EXHIBIT A - EXECUTIVE SUMMARY

SUMMARY STATEMENT

EXPERT PANEL REGARDING THE "GENERAL RECOGNITION" OF BIFIDOBACTERIUM, LACTOBACILLUS AND *STREPTOCOCCUS THERMOPHILUS* FOR USE IN INFANT FORMULA

Members of the Expert Panel were requested by Nestlé U.S.A. to independently review the safety of two genera and one species of bacteria for addition to infant formula. The training and experience of members of the Expert Panel is provided in Attachment 1.

To assist in this review, original scientific articles were provided to the Panel. A list of these articles is contained in Attachment 2.

Following review of the above articles and of other information considered relevant by individual members of the Expert Panel, a meeting was held in Washington, D.C. on April 18-19, 1996 at the law offices of King & Spalding, 1730 Pennsylvania Avenue, N.W. The focus throughout the discussions was on the safety for use in infants and children of formulas containing *Bifidobacterium*, *Lactobacillus* and *Streptococcus thermophilus*. Scientific literature was critiqued and discussion ensued regarding *Bifidobacterium*, *Lactobacillus*, and *Streptococcus thermophilus* and the following issues were addressed:

a) history and taxonomy;

b) prior use in foods;

c) infant's and young child's evolving intestinal microflora;

d) potential host growth and nutritional consequences; and

e) adverse effects; in the areas of allergenic potential, virulence and toxicity.

The Expert Panel has come to a consensus on the following points, whereas:

I. Addition to infant formula of bacterial strains of two genera, namely,

Bifidobacterium and *Lactobacillus*, and strains of one species, *Streptococcus thermophilus* result in a "new" infant formula for the purpose of improving the overall wholesomeness of food for infants and children. Such formulas are not "new" in Europe and Asia where they have been used for decades.

II. The use of these ingredients in infant formula was not contemplated when the "functionality" categories delineated in 21 CFR § 170.3 were developed; nevertheless, arguments can be made that the following categories may apply: synergist, antimicrobial agent, enzyme, and nutrient supplement.

III. The literature and evolving science support the rationale for adding these organisms to infant formula in light of:

a) the ontogeny of infant intestinal microflora;

b) the developmental expression of intestinal carbohydrate enzyme activity in the infant and child, and

c) the mechanisms for the prevention of common infectious diarrheal agents.

IV. Similarly, the literature and evolving science support the value of these uses of *Bifidobacterium, Lactobacillus,* and *Streptococcus thermophilus* in infant formula. Although at this time definitive conclusions about their efficacy for specific indications cannot be reached, assessments of the safety and GRAS status of these organisms can be appropriately made.

V. Concerning the safety and GRAS status of *Bifidobacterium, Lactobacillus,* and *Streptococcus thermophilus* for addition to infant formula:

Bifidobacterium: Organisms of this genus, with the exception of *B. dentium*, may be viewed as safe and as GRAS on the basis of available scientific data. *B. dentium* is

an identified agent of dental carries and, accordingly, should not be used in infant formula. Note was taken of the recent study of Moore, W. E. C. and Moore, L. H., Appl. Environ. Microbiol. 1995; 61:3202-3207, in which certain *Bifidobacterium* species were identified as being associated with populations at risk of colonic cancer. The panel's consensus was that neither the data presented nor the scientific issues posed by this publication are of sufficient strength to warrant concern.

Lactobacillus: No reservations concerning the safety or GRAS status of members of this genus were raised by the Panel. In reaching this conclusion, the Panel considered the position taken by the European Union sponsored workshop organized by the *Lactobacillus* Industrial Platform (LABIP) concerning the role of *L. rhamnosus, L. casei* (*L. paracasei*), and *L. plantarum* in opportunistic infections in compromised hosts (Adams, M.R., Martue, P., Int. J. Food Microbiol. 1995; 27: 263-264).

Streptococcus thermophilus: No reservations were raised about the safety or GRAS status of this species.

VI. Unlike the case with normal newborns and infants, insufficient information is available to assess the use and safety of such infant formulas fed to severely immunocompromised infants and young children (e.g., those suffering from AIDS or SCIDS and those receiving chemotherapy or radiation such that entry into the systemic compartment might reasonably occur). Information from studies on the risks or possible benefits of such preparations of foods in these few groups is too limited at this time to draw definitive conclusions and, thus, any such use should be under medical supervision.

CONCLUSIONS

Assuming that current good manufacturing practice, including appropriate stability assessment, is followed in the production of the contemplated finished infant formulas:

In our opinion the addition of *Bifidobacterium* (with the exception of *B. dentium*), *Lactobacillus,* and *Streptococcus thermophilus* to infant formula is safe;

Moreover, in our opinion, experts qualified by adequate training and experience, would generally recognize on the basis of the available scientific information ("scientific procedures") *Bifidobacterium* (with the exception of *B. dentium*), *Lactobacillus*, and *Streptococcus thermophilus* as safe for addition to infant formula.

Each of the foregoing conclusions incorporates the limitation noted in paragraph VI above.

TABLES 1 - 13

TABLE-1: CHRONOLOGICAL DISTRIBUTION OF HUMAN CLINICAL TRIALS WITH PROBIOTIC LAB

Years	Single strain		Mixed strains		Total	
	Trials	Subjects	Trials	Subjects	Trials	Subjects
1961-70	5	309	2	95	7	404
1971-80	5	280	7	441	12	721
1981-90	26	1,874	20	1,205	45	3,079
1991-98	61	2,543	24	1,116	85	3,659
Total	**97**	**5,005**	**53**	**2,857**	**150**	**7,863**

TABLE-2: DISTRIBUTION OF HUMAN CLINICAL TRIALS WITH PROBIOTIC LAB BASED ON SUBJECTS AND REGIMEN

Group	Prophylactic Regimen		Therapeutic Regimen		Total	
	Trials	Subjects	Trials	Subjects	Trials	Subjects
Infants	12	532	9	430	21	962
Children	9	697	30	1,520	39	2,217
Adults	43	2,535	47	2,149	90	4,684
Total	**64**	**3,764**	**86**	**4,099**	**150**	**7,863**

TABLE 3a. HUMAN CLINICAL TRIALS WITH PROBIOTICS – EFFICACY, SAFETY AND TOLERANCE (1961-1986)

Subjects	(n =)	LAB Culture (Dosage)	Goal	Effect	Adverse Event	Reference
Adult patients	59	*L. acidophilus*	Therapeutic for diarrhea	(+)	None	Beck & Necheles (1961)
Pediatric patients	45	*L .bulgaricus / S. thermophilus*	Therapeutic for infantile diarrhea	(+)	None	Niv *et al.* (1963)
Adult patients	40	*L. acidophilus/ L. bulgaricus*	Therapeutic for oral ulcerations	4+	None	Rapoport & Levine (1965)
Pediatric patients	150	*B. bifidum*	Therapeutic for *E. coli* diarrhea	(+)	None	Schneegans *et al.* (1966)
Adult patients	18	Bifidobacteria *sp.*	Therapeutic for chronic liver disease		None	Muting *et al.* (1968)
Pediatric patients	2	Bifidobacteria *sp.*	Therapeutic for chronic liver disease		None	Muting *et al.* (1968)
Adult patients	80	*L. acidophilus*	Therapeutic for oral ulcerations	0	None	Gertenrich & Hart (1970)
Pediatric patients	94	*S. thermophilus / L. acidophilus L. bulgaricus*	Therapeutic for diarrhea	0	None	Pearce & Hamilton (1974)
Healthy adults	24	LAB cultures	Hypocholesterolemic effect	(+)	None	Mann (1974)
Adult patients	17	LAB culture (Lactovagil)	Therapeutic for vaginitis	(+)	None	Vazquez & Rivera (1976)
Adult patients	5	*Lactobacillus sp.*	Therapeutic for neutropenia		None	Hensgens & Klasterski (1976)
Healthy adults	50	*Lactobacillus sp.* (3-6 x 10^8)	Prophylactic for travelers diarrhea	0	None	Pozo-Olano *et al.* (1978)
Healthy adults	54	LAB cultures	Hypocholesterolemic effect	(+)	None	Hepner *et al.* (1979)
Adult patients	98	*L. acidophilus / L. bulgaricus*	Prophylactic for AB-induced diarrhea	2+	None	Gotz *et al.* (1979)
Adult patients	150	*L. sporogenes* (6 x 10^7)	Therapeutic for oral ulcerations	3+	None	Sharma *et al.* (1980)
Adult patients	50	*L. acidophilus / B. bifidum*	Therapeutic for enterocolitis		None	Satta *et al.* (1980)
Healthy adults	7	*L. acidophilus* (10^{10} cfu/ml)	Elimination of procarcinogens	(+)	None	Goldin *et al.* (1980)
Infants	104	*S. faecium SF68 / L. lactis L. bulgaricus / L .acidophilus*	Therapeutic for infantile diarrhea	3+	None	Bellomo *et al.* (1980)
Pediatric patients	68	*Lactobacillus sp.*	Therapeutic for neutropenia		None	Ekert *et al.*, (1980)
Healthy adults	32	LAB cultures	Hypocholesterolemic effect	0	None	Rossouw *et al.* (1981)
Adult patients	49	*S. faecium SF68*	Therapeutic for enteritis	(+)	None	Bruno & Grigerio (1981)
Healthy adults	11	*L. acidophilus / L. bulgaricus*	Tolerance study	0	None	Robins-Browne & Levine (1981)
Healthy adults	23	*L. acidophilus / L. bulgaricus*	Prophylactic for ETEC diarrhea	0	None	Clements *et al.* (1981)
Healthy adults	48	*L. acidophilus / L. bulgaricus*	Prophylactic for ETEC diarrhea	0	None	Clements *et al.* (1981)
Healthy adults	68	LAB cultures	Hypocholesterolemic effect	0	None	Thompson *et al.* (1982)
Adult patients	100	*L. bulgaricus LB51*	Antitumor activity		None	Bogdanov (1982)
Adult patients	12	*L. acidophilus*	Alleviation of lactose intolerance	0	None	Kim & Gilliland (1983)
Healthy adults	23	*L. acidophilus / L. bulgaricus*	Prophylactic for ETEC diarrhea	(+)	None	Clements *et al.* (1983)
Adult patients	36	*L. acidophilus / L. bulgaricus*	Therapeutic for ETEC diarrhea		None	Clements *et al.* (1983)
Healthy adults	16	*B. breve*	Prophylactic study		None	Tanaka *et al.* (1983)
Healthy adults	21	LAB cultures	Hypocholesterolemic effect	(+)	None	Bazzare *et al.* (1983)
Adult patients	61	*L. acidophilus* (4 x 10^6 /ml)	Therapy for Irritable bowel syndrome	0	None	Newcomer *et al.* (1983)
Adult patients	18	*L. acidophilus* (4 x 10^6 /ml)	Therapy for Lactase deficiency	0	None	Newcomer *et al.* (1983)
Healthy adults	10	*L. acidophilus* (4 x 10^6 /ml)	Control group	0	None	Newcomer *et al.* (1983)
Healthy adults	10	*L. bulgaricus / L. thermophilus*	Hypocholesterolemic effect	0	None	Jaspers *et al.* (1984)
Adult patients	10	LAB culture	Alleviation of lactose intolerance	(+)	None	Kolars *et al.* (1984)
Adult patients	9	*L. acidophilus*	Alleviation of lactose intolerance	(+)	None	Savaiano *et al.* (1984)
Healthy adults	21	*L. acidophilus* (10^9 cfu/day)	Elimination of procarcinogens	(+)	None	Goldin & Gorbach (1984)
Infants	30	*L. acidophilus* (5 x 10^{10})	Prophylactic study	0	None	Reuman *et al.* (1986)
Healthy adults	6	*L. acidophilus* (10^8 cfu/g)	Tolerance study	(+)	None	Prajapati *et al.* (1986)

Efficacy scores: 0 = No effect; (+) = Undefined effect; 1+ = <25% efficacy; 2+ = 26-50% efficacy; 3+ = 51-75% efficacy; 4+ = >75% efficacy

TABLE 3b. HUMAN CLINICAL TRIALS WITH PROBIOTICS – EFFICACY, SAFETY AND TOLERANCE (1987-1991)

Subjects	(n =)	LAB Culture (Dosage)	Goal	Effect	Adverse Event	Reference
Pediatric patients	15	*B. breve / L. casei* (10^9/g)	Therapeutic for Intractable diarrhea	(+)	None	Hotta *et al.* (1987)
Pediatric patients	50	LAB culture	Therapeutic for diarrhea	(+)	None	Singh (1987)
Pediatric patients	133	*B. breve*	Therapeutic for *Campylobacter* enteritis	(+)	None	Tojyo *et al.* (1987)
Pediatric patients	9	LAB culture	Therapeutic for diarrhea	(+)	None	Dewit *et al.* (1987)
Adult patients	5	*L. casei GG* (10^{10})	Therapeutic for colitis with *C. difficile*	(+)	None	Gorbach *et al.* (1987)
Healthy adults	10	*B. longum* in yogurt	Prophylactic for AB-induced diarrhea	(+)	None	Colombel *et al.* (1987)
Adult patients	40	*Streptococcus faecium SF68*	Therapeutic for hepatic encephalopathy		None	Loguercio *et al.* (1987)
Adult patients	14	*L. acidophilus*	Alleviation of lactose intolerance	(+)	None	McDonough *et al.* (1987)
Adult patients	24	*L. acidophilus* (2 x 10^9)	Against radiation-induced GI disorder	(+)	None	Salminen *et al.* (1988)
Adult patients	8	LAB culture	Alleviation of lactose intolerance	(+)	None	Wytock & DiPalma (1988)
Healthy adults	94	*L. acidophilus / B. bifidum* *L. bulgaricus / S. thermophilus*	Prophylactic for travelers diarrhea	(+)	None	Black *et al.* (1989)
Adult patients	45	*Streptococcus faecium SF68*	Therapeutic for AB-induced diarrhea	2+	None	Wunderlich *et al.* (1989)
Adult patients	78	*Streptococcus faecium SF68*	Therapeutic for acute diarrhea		None	Wunderlich *et al.* (1989)
Healthy adults	334	*L. acidophilus / L. bulgaricus*	Hypocholesterolemic effect	0	None	Lin *et al.* (1989)
Cancer Patients	61	*L. casei*	Protection against leukopenia		None	Okawa *et al.* (1989)
Children	82	*L. helvaticus / S. thermophilus*	Prophylactic for Infantile diarrhea	(+)	None	Brunser *et al.* (1989)
Healthy adults	16	*L. casei GG*	Prophylactic for AB-induced diarrhea	3+	None	Siitonen *et al.* (1990)
Adult patients	183	*Streptococcus faecium SF68*	Therapeutic for ETEC & Vibrio cholera	0	None	Mitra & Rabbani (1990)
Healthy adults	820	*L. casei GG*	Prophylactic for traveler's diarrhea	1+	None	Oksanen *et al.* (1990)
Infants	24	LAB culture	Therapeutic for a specific diarrhea		None	Roggero *et al.* (1990)
Infants	54	LAB culture	Therapeutic for infantile diarrhea	(+)	None	Beau *et al.* (1990)
Children	245	Heat killed LAB mixture	Prophylactic for dental caries		None	Bayona-G *et al.* (1990)
Pediatric patients	52	*S. thermophilus / L. bulgaricus*	Therapeutic for persistent diarrhea	(+)	None	Boudraa *et al.* (1990)
Children	60	*L. acidophilus / L. bulgaricus*	Prophylactic for AB-induced diarrhea	0	None	Tankanow *et al.* (1990)
Healthy adults	9	*L. acidophilus* (10^7 cfu/g) *B. bifidum* (10^8 cfu/g)	Elimination of procarcinogens	(+)	None	Marteau *et al.* (1990a)
Pediatric patients	71	*L. casei GG* (10^{10-11} cfu)	Therapeutic for Rota viral diarrhea	(+)	None	Isolauri *et al.* (1991)
Healthy adults	12	*Bifidobacterium sp.*	Tolerance study	0	None	Berrada (1991)
Healthy adults	10	*Bifidobacterium sp.*	Tolerance study	0	None	Tomoda *et al.* (1991)
Healthy adults	20	*L. acidophilus / B. bifidum*	Prophylactic for AB-induced diarrhea	(+)	None	Black *et al.* (1991)
Healthy adults	24	LAB strains	Hypocholesterolemic effect	0	None	Halpern *et al.* (1991)
Adult patients	10	*L. acidophilus* (10^8 cfu/g)	Alleviation of lactose intolerance	(+)	None	Lin *et al.* (1991)
Adult patients	21	*L. acidophilus / L. bulgaricus*	Alleviation of lactose intolerance	(+)	None	Martini *et al.* (1991a)
Adult patients	22	LAB strains	Alleviation of lactose intolerance	(+)	None	Martini *et al.* (1991b)
Infants	40	LAB strain	Therapeutic for AB-induced diarrhea		None	Contardi (1991)

Efficacy scores: 0 = No effect; (+) = Undefined effect; 1+ = <25% efficacy; 2+ = 26-50% efficacy; 3+ = 51-75% efficacy; 4+ = >75% efficacy

TABLE 3c. HUMAN CLINICAL TRIALS WITH PROBIOTICS – EFFICACY, SAFETY AND TOLERANCE (1992-1994)

Subjects	(n =)	LAB Culture (Dosage)	Goal	Effect	Adverse Event	Reference
Infants	78	*L. bulgaricus / S. thermophilus*	Therapeutic for infantile diarrhea		None	Touhami *et al.* (1992)
Adult patients	24	*L. acidophilus / B. bifidum*	Therapeutic for constipation	(+)	None	Alm *et al.* (1992)
Healthy adults	11	*L. acidophilus*	Reduction in mutagen excretion	(+)	None	Lidbeck *et al.* (1992)
Healthy adults	12	L. casei GG	Improved bowel function	0	None	Ling *et al.* (1992)
Infants	11	*B. longum / B. breve* *L. acidophilus*	Adjunct to AB-treatment	0	None	Bennet *et al.* (1992)
Healthy adults	5	*B. longum* (2-5 x 10^9 cfu/g)	Improved fecal microflora	(+)	None	Benno & Mitsuoka (1992)
Healthy adults	76	*L. casei GG*	Tolerance study	0	None	Goldin *et al.* (1992)
Adult patients	30	*L. acidophilus / Bifidobacterium*	Infection prophylaxis in neutropenia	0	None	Ellegaard *et al.* (1992)
Pediatric patients	40	*L. acidophilus*	Therapeutic for diarrhea		None	Balli *et al.* (1992)
Pediatric patients	39	*L. casei GG* (10^{10-11} cfu)	Therapeutic for acute diarrhea	2+	None	Kaila *et al.* (1992)
Cancer patients	58	*Lactobacillus sp.* (Biolactis)	Prophylaxis for recurrence		None	Aso & Akazan (1992)
Adult patients	33	*L. acidophilus*	Prophylaxis for Candidal vaginosis	4+	None	Hilton *et al.* (1992)
Healthy adults	15	*L. acidophilus / B.bifidum*	Modulation of immune responses	(+)	None	De Simone *et al.* (1992)
Healthy adults	98	LAB culture	Decrease in allergic symptoms	(+)	None	Trapp *et al.* (1993)
Infants	20	*L. casei GG* (2 x 10^8 cfu/day)	Prophylaxis for enteropathogens	0	None	Millar *et al.* (1993)
Healthy adults	44	*L. casei GG*	Dose response study	0	None	Saxelin *et al.* (1993)
Cancer patients	228	*L. casei LC9018*	Adjuvant immunotherapy		None	Okawa *et al.* (1993)
Healthy adults	6	*L. casei*	Antimutagenic effect		None	Hayatsu & Hayatsu (1993)
Infants	20	*L. casei GG*	Tolerance study	0	None	Stansbridge *et al.* (1993)
Adult patients	9	LAB culture (4.2 x 10^{10} cells)	Reduce lactose malabsorption	0	None	Hove *et al.* (1994)
Healthy adults	6	LAB culture	Improved nitrogen/electrolyte mobility	3+	None	Mahé *et al.* (1994)
Pediatric patients	51	LAB culture	Therapeutic for persistent diarrhea	3+	None	Bhutta *et al.* (1994)
Adult patients	64	*L. casei GG*	Regulation of intestinal enzymes	2+	None	Ling *et al.* (1994)
Healthy adults	60	*L. casei GG* (10^{11} cfu/L)	Decrease in reductive enzymes	(+)	None	Ling *et al.* (1994)
Adult patients	47	*L. casei ssp. Rhamnosus*	Reduced reinfection during cystitis		None	Baerheim *et al.* (1994)
Pediatric patients	42	*L. casei GG* (10^{11} cfu/day)	Therapeutic for Rota viral diarrhea	4+	None	Isolauri *et al.* (1994)
Healthy adults	30	*L. acidophilus / B. bifidum*	Modulation of immune responses	4+	None	Link-Amster *et al.* (1994)
Infants	55	*B. bifidum / S. thermophilus*	Prophylaxis for Rota viral diarrhea	(+)	None	Saavedra *et al.* (1994)

Efficacy scores: 0 = No effect; (+) = Undefined effect; 1+ = <25% efficacy; 2+ = 26-50% efficacy; 3+ = 51-75% efficacy; 4+ = >75% efficacy

TABLE 3d. HUMAN CLINICAL TRIALS WITH PROBIOTICS – EFFICACY, SAFETY AND TOLERANCE (1995-1997)

Subjects	(n =)	LAB Culture (Dosage)	Goal	Effect	Adverse Event	Reference
Healthy adults	28	*L. acidophilus / B. bifidum*	Modulation of immune responses	3+	None	Schiffrin *et al.* (1995)
Adult patient	10	*S. thermophilus / L. gasseri*	Improve intestinal microecology	(+)	None	Pedrosa *et al.* (1995)
Healthy adults	23	*L. bulgaricus*	Improve intestinal microecology	(+)	None	Pedrosa *et al.* (1995)
Healthy adults	282	*L. fermentum* (10^{11} *cfu*) *L. acidophilus* (10^{11} *cfu*)	Prophylaxis for travelers diarrhea	0	None	Katelaris *et al.* (1995)
Children	24	*L. casei GG* (5 x 10^8 / dose)	Tolerance study	(+)	None	Sheen *et al.* (1995)
Pediatric patients	23	*L. bulgaricus / S. thermophilus* (1.6 x 10^8 cfu /g)	Alleviation of lactose malabsorption	(+)	None	Shermak *et al.* (1995)
Pediatric patients	20	*L. acidophilus* (10^{10} cells) OR *L. lactis / S. thermophilus*	Alleviation of lactose malabsorption	4+	None	Montes *et al.* (1995)
Infants	20	*B. bifidum* (10^6 cfu/g)	Tolerance study	(+)	None	Langhendries *et al.* (1995)
Pediatric patients	12	*L. casei GG* (viable)	Therapeutic for Rota viral diarrhea	4+	None	Kaila *et al.* (1995)
Pediatric patients	13	*L. casei GG* (heat-killed)	Therapeutic for Rota viral diarrhea	1+	None	Kaila *et al.* (1995)
Infants	40	*L. casei GG*	Therapeutic for infantile diarrhea	4+	None	Raza *et al.* (1995)
Healthy adults	30	*L. reuteri* (10^{11} *cfu/day*)	Tolerance study	0	None	Wolf *et al.* (1995)
Adult patients	138	*L. casei LC9018*	Prophylaxis for recurrence		None	Aso *et al.*, (1995)
Adult patients	30	*L. acidophilus*	Alleviation of lactose intolerance	(+)	None	Gaon *et al.* (1995)
Healthy adults	20	*L. casei GG*	Tolerance study	0	None	Saxelin *et al.* (1995)
Pediatric patients	16	*L. casei GG* (5 x 10^9)	Therapeutic for acute diarrhea	(+)	None	Majamaa *et al.* (1995)
Pediatric patients	14	*L. casei / L. rhamnosus* (2 x 10^8)	Therapeutic for acute diarrhea	(+)	None	Majamaa *et al.* (1995)
Pediatric patients	19	*S. thermophilus / L. delbrucki* *L. casei* (2.8 x 10^9)	Therapeutic for acute diarrhea	(+)	None	Majamaa *et al.* (1995)
Infants	55	*L. casei GG*	Immunostimulating effect of oral vaccine	4+	None	Isolauri *et al.* (1995)
Infants	28	*L. casei GG*	Modulation of immune responses	4+	None	Isolauri *et al.* (1995)
Pediatric patients	4	*L. casei* GG	Therapeutic for *C. difficile* diarrhea	4+	None	Biller *et al.* (1995)
Pediatric patients	31	*L. casei* GG (10^{10}-10^{11} cfu/d)	Therapeutic for Shigellosis	4+	None	Sepp *et al.* (1995)
Adult patients	28	*L. casei* GG (10^9 cfu/d)	Therapeutic for vaginitis	4+	None	Hilton *et al.* (1995)
Infants	17	*Bifidobacterium* sp. (10^6 cfu/g)	Tolerance study	0	None	Kok *et al.* (1996)
Children	72	*L. reuteri / L. acidophilus* *B. infantis*	Prophylactic		None	Ruiz-Palacios *et al.* (1996)
Infants	39	*L. casei GG*	Therapeutic for infantile diarrhea	0	None	Pant *et al.* (1996)
Adult patients	32	*L. casei GG*	Therapeutic for *C. difficile* diarrhea	4+	None	Bennet *et al.* (1996)
Pediatric patients	63	*L. acidophilus*	Therapeutic for acute diarrhea		None	Michielutti *et al.* (1996)
Adult patients	32	*L. acidophilus*	Therapeutic for bacterial vaginosis		None	Parent *et al.* (1996)
Adult patients	6	*L. acidophilus*	Antimutagenic effect		None	Simenhoff *et al.* (1996)
Healthy adults	6	*L. acidophilus LA-2*	Antimutagenic effect		None	Hosoda *et al.* (1996)
Adult patients	15	*B. longum*	Alleviation of lactose intolerance	(+)	None	Jiang *et al.* (1996)
Pediatric patients	30	*L. casei GG*	Modulation of immune response	(+)	None	Malin *et al.* (1996)
Adult patients	14	*L. casei GG*	Protection against dietary antigens	(+)	None	Sütas *et al.* (1996)
Adult patients	28	*L. casei GG*	Management of juvenile chronic arthritis	(+)	None	Malin *et al.* (1996)
Infants	27	*L. casei GG*	Management of food allergy	(+)	None	Majamaa & Isolauri (1997)
Infants	40	*L. reuteri* (10^{11} *cfu/day*)	Therapeutic for acute viral diarrhea	3+	None	Shornikova *et al.* (1997)
Pediatric patients	100	*L. casei GG*	Therapeutic for acute viral diarrhea	(+)	None	Guarino *et al.* (1997)
Children	104	*Bifidobacterium* Bb12 + *S. thermophilus*	Intestinal colonization study	4+	None	van't Hof & Haschke (1997)

Efficacy scores: 0 = No effect; (+) = Undefined effect; 1+ = <25% efficacy; 2+ = 26-50% efficacy; 3+ = 51-75% efficacy; 4+ = >75% efficacy

TABLE 3e. HUMAN CLINICAL TRIALS WITH PROBIOTICS – EFFICACY, SAFETY AND TOLERANCE (1998)

Subjects	(n =)	LAB Culture (Dosage)	Goal	Effect	Adverse Event	Reference
Infants	39	*L. casei*	Tolerance study	(+)	None	Guerin-Danan *et al.* (1998)
Children	71	*L. plantarum 299V*	Prophylactic for diarrheal illness	2+	None	Ribeiro Jr. & Vanderhoof (1998)
Pediatric patients	3	*L. casei GG*	Therapeutic for *C. difficile* colitis	4+	None	Loret de Mola *et al.* (1998)
Pediatric patients	24	*L. casei GG*	Therapeutic for cystic fibrosis	(+)	None	Di Benedetto *et al.* (1998)
Infants	90	*B. bifidum*	Prophylactic for Infantile diarrhea	(+)	None	Chouraqui *et al.* (1998)
Pediatric patients	287	*L. casei GG*	ORS-therapeutic for acute diarrhea	0	None	Guandalini, et al. (1998)
Children	19	*Bifidobacterium* Bb12 (10^8 cfu/g)	Safety study	4+	None	Haschke *et al.* (1998)
Children	20	*Bifidobacterium* Bb12 (10^8 cfu/g) + *S. thermophilus*	Safety study	4+	None	Haschke *et al.* (1998)
Infants	131	*Bifidobacterium* Bb12 + *S. thermophilus* (10^6/10^7 cfu/g)	Safety and Tolerance	4+	None	Abi-Hanna *et al.* (1998)

Efficacy scores: 0 = No effect; (+) = Undefined effect; 1+ = <25% efficacy; 2+ = 26-50% efficacy; 3+ = 51-75% efficacy; 4+ = >75% efficacy

TABLE-4: PROBIOTIC LAB USED AS SINGLE OR MIXED CULTURES IN HUMAN CLINICAL TRIALS

Probiotic Species	Single strain		Mixed strains		Total	
	Trials	Subjects	Trials	Subjects	Trials	Subjects
Lactobacillus acidophilus	22	582	24	1,577	46	2,159
Lactobacillus casei	39	2,629	1	19	40	2,648
Lactobacillus bulgaricus	0	0	20	1,327	20	1,327
Lactobacillus reuteri	2	70	1	72	4	142
Lactobacillus sp.	3	123	0	0	3	123
Lactobacillus lactis	0	0	2	124	2	124
Lactobacillus fermentum	0	0	1	282	1	282
Lactobacillus sporogenes	1	150	0	0	1	150
Lactobacillus plantarum	1	71	0	0	1	71
Lactobacillus helveticus	0	0	1	82	1	82
Lactobacillus delbruckii	0	0	1	19	1	19
Lactobacillus gasseri	0	0	1	33	1	33
LAB strains (undefined)	0	0	18	621	18	621
Streptococcus thermophilus	0	0	12	605	12	605
Streptococcus faecium	5	395	1	104	5	499
Bifidobacterium bifidum	2	240	10	345	12	585
Bifidobacterium breve	3	164	1	11	4	175
Bifidobacterium longum	3	30	1	11	4	41
Bifidobacterium animalis	0	0	1	72	1	72
Bifidobacterium infantis	0	0	1	72	1	72
Bifidobacterium sp.	5	69	1	30	6	99

TABLE-5: FACTORS PROMOTING GROWTH OF BIFIDOBACTERIA IN HUMAN GUT

Bifidogen factor	Source	Reference
N-acetyl-lactosamine	Porcine gastric mucin	Zilliken *et al.* (1954)
β-ethyl-N-acetyl-D-glucosamine	Aminosugar-based	Lambert & Zilliken (1965)
N-carboehoxy-D-glucosamine	Aminosugar-based	Lambert & Zilliken (1965)
N-acetylmannosamine	Aminosugar-based	Jao (1974)
N-acetylneuraminic acid	Aminosugar-based	Jao *et al.* (1978)
Polyfructans	*Helianthus tuberosus*	Fleming & Groot-Wassink (1979)
N-aacetylgalactosamine	Aminosugar-based	Kawase *et al.* (1983)
N-acetylglucosamine	Aminosugar-based	Rasic & Kurmann (1983)
Lacto-N-difucodecaose	Human milk	Rasic & Kurmann (1983)
N-N-diacetylchitobiose	Chitin	Rasic & Kurmann (1983)
1-kestose (GF2)	Fructooligosaccharide (FOS)	Hidaka *et al.* (1984)
Nytose (GF3)	Fructooligosaccharide (FOS)	Hidaka *et al.* (1984)
1^F-β-fructose-furanosyl nystose (GF5)	Fructooligosaccharide (FOS)	Hidaka *et al.* (1984)
4-O-β-D-galactopyranosyl-D-fructose	Lactulose	Mizota *et al.* (1987)
Lactitol	Sugar alcohol from lactose	Harju (1988)
α-lactalbumin	Human milk	Petschow & Talbott (1991a)
Lactoferrin	Human milk	Petschow & Talbott (1991)
Lacto-N-tetraose	Human milk	Kunz & Rudloff (1996)
Lacto-N-fucopentose	Human milk	Kunz & Rudloff (1996)
2-amino-3-carboxy-1,4-naphthoquinone	*Propionibacterium freudenreichii*	Mori *et al.* (1997)
Transgalacto-oligosaccharides	Glucose and galactose	Bouhnik *et al.* (1997)
β-glucooligomers	Hydrolysis of β-glucan and xylan	Jaskari *et al.* (1998)
xylooligomers	Hydrolysis of β-glucan and xylan	Jaskari *et al.* (1998)
Chicory inulin	*Cichorium intybus*	Roberfroid *et al.* (1998)

TABLE-6: LAB – TYPES OF BACTERIOCIN PRODUCTION

LAB Strain	Bacteriocin	Genetic Determinant Size			Reference
		Plasmid	**Chromosome**	**M_r**	
Small Heat-stable bacteriocins					
Lactococcus lactis ssp. *lactis LMG2130*	Lactococcin A	55 kb		5.8 kD	Holo *et al.* (1991)
Lactococcus lactis ssp. *cremoris 9B4*	Lactococcin B	60 kb			van Belkum *et al.* (1992)
Lactococcus lactis ssp. *lactis*	Lactococcin M	60 kb			van Belkum *et al.* (1991)
Lactobacillus acidophilus 11088	Lactacin F	110 kb		6.3 kD	Klaenhammer, (1993)
Leuconostoc gelidium UAL187	Leucocin A	11.6 kb		3.9 kD	Hastings & Stiles (1991)
Pediococcus acidilactici PAC1.0	Pediocin PA-1	9.3 kb		4.6 kD	Gonzalez & Kunka (1987)
Large Heat-labile Bacteriocins					
Lactobacillus helveticus 481	Helvetican J		+	37 kD	Joerger & Klaenhammer (1986)
Lactobacillus delbrukii JCM1106	Lacticin A				Toba *et al.* (1991a)
Lactobacillus delbrukii JCM1248	Lacticin B				Toba *et al.* (1991a)
Lactobacillus acidophilus LAPT1060	Acidophilucin A				Toba *et al.* (1991b)
Lactobacillus casei B80	Caseicin 80			42 kD	Rammelsberg & Radler (1990)
Lantibiotics					
Lactococcus lactis ssp. lactis ATCC114	Nisin A	+	+	3.35 kD	Buchman *et al.* (1988)
Lactococcus lactis ssp. lactis NIZO22186	Nisin Z	+			Mulders *et al.* (1991)
Lactobacillus sake L45	Lactosin S	50 kb		3.8 kD	Mortvedt & Nes (1990)
Lactococcus lactis ssp. lactis CNRZ481	Lacticin 481	70 kb		2.9 kD	Piard *et al.* (1990)
Carnobacterium ssp. U149	Carnocin U149			4.6 kD	Stoffels *et al.* (1992)
Lactobacillus plantarum LPCO10	Plantaricin S				Jimenez-Diaz *et al.* (1995)
Enterococcus faecium T136	Enterocin A			4.8 kD	Casaus *et al.* (1997)
	Enterocin B				

TABLE-7: LAB BACTERIOCINS - INHIBITORY SPECTRUM AGAINST NON-LACTIC MICROORGANISMS[a]

Susceptible Organism	Bacteriocin
Aeromonas hydrophila	Sakacin A
Bacillus cereus	Lactocin-S, Lactostrepcin-5, Nisin, Pediocin-A, Pediocin-AcH, Sakacin-A
Bacillus coagulans	Nisin
Bacillus licheniformis	Nisin
Bacillus pumilis	Thermophillin
Bacillus stearothermophilus	Nisin
Bacillus subtilis	Lacticin-481, Nisin, Thermophillin
Bronchothrix thermospacta	Curvacin-A, Pediocin-AcH, Sakacin-A, Sakacin-P
Clostridium bifermentans	Nisin
Clostridium botulinum	Nisin, Pediocin-A, Reuterin, Sakacin-A
Clostridium butyricum	Nisin, Reuterin
Clostridium perfringens	Nisin, Pediocin-A, Pediocin-AcH, Pediocin-VTT, Reuterin, Thermophillin
Clostridium sporogenes	Nisin, Pediocin-A
Clostridium tyrobutricum	Lacticin-481, Lactocin-S, Pediocin-AcH
Escherichia coli	Reuterin, Thermophillin
Listeria innocua	Lacticin-481, Lactosin-S, Pediocin-A, Pediocin-AcH,
Listeria ivanovii	Pediocin-A, Pediocin-AcH, Pediocin-PAC10
Listeria monocytogenes	Carnobacteriocin A&B, Curvacin-A, Enterocin-1146, Lactacin-B, Lacticin-481, Leucocin-A, Nisin, Pediocin-A, Pediocin-AcH, Pediocin-JD, Pediocin PA-1, Pediocin-PAC10, Pediocin-VTT, Piscicolin-61, Reuterin, Sakacin-A, Sakacin-P
Listeria seeligeri	Pediocin-A,
Listeria welchii	Lacticin-481, Pediocin-A
Proteus mirabilis	Nisin
Pseudomonas aeruginosa	Thermophillin
Pseudomonas fluorescens	Thermophillin
Salmonella enteritidis	Reuterin, Thermophillin
Salmonella infantis	Pediocin-VTT, Reuterin,
Salmonella typhimurium	Reuterin, Thermophillin
Shigella sp.	Reuterin, Thermphillin
Staphylococcus aureus	Nisin, Lacticin-481, Pediocin-A, Pediocin-AcH, Plantaricin-SIK83, Sakacