatment Group 2: challenged at hatch and treated with *L. reuteri* and whey Group 3: challenged at day 1 (one day of age) with no *L. reuteri* or whey treatment Group 4: challenged at day 1 (one day of age) and treated with *L. reuteri* and whey Group 5: challenged at day 5 (5 day of age) with no *L. reuteri* or whey treatment Group 6: challenged at day 5 (5 day of age) and treated with *L. reuteri* and whey
Duration	12 days
Safety Results	*L. reuteri* treatment was well-tolerated, and no adverse effects related to *L. reuteri* treatment were observed. High dose of salmonella caused considerable mortalities and stunted growth. Birds treated with *L. reuteri* were protected from mortality and growth repression compared to the control birds. The protective effect on mortality was more pronounced in the birds challenged at hatch. *L. reuteri* treatment improved the "weight-survival ratio" but could not overcome these stresses completely if the birds were challenged with salmonella at hatch or continuously. However, in the birds challenged at day 5, these stresses were reversed. At the lower dose of salmonella, the mortality in the *L. reuteri* group was lower compared to the control groups. The repressive effect on growth was significantly reversed with *L. reuteri* treatment.
Safety Conclusion	*L. reuteri* treatment was well-tolerated, reversed the growth suppression, and reduced the mortality caused by salmonella infection.

TABLE 6 (continued) Studies of *L. reuteri* Administration in Animals	
Ratcliffe et al. 1986 *Food Microbiology* 1986;3:203-211 UK	
Study design	Controlled experiment
Species and Strain	Large White piglets weaned at day 2.
Dose	Five experiments were conducted, two of which involved administration to pigs of a milk fermented with *L. reuteri* (poghurt). In one of the *L. reuteri* treatments, seven pairs of pigs were fed base milk fermented with *L. reuteri* strain 14 (poghurt) and fortified with casein hydrolysate or fortified base milk without *L. reuteri.* In the second experiment, ten pigs were given poghurt or fortified base milk for 14 days of age. Five pigs from each group were killed at this stage and the remaining pigs were fed their respective diets for additional 3 days and killed at 17 days of age. The second experiment also included another group of five pigs that was given poghurt for 14 days and then fortified base milk for 3 days.
Duration	12 days for the first *L. reuteri* experiment and 15 days for the second *L. reuteri* experiment.
Safety Results	In the first *L. reuteri* experiment, the performance of pigs fed poghurt was similar to the pigs fed yogurt. Pigs consuming poghurt showed less weight gain compared to the pigs on base milk diet. Earlier experiments in this series showed a decrease in weight gain in pigs fed fermented milk or base milk acidified with lactic acid, in comparison to unacidified base milk. Therefore, the decrease in weight gain was not attributable to *L. reuteri.* The number of pigs which scoured were 6/7 in both treatment groups. In the second *L. reuteri* experiment, two pigs fed base milk died, one at 13 days and the other at 17 days of age. Scouring prior to 14 days of age was 7/9 and 8/14 in base milk and poghurt group, respectively. One pig who was changed from poghurt to base milk diet scoured after 14 days.
Safety Conclusion	The results show that fermented milk with *L. reuteri* was well-tolerated. It had beneficial effects on *Lactobacillus* (increased) and coliform (decreased) counts and on pH and these effects were similar to the effects of yogurt and acidified milk. All treatment diets i.e. yogurt, acidified milk with lactic acid, and *L. reuteri* fermented milk caused a decrease in weight gain compared to the base-milk diet. Hence, the effect on weight gain cannot be attributed to *L. reuteri* since all fermented products regardless of the method used for fermentation had a depressing effect on weight gain.

TABLE 6 (continued) Studies of *L. reuteri* Administration in Animals	
Fabia et al. 1993 *Scand J Gastroenterol* 1993;28:155-162 Sweden	
Study design	Controlled experiment
Species and Strain	Female Sprague-Dawley rats weighing 240 g. Colitis was induced in the exteriorized segment of the colon by administration of 4% acetic acid.
Dose	Nine groups of rats. The rats in the control groups were treated with saline at the end of the operation. The treatment was administered directly into the exteriorized colonic segment. Group 1: control normal rats (surgically prepared but without acetic acid treatment) Group 2: control colitis rats Group 3: fermented oatmeal soup given as a single dose of 5 ml of $7x10^7$ CFU/ml of *L. reuteri* R2LC strain 156 immediately after colitis induction Group 4: fermented oatmeal soup given as a single dose of 5 ml of $7x10^7$ CFU/ml of *L. reuteri* R2LC strain 156 one day after colitis induction Group 5: 3 doses of fermented oatmeal soup (1 ml of $7x10^7$ CFU/ml of *L. reuteri* R2LC strain 156), one dose given immediately after acetic acid administration and two doses given on the following two days Group 6: a suspension of *L. reuteri* R2LC strain 156 in physiologic saline given as a single dose of 5 ml of $7x10^7$ CFU/ml immediately after colitis induction Group 7: 3 doses of a suspension of *L. reuteri* R2LC strain 156 in physiologic saline (1 ml of $7x10^7$ CFU/ml), one dose given immediately after colitis induction and two doses given on the following two days Group 8: a suspension of *L. reuteri* HLC given as a single dose of 5 ml of $7x10^7$ CFU/ml immediately after colitis induction Group 9: 5 ml of unfermented oatmeal given immediately after acetic acid administration
Duration	1-3 days
Safety Results	Administration of a rat strain of *L. reuteri* limited the development of colitis in this acid-induced model. No adverse effects were associated with administration of a rat or human *L. reuteri* strain in this model. On day 4 after acetic acid administration, colitis had developed in the exteriorized colonic segment in the control colitis rats. The exteriorized colonic segment from the control normal rats showed normal colonic mucosa with mild edema and few dilated blood vessels. The colonic mucosa was well preserved and the development of colitis was prevented in rats treated with fermented oatmeal soup immediately after acetic acid administration. The colonic mucosa was less preserved if the treatment with fermented oatmeal soup was given one day after colitis induction or in three smaller doses, one given immediately after acetic acid administration and two on the following days. Intracolonic administration of pure *L. reuteri* R2LC suspension preserved the colonic mucosa and prevented the development of colitis when administered immediately after acetic acid administration but it was less effective when administered in three small doses. Treatment with human *L. reuteri* HLC immediately after acetic acid administration did not prevent the development of colitis and the colitis mucosa of this group was similar to the control untreated colitis rats. Unfermented oatmeal soup did not prevent the development of colitis.
Safety Conclusion	The results show that *L. reuteri* administration was beneficial in preserving the colonic mucosa and preventing acetic acid induced colitis in rats. No adverse effects were associated with administration.

TABLE 6 (continued) Studies of *L. reuteri* Administration in Animals	
Alak et al. 1997 *Gastroenterology* 1997;110(4) (abstract) US	
Study design	Controlled experiment
Species and Strain	C57BL/6 female mice. The mice were inoculated i.p. with 0.3 ml of LP-BM5 which had an ecotropic titer of 4.5 $\log_{19}$ PFU/ml in an XC-cell line. The mice developed immunodysfunction 4 months after the inoculation.
Dose	The mice were primed with *L. reuteri* for 10 days and then challenged with *Cryptosporidium parvum* parasite ($6.5x10^6$). The *L. reuteri* treatment was continued throughout the experimental period.
Duration	Greater than 10 days
Safety Results	*L. reuteri* was well tolerated, and body weight did not differ between the two groups. Mice supplemented with *L. reuteri* and challenged with *C. parvum* cleared the infection and no *C. parvum* parasites were detected in the intestinal epithelium compared to the untreated mice. Fecal levels of *L. reuteri* were higher in the supplemented mice as compared to the unsupplemented mice. Mice not treated with *L. reuteri* developed persistent cryptosporidiosis and shed high levels of oocysts in the feces.
Safety Conclusion	*L. reuteri* supplementation was well-tolerated and was not associated with adverse effects in this mouse model.

TABLE 6 (continued) Studies of *L. reuteri* Administration in Animals	
De Smet et al. 1998 *British Journal of Nutrition* 1998;79:185-194 Belgium	
Study design	Controlled experiment
Species and Strain	Twenty Seghers hybrid (sow) x Pietrain (boar) pigs (ten females and ten castrated males, age 10 weeks)
Dose	1.18×10^{11} *L. reuteri* cells were added to the morning and afternoon feeds
Duration	Four weeks on *L. reuteri* treatment; total duration of the study was 13 weeks Period 1: Three week acclimatization period during which the animals received 'Western type' diet (high fat, high cholesterol, low fiber) + 2g cholesterol/kg. Period 2: The treatment group received 'Western type' diet + 2g cholesterol/kg + *L. reuteri* for first 2 weeks, then 'Western type' diet + 4g cholesterol/kg + *L. reuteri* for the last two weeks. Period 3: Post-treatment follow-up period on 'Western type' diet + 4g cholesterol/kg for 3 weeks. Period 4: Regular pig diet for 3 weeks.
Safety Results	One pig in the control group showed an aberrantly high food efficiency ratio during the normalization period, which was not related to the probiotic feeding or high cholesterol diet. One pig in the control group died during week 10 for an unknown reason. No significant differences in body weight were observed between the two groups during the duration of the experiment and there were no illnesses or adverse effects due to the *L. reuteri* treatment. Total and LDL-cholesterol was significantly reduced by 11% and 26% in the pigs treated with *L. reuteri* compared to the control group after 2 weeks of probiotic supplementation. After 4 weeks, the reductions were 15% and 24% in total and LDL-cholesterol, respectively in the treated group. The reductions were 18% (total) and 34% (LDL-cholesterol) in the treated group during the 3 weeks post-treatment period. HDL-cholesterol was not significantly affected by *L. reuteri* treatment. Triglyceride levels were not significantly different between the groups during the duration of the experiment. Total bile salt excretion increased in the *L. reuteri* treated group compared to the control group after 1 week of supplementation and it lasted until the end of the treatment. The cholesterol, coprostanol and total neutral sterol excretion was significantly higher at 12 week in the treated group compared to the control group. These biochemical changes were not deleterious, and are of no toxicologic concern.
Conclusion	The results show that *L. reuteri* treatment reduced total and LDL-cholesterol levels in serum and reduced the numbers of streptococci and enterobacteriaceae in the feces. *L. reuteri* treatment was well-tolerated and did not cause proliferation of pathogenic microorganisms in the gastrointestinal tract.

TABLE 6 (continued) Studies of *L. reuteri* Administration in Animals	
Molin et al. 1992 *Antonie van Leeuwenhoek* 1992;61:167-173 Sweden	
Study design	Controlled experiment
Species and Strain	Male Sprague-Dawley rats
Dose	Six *Lactobacillus* strains were administered. Three of them were *L. reuteri* (*L. reuteri* R21c, *L. reuteri* Hj108, *L. reuteri* Hj108^{ery-}). A mixture of freeze-dried fermented oatmeal soup (FOS) containing these lactobacilli was supplemented with 20% (w/w) soya meal. The CFU/g of the product varied between $3x10^7$ and $1x10^9$. The mixture had a total *Lactobacillus* count of $1x10^7$ CFU/g. The rats on an average consumed 23 g of the freeze-dried powder/day. Unfermented oatmeal soup was used as a control. 90 rats were assigned to the following groups (10/group): Group 1: FOS feeding for 9 days + 1 day on unfermented oatmeal soup Group 2: FOS feeding for 9 days + 1 day on unfermented oatmeal soup. These animals were administered antibiotic treatment (cefuroxime and metronidazole) 3 times/day i.p. on the day before the trial. Group 3: FOS feeding for 9 days + 7 days on unfermented oatmeal soup Group 4: FOS feeding for 9 days + 7 days on unfermented oatmeal soup. These animals were administered antibiotic treatment (cefuroxime and metronidazole) 3 times/day i.p. on the day before the trial. Group 5: FOS feeding for 9 days + 24 days on unfermented oatmeal soup Group 6: FOS feeding for 9 days + 24 days on unfermented oatmeal soup. These animals were administered antibiotic treatment (cefuroxime and metronidazol) 3 times/day i.p. on the day before the trial. Group 7: FOS feeding for 0 days + 10 days on unfermented oatmeal soup Group 8: FOS feeding for 0 days + 10 days on unfermented oatmeal soup. These animals were administered antibiotic treatment (cefuroxime and metronidazole) 3 times/day i.p. on the day before the trial. Group 9: Animals were fed commercial diet, Biosorb Sond.
Duration	9 days on *L. reuteri*
Safety Results	*L. reuteri* treatment had no effects on weight gain, variation in clinical condition, and serum cholesterol levels. Oatmeal feeding decreased serum cholesterol levels but lactobacilli feeding caused no further decrease in serum cholesterol levels. Of the three strains of *L. reuteri*, only *L. reuteri* strain R21c effectively colonized the intestinal mucosa.
Safety Conclusion	The lactobacilli administration was well-tolerated and no adverse effects were noted by the authors.

J. Human Clinical Studies on *L. reuteri*

The use of *L. reuteri* as a probiotic has been studied in infants, children, healthy adults and in individuals with immunodeficiency virus (Wolf et al. 1998, 1995; Shornikova et al. 1997a and 1997b; Johansson et al. 1993; Jacobsen et al. 1999; Casas et al. (unpublished abstract); Ruiz-Palacios et al. 1996a and 1996b). Details of these studies are presented in Table 7 and discussed below.

Wolf et al. (1995) studied the safety and tolerance of *L. reuteri* ingestion in healthy males in a randomized, double-blinded placebo controlled trial. Thirty healthy males (n=15/group) were randomly assigned to receive either *L. reuteri* SD2112 ($1x10^{11}$) or placebo capsules for 21 days with a 7 day washout period. Serum chemistry, hematology, and urinalysis parameters were measured on days 7, 14, 21 and 28. Although the changes from baseline in several serum chemistry, hematology and urinalysis variables were significantly different between the two groups, these changes were sporadic and were not considered to be clinically significant. Furthermore, all the values were within the normal range for healthy adult males. The incidence of subjective tolerance factors such as flatulence, diarrhea and cramping were infrequent and similar between the groups. *L. reuteri* levels in the feces and the ratio of *L. reuteri* to *Lactobacillus* spp. was significantly higher in the *L. reuteri* supplemented group on days 7, 21, 14 and 28 as compared to the control group. The results demonstrate that *L. reuteri* can be ingested at a level of $1x10^{11}$ CFU/day without any clinically significant safety or tolerance problems.

Wolf et al. (1998) also examined the safety and tolerance to *L. reuteri* SD2112 in individuals with HIV infection in a randomized, double-blinded placebo controlled trial. The subjects were supplemented either with *L. reuteri* (10^{10} CFU/day, n=15) or placebo (n=20) capsules for 21 days followed by a 14 day washout period. Serum chemistry, hematology, and urinalysis parameters were measured on the screening day and on days 21 and 35. Sporadic changes from baseline in some hematology, immunology, and urinalysis variables were seen between the groups. These changes were considered clinically insignificant and not related to treatment. Flatulence was frequent in both groups, and the incidence was similar between the groups. There was a trend toward more complaints of mild nausea in the *L. reuteri* group. *L. reuteri* supplementation caused an increase in fecal levels of *L. reuteri* on days 7, 14 and 21 compared to baseline. These results demonstrate that *L. reuteri* can be ingested at $1x10^{10}$ CFU/day by HIV positive individuals without any clinically significant safety and tolerance problems.

Although the supplement will not be recommended for use by children, several studies demonstrate the excellent safety profile among this group. Casas et al. (unpublished abstract) reported on the tolerability of various levels of *L. reuteri* SD2112 in a population of children aged 6 to 36 months who were hospitalized for presumed viral or mild bacterial infections. *L. reuteri* was administered orally in 75 ml of liquid; the placebo consisted of the liquid base.

Subjects (number not provided) were randomized to four treatment groups. One group received *L. reuteri* SD2112 at a level of 1 x 10^{10} to 5 x 10^{10} cfu for three to five days. A second group received a single administration of this level of bacteria. A third group received 1 x 10^{8} to 5 x 10^{8} CFU for three to five days. The fourth group received the placebo. *L. reuteri* colonized the gastrointestinal tracts of all three groups receiving bacteria. The investigators reported that no clinical or tolerance effects were associated with the administration of *L. reuteri.*

Ruiz-Palacios et al. (1996a) established the tolerance and dose response of a probiotic mixture containing *L. reuteri, L. acidophilus* and *B. infantis* in children (n=72), ages 12 to 36 months. The children were randomly assigned to the control, low probiotic (10^{6} CFU/day), medium probiotic (10^{8} CFU/day) or high probiotic (10^{10} CFU/day) group for 3 weeks. No significant differences in the incidences of vomiting, abdominal discomfort, gas, and stool characteristics were observed among the groups. The *L. reuteri* supplementation caused an increase in fecal *L. reuteri* levels in a dose dependent manner. The results show that *L. reuteri* supplementation was well tolerated by the children.

In another study, children (ages 12 to 35 months) were randomly assigned to a probiotic blend treatment group (n=119) or to a control group (n=120) for 14 weeks to determine the effect of this blend in the prevention of diarrhea in healthy children. The number of organisms administered was not reported. The number of children with diarrhea and the incidence of diarrhea was lower in the treatment group compared to the control group, but the severity was not different between the groups. No adverse health effects were reported (Ruiz-Palacios et al. 1996b).

Two additional studies using children (ages 6 to 36 months) examined the effects of *L. reuteri* SD2112 supplementation on colonization of the gut in subjects with mild viral or mild bacterial diarrhea, as well as its use as a therapeutic agent for rotavirus-associated diarrhea (Shornikova et al. 1997a and 1997b). In both studies, the children were randomly assigned to receive either freeze-dried *L. reuteri* SD2112 or a placebo in a double-blind manner. In one study, children received 10^{10} to 10^{11} cfu of *L. reuteri* suspended in liquid once per day for five days or until release from the hospital, if earlier than five days. In the second study, children received either 10^{7} or 10^{10} CFU under treatment conditions that were otherwise identical to the first study. Both studies showed no adverse effects of *L. reuteri* supplementation on either weight gain, consumption of oral rehydration solution or electrolyte, or on acid-base balance. The frequency and the duration of watery diarrhea and the incidence of vomiting was reduced in children receiving *L. reuteri* compared to the children given placebo. Increased colonization of the gut with *L. reuteri* occurred as a result of supplementation, as evidenced by the increase in fecal *L. reuteri* counts (Shornikova et al. 1997a and 1997b). The extent of colonization was dependent on the dose and frequency of administration of *L. reuteri* (Shornikova et al. 1997b). Urease levels decreased in the *L.* reuteri group. Rotavirus IgA antibodies, beta-glucuronidase

and beta-glucosidase activities were not affected by *L. reuteri* treatment (Shornikova et al. 1997a and 1997b). These three studies demonstrate that *L. reuteri* may be safely ingested by children at levels up to 10^{11} CFU/day.

The ability of *Lactobacillus* spp. to survive *in vivo* was examined in 12 healthy men in a double-blind cross-over trial (Jacobsen et al. 1999). Three groups were enrolled in a three period crossover trial. One treatment consisted of L. *reuteri* DSM 12246 (10^{10} CFU/day/dose) and L. *rhamnosus* 19070-2 (10^{10} CFU/day/dose). A second treatment consisted of L. *rhamnosus* LGG (10^{10} CFU/day/dose), L. *delbrueckii* subsp. *lactis* CHCC 2329 (10^{10} CFU/day/dose) and L. *casei* subsp. *alactus* CHCC3137 (10^{10} CFU/day/dose). The third treatment was a placebo. Each treatment lasted for 18 days with a 17 day washout period after each treatment. The investigators did not report any adverse health effects.

The ability of *Lactobacillus* strains to colonize the human intestinal mucosa was also studied in thirteen healthy volunteers (nine women, four men) (Johansson et al. 1993). Nineteen different strains of multiple species of lactobacilli (two of which were *L. reuteri* 108 and *L. reuteri* 47 (=R2LC)) were administered as freeze-dried fermented oatmeal soup for 10 days. Biopsy samples of the gut were taken for microbial identification. The *Lactobacillus* numbers increased in the gut, and both phenotypic and genotypic identification showed colonization by *L. reuteri* 108 and four other strains of *Lactobacillus*. The investigators did not report any adverse effects.

Summary

The human clinical trials show that *L. reuteri* effectively colonizes the gut. These trials have shown that there were no problems with gastrointestinal tolerance after *L. reuteri* was administered at levels of up to 10^{11} CFU/day. No significant adverse effect on any safety parameter such as physical signs, serum chemistry, urinalysis or hematology variables was noted at levels of *L. reuteri* administration up to 10^{11} CFU/day. In conclusion, the safety of, and tolerance to, *L. reuteri* administration at levels up to 10^{11} CFU/day has been demonstrated in healthy adults, individuals with HIV infection, and children.

TABLE 7 **Clinical Studies of *L. reuteri* in Humans**	
Wolf et al. 1995 *Microbial Ecology in Health and Disease* 1995;8:41-50 US	
Study design	Randomized, double-blind placebo controlled trial
Subjects	Thirty healthy males The subjects were asked to maintain their normal diet and avoid alcohol. Placebo: n=15 L. *reuteri* (SD2112): n=15
Dose	Freeze-dried 1 x 10^{11} CFU/day *L. reuteri* (2 capsules each 5 X 10^{10} CFU). Placebo capsules were filled with cryoprotectant
Duration	21 days of treatment with 7 days washout period
Safety Results	Few individuals had detectable levels of *L. reuteri* in the feces at baseline. Intake of *L. reuteri* increased the fecal levels of *L. reuteri* on days 7, 14, 21 and 28 compared to baseline levels. Total *Lactobacillus* spp. in the feces was not affected by *L. reuteri* supplementation and the ratio of *L. reuteri*/total *Lactobacillus* spp. was higher in the *L. reuteri* group on days 7, 14, 21 and 28. Serum chemistry, hematology, and urinalysis parameters were measured on days 7, 14, 21 and 28. Physical parameters: No changes were noted between the groups, except that the respiratory rate was lower in the placebo group at day 28. Serum chemistry: Serum chemistries evaluated heart, liver and kidney function and protein balance. The change from baseline was significantly different between the groups for several variables. Iron (day 28 only) and GGT (day 7 only) were greater in the treatment group compared to the placebo group. Calcium (day 14 only), creatinine (day 28 only) and potassium (day 7 only) were higher in the placebo group compared to *L. reuteri* group. Hematology: The change from baseline was significantly different between the groups for the following variables: the percentage of lymphocytes (day 7 and 28) was higher in the *L. reuteri* group and the percentage of neutrophils (day 28 only) was higher in the placebo group. The change from baseline in MCHC at day 28 was significantly higher in the placebo group compared to the treatment group. Urinalysis: The changes from baseline in urinary pH, specific gravity and other qualitative parameters were not different between the groups. Urinary indican excretion decreased at day 7 in the *L. reuteri* group, but no effect was observed at subsequent collections. GI intolerance: Mild flatulence was reported on 2.3 per cent of the study days in the placebo group. The percentage of flatulence in the treatment group was 5.61 (mild), 0.51 (moderate) and 0.51 per cent (severe) of the study days in the *L. reuteri* group. Diarrhea was noted on 0.51 per cent of the study days as severe in *L. reuteri* group but not in the placebo group. Cramping was noted on 0.77 per cent of the study days in the placebo group but not in the *L. reuteri* group. Fecal fat analysis showed not effect of treatments on fat absorption. Cold symptoms and headache were also observed but not treatment related.
Safety Conclusion	Although the changes from baseline in several serum chemistry and hematology variables were statistically different between the two groups, these differences were not considered clinically significant by the authors and all values were within the normal range for healthy male adults during the duration of the study. The authors concluded "healthy adults can be fed *L. reuteri* at 1 x 10^{11} CFU/day with no clinically significant safety or tolerance problems".

TABLE 7 (continued) Clinical Studies of *L. reuteri* in Humans	
Wolf et al. 1998 *Food and Chemical Toxicology* 1998;36:1085-1094 US	
Study design	Randomized, double-blind placebo controlled trial
Subjects	Thirty-nine subjects with HIV (37 males and 2 females) were randomized within each block i.e. antiretroviral therapy (zidovundine) or no therapy based on the assumption that some subjects might be on antiretroviral therapy. One subject in the placebo group and three in the *L. reuteri* group dropped out due to reasons not related to the treatment. Thirty-five subjects completed the entire study. The subjects were asked to maintain their normal diet and avoid alcohol. Placebo: n=20 *L. reuteri*: n=15
Dose	Freeze-dried *L. reuteri* (strain SD2112)10^{10} CFU/day (2 packets each 5 x 10^{9}/day CFU) mixed with beverages such as tap water, milk, orange juice, apple juice, cranberry juice and regular or diet 7-up at temperatures less than 37 °C. The placebo contained all other ingredients except *L. reuteri.*
Duration	21 days of treatment with 14 days washout period
Safety Results	Intake of *L. reuteri* increased the fecal levels of *L. reuteri* on days 7, 14 and 21 compared to the baseline levels. Some individuals in the placebo group also had detectable levels of *L. reuteri* in the feces confirming the indigenous nature of *L. reuteri.* The changes from baseline in the treatment group were compared to the changes from baseline in the placebo group for all variables measured. Serum chemistry, hematology, and urinalysis parameters were measured on the screening day and on days 21 and 35. Physical parameters: No differences in physical parameters (temperature, pulse rate, respiratory rate, SBP, body weight) between the two groups were observed except for an increase in DBP in *L. reuteri* group at day 21. Serum chemistry: No differences in serum chemistries between the two groups, when evaluated for metabolites, renal function and hepatic function. Hematology: No differences in the hematology variables between the two groups except for a significant change from baseline for differential percent lymphocytes in the placebo group at day 35. Immunology profile: No differences in the immunology parameters between the two groups except for an increase in the change from baseline for CD4+ lymphocytes in the placebo group at day 35. Urinalysis: At day 21 the change from baseline for specific gravity was higher in the *L. reuteri* group, but other variables were not different between the groups. A few urine samples showed bacterial growth but these were considered non-significant. GI intolerance: The frequency of flatulence was similar in both groups. More complaints for mild nausea were reported in the *L. reuteri* group. Bowel movements and fecal consistency were similar between the two groups. Respiratory infections did not occur during the study because the subjects were unable to provide a sputum sample.
Safety Conclusion	*L. reuteri* at 1 x 10^{10} CFU/day was well tolerated and may be fed to HIV-positive individuals without any clinically significant safety or tolerance problems. The changes from baseline in hematology, immunology and urinalysis variables were not considered to be clinically significant by the authors.

TABLE 7 (continued) Clinical Studies of *L. reuteri* in Humans	
Shornikova et al. 1997a *Journal of Pediatric Gastroenterology and Nutrition* 1997a;24:399-404 Finland	
Study design	Randomized, double-blind placebo controlled trial.
Subjects	40 well-nourished children between the ages of 6 and 36 months with acute diarrhea of less than seven days duration were recruited. The study was conducted between January 29 and July 3, 1995 and the subjects were recruited from the Department of Pediatrics, Tampere University Hospital. Placebo: n=21 *L. reuteri*: n=19 One subject of the 41 enrolled was dropped because of cross-contamination with the treatments.
Dose	Freeze-dried *L. reuteri* (strain SD 2112)10^{10}- 10^{11} CFU/day was reconstituted in 50-100 ml of fluid. Placebo consisted of nonfat dry milk powder.
Duration	Five days or for the duration of hospitalization if shorter.
Safety Results	Both treatments had similar effects on weight gain, acidosis and electrolyte levels.
Safety Conclusion	*L. reuteri* may be safely used in children with acute rotavirus diarrhea.
Shornikova et al. 1997b *Pediatr Infect Dis J* 1997b;16:1103-7 Finland	
Study design	Randomized, double-blind placebo controlled trial
Subjects	Sixty-six children between the ages of 6 to 36 months with acute diarrhea for < 7 days were recruited from two pediatric infectious disease wards at Tampere University Hospital. Fermented milk products were not allowed during the treatment period.
Dose	Freeze dried *L. reuteri* High dose of *L. reuteri*: 5 x 10^8 to 2.5 x 10^9 CFU/ml (10^{10} CFU/day) (n=21) Low dose of *L. reuteri*: 2.5 x 10^6 to 5 x 10^6 CFU/ml (10^7 CFU/day) (n=20) Placebo (n=25) The capsules were reconstituted in 20-50 ml of infant formula or breast milk.
Duration	5 days
Safety Results	*L. reuteri* treatment significantly decreased the duration of watery diarrhea. There were no differences in weight gain, consumption of oral rehydration solution or electrolyte and acid-base balance between the groups.
Safety Conclusion	*L. reuteri* given daily is safe in children with acute rotavirus diarrhea.

TABLE 7 (continued) Clinical Studies of *L. reuteri* in Humans	
Jacobsen et al. 1999 *Applied and Environmental Microbiology* 1999;65(11):4949-4956 Denmark	
Study design	Double-blind cross-over trial
Subjects	Twelve healthy men were given either a combination of *L. reuteri* DSM 12246 and *L. rhamnosus* 19070-2, or a combination of *L. rhamnosus* LGG, *L. delbrueckii* subsp. *lactis* CHCC 2329, and *L. casei* subsp. *alactus* CHCC3137, or placebo during a three period crossover trial. Subjects were asked to consume their normal diet and abstain from fermented milk products.
Dose	2×10^{10} CFU/strain
Duration	Total duration for each treatment was 18 days with washout period of 17 days after each treatment.
Safety Results	No adverse effects were reported.
Safety Conclusion	Selected spp. of *Lactobacillus* can effectively colonize the gut.
Ruiz-Palacios et al. 1996a *Pediatric Research* 1996a;39(4):1090 (abstract) Mexico	
Study design	Randomized, blinded controlled trial.
Subjects	Children (n=72) between the ages of 12 to 36 months were randomized to four treatment groups. The study consisted of an entry evaluation period, a three week feeding period and a post feeding period.
Dose	Group 1: control feeding with no probiotic Group 2: low (10^6 CFU) probiotic Group 3: medium (10^8 CFU) probiotic Group 4: high (10^{10} CFU) probiotic The probiotic was a blend of *L. reuteri* SD2112, *L. acidophilus* and *B. infantis* added to liquid beverage (PediaSure®).
Duration	3 weeks
Safety Results	No significant differences between the groups were observed for intake, incidence of vomiting, abdominal discomfort, gas and stool characteristics.
Safety Conclusion	Probiotic mixtures containing *L. reuteri* were well tolerated. The probiotic administration resulted in a transient and dose dependent increase in fecal lactobacillus levels.

TABLE 7 (continued) Clinical Studies of *L. reuteri* in Humans	
Ruiz-Palacios et al. 1996b *Pediatric Research* 1996b;39(4):1089 (abstract) Mexico	
Study design	Randomized, blinded controlled trial.
Subjects	Children (n=243) between the ages of 12 to 35 months living in the Mexico city were recruited. *L. reuteri*: n=123 Control: n=120 Four children in the *L. reuteri* group were dropped due to protocol transgressions.
Dose	*L. reuteri* given in beverage
Duration	Fourteen weeks
Safety Results	Higher number of children in the *L. reuteri* group (90/119) were free of diarrhea compared to the control group (77/120, p=0.04). The incidence of diarrhea was significantly lower in the probiotic group (0.27 vs. 0.42 episodes/child, RR=0.59; 95% CI 0.36-0.97, p=0.03) compared to the control group. No adverse effects were reported.
Safety Conclusion	Daily intake of beverage containing L. *reuteri* may be safely consumed by children.

TABLE 7(continued) Clinical Studies of *L. reuteri* in Humans	
Johansson et al. 1993 Applied and Environmental Microbiology *1993;59(1):15-20* Sweden	
Study design	Clinical trial
Subjects	Thirteen healthy volunteers (9 women and 4 men) between the ages of 31 and 56 years. The subjects were not on antibiotic therapy for two months prior and during the study. The subjects were asked to refrain from any lactic acid fermented products before and during the study period. Biopsy samples of the gut were taken for microbial analysis.
Dose	*Lactobacillus reuteri* 108 *Lactobacillus reuteri* 47 Nineteen different strains of *Lactobacillus* were administered to healthy human volunteers. Seventeen strains were isolated from human intestinal mucosa, one from rat intestinal mucosa and one from sourdough. Freeze-dried fermented oatmeal soup was mixed with cold water and 100 ml of this preparation containing 19 strains was given to the subjects for 10 days. The daily intake of each strain was $5x10^8$ CFU. Samples of the upper jejunum and rectum were taken on the day before the treatment, and 1 and 11 days after the termination of the treatment.
Duration	10 days
Safety Results	No adverse effects were reported.
Safety Conclusion	The authors concluded that, "certain *Lactobacillus* strains have the ability to colonize human intestinal mucosa, independent of dietary and physiological differences among individuals".

REFERENCE LIST

V. REFERENCES

Alak, J.I.B., B.W. Wolf, et al. 1997. Effect of *Lactobacillus reuteri* on intestinal resistance to *Cryptosporidium parvum* infection in C57BL/6 female mice rendered immunodeficient by prior infection with LP-BM5, a murine retrovirus (abstract). *Gastroenterology* vol. 110.

Anonymous. 1998. Basics Plus

Axelsson, L., T. Chung, W. Dobrogosz, and S. Lindgren. 1989. Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri. Microbial Ecology Health Disease* 2:131-136.

Blom, H., B.F. Hagen, B.O. Pedersen, A.L. Holck, L. Axelsson, and H. Naes. 1996. Accelerated production of dry fermented sausage. *Meat Sci.* 43:S229-S242

Casas, I.A., Dobrogosz, W.J. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad spectrum protection against disease in humans and animals. Manuscript submitted to *Microbial Ecology in Health and Disease.*

Casas, I.A., A. Shornikova, E. Isolauri, S. Salminen, and T. Vesikari. Colonization of *Lactobacillus reuteri* in the gastrointestinal tract of children. Unpublished abstract.

Chen, C., J. Chen, and S. Lee. 1999. Growth inhibition of glycerol metabolites of *Lactobacillus reuteri* on microorganisms and human cancer cell lines. *J.Chinese Agricultural Chemical Society* 37:117-125.

Chung, T.C., L. Axelsson, S.E. Lindgren, and W.J. Dobrogosz. 1989. In vitro studies on reuterin synthesis by *Lactobacillus reuteri. Microb.Ecol.Health Dis.*

Cogan, T.M. 1996. History and taxonomy of starter cultures. In: Dairy Starter Cultures. T.M. Cogan and J.-P. Accolas, editors. VCH Publishers, New York. 1-23.

De Smet, I., P. De Boever, and W. Verstraete. 1998. Cholesterol lowering in pigs through enhanced bacterial bile salt hydrolase activity. *British Journal of Nutrition* 79:185-194.

Donohue, D.C. and S. Salminen. 1996. Safety of probiotic bacteria. *Asia Pacific J.Clin.Nutr.* 5:25-28.

Edens, F.W., C. Parkhurst, and I.A. Casas. 1991. *Lactobacillus reuteri* and whey reduce salmonella colonization in the ceca of turkey poults. In: Presented at the Southern Poultry Science Society Annual Meeting. January 28-29, 1981, Atlanta, GA. Anonymous1-8.

Fabia, R., A. Rajab, M. Johansson, R. Willen, and W. Andersson. 1993. The effect of exogenous administration of *Lactobacillus reuteri* R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand.J.Gastroenterol.* 28:155-162.

Fuller, R. 1991. Probiotics in human medicine. *Gut* 32:439-442.

Gardiner, G., R.P. Ross, J.K. Collins, G. Fitzgerald, and C. Stanton. 1998. Development of a probiotic cheddar cheese containing human-derived *Lactobacillus paracasei* strains. *Appl.Environ.Microbiol.* 64:2192-2199.

Gorbach, S.L. 1990. Lactic acid bacteria and human health. *Ann.Med.* 22:37-41.

Gorski, D. 1998. Probiotic revolution. *Dairy Foods* 99:41

Hammes, W.P. and P.S. Tichaczek. 1994. The potential of lactic acid bacteria for the production of safe and wholesome food. *Z.Lebensm.Unters.Forsch.* 198:193-201.

Hammes, W.P., C. Hertel, and C. Cavadini. 1995. Safety aspects of genetically modified lactic acid bacteria. In: ACS Symposium Series 605: Genetically Modified Foods. Safety Issues. K. Engel, G.R. Takeoka, and R. Teranishi, editors. American Chemical Society, Washington, DC.

Holzapfel, W.H., R. Geisen, and U. Schillinger. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International J.Food Microbiol.* 24:343-362.

Jacobsen, C., V. Nielsen, A. Hayford, P. Moller, and et al. 1999. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. *Appl.Environ.Microbiol.* 65:4949-1956.

Johansson, M., G. Molin, B. Jeppsson, S. Nobaek, S. Ahrne, and S. Bengmark. 1993. Administration of different *Lactobacillus* strains in fermented oatmeal soup: *In vivo* colonization of human intestinal mucosa and effect on the indigenous flora. *Appl. Environ. Microbiol.* 59:15-20.

Kandler, O. and N. Weiss. 1986. Regular, nonsporing gram-positive rods. In: Bergey's Manual of Systematic Bacteriology (Volume 2). P.H. Sneath, N.S. Mair, M.E. Sharpe, and J.G. Holt, editors. 1208-1234.

Lee, Y.-K. and S. Salminen. 1995. The coming age of probiotics. *Trends in Food Sci.Technol.* 6:241-245.

Marklinder, I. and C. Loenner. 1992. Fermentation properties of intestinal strains of Lactobacillus, of a sourdough and of a yogurt starter culture in an oat-based nutritive solution. *Food Microbiol.* 9:197-205.

Marklinder, I. and C. Loenner. 1994. Fermented oatmeal soup influence of additives on the properties of a nutrient solution for enteral feeding. *Food Microbiol.* 11:505-513.

Molin, G., R. Andersson, S. Ahrne, C. Lonner, I. Marklinder, M. Johansson, B. Jeppsson, and S. Bengmark. 1992. Effect of fermented oatmeal soup on the cholesterol level and the *Lactobacillus* colonization of rat intestinal mucosa. *Antonie van Leeuwenhoek* 61:167-173.

Okada, S., M. Ishikawa, I. Yoshida, T. Uchimura, N. Ohara, and M. Kozaki. 1992. Identification and characteristics of lactic acid bacteria isolated from sour dough songes. *Biosci. Biotech. Biochem.* 56:572-575.

Piva, A. and L. Morelli. 1997. Role of swine isolated *Lactobacillus reuteri* and *L. acidophilus* on the microbial ecology and fermentation pattern in the gastrointestinal tract of the adult pig. *Zoot.Nutr.Anim.* 23:147-155.

Rafter, J.J. 1995. The role of lactic acid bacteria in colon cancer prevention. *Scand. J. Gastroenterol.* 30:497-502.

Ratcliffe, B., C.B. Cole, and R. Fuller. 1986. The effect of yoghurt and milk fermented with a porcine intestinal strain of *Lactobacillus reuteri* on the performance and gastrointestinal flora of pigs weaned at two days of age. *Food Microbiol.* 3:203-211.

Ruiz-Palacios, G., F. Tuz, F. Arteaga, M. Guerrero, M. Dohnalek, and M. Hilty. 1996a. Tolerance and fecal colonization with *Lactobacillus reuteri* in children fed a beverage with a mixture of *Lactobacillus* spp. *Pediatr.Res.* 39: Abstract no. 1090.

Ruiz-Palacios, G., M. Guerrero, M. Hilty, M. Dohnalek, P. Newton, J. Calva, T. Costigan, F. Tuz, and F. Arteaga. 1996b. Feeding of a probiotic for the prevention of community-acquired diarrhea in young Mexican children. *Pediatr.Res.* 39:Abstract no. 1089.

Salminen, S. 1996a. Functional dairy foods with *Lactobacillus* strain GG. *Nutr.Rev.* 54:S99-S101

Salminen, S., E. Isolauri, and E. Salminen. 1996b. Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. *Antonie van Leeuwenhoek* 70:347-358.

Saloff-Coste, C.J. Kefir. *Danone World Newsletter* 11, April 1996.

Sanders, M.E. and J.H.J. Huis in't Veld. 1999. Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. *Antonie van Leeuwenhoek* 76:293-315.

Saxelin, M., N.-H. Chuang, B. Chassy, H. Rautelin, P.H. Makela, S. Salminen, and S.L. Gorbach. 1996a. Lactobacilli and bacteremia in Southern Finland, 1989-1992. *Clin. Infect. Dis.* 22:566

Saxelin, M., H. Rautelin, S. Salminen, and P.H. Makela. 1996b. Safety of commercial products with viable *Lactobacillus* strains. *Infect.Dis.Clin.Prac.* 5:331-335.

Scheinbach, S. 1998. Probiotics: Functionality and commercial status. *Biotechnol.Adv.* 16:581-608.

Shornikova, A., I.A. Casas, E. Isolauri, H. Mykkanen, and T. Vesikari. 1997a. *Lactobacillus reuteri* as a therapeutic agent in acute diarrhea in young children. *J.Ped.Gastro.Nutri.* 24:399-404.

Shornikova, A., I.A. Casas, H. Mykkanen, E. Salo, and T. Vesikari. 1997b. Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. *Pediatr.Infect.Dis.J.* 16:1103-1107.

Stiles, M.E. 1996. Biopreservation by lactic acid bacteria. *Antonie van Leeuwenhoek* 70:331-345.

Talarico, T. and W. Dobrogosz. 1989. Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. *Antimicrobial Agents Chemotherapy* 33:674-679.

Wolf, B.W., K. Garleb, D. Ataya, and I.A. Casas. 1995. Safety and tolerance of *Lactobacillus reuteri* in healthy adults male subjects. *Microbial Ecology Health Disease* 8:41-50.

Wolf, B.W., K. Wheeler, D. Ataya, and K. Garleb. 1998. Safety and tolerance of *Lactobacillus reuteri* supplementation to a population infected with the human immunodeficiency virus. *Food Chem.Toxicol.* 36:1085-1094.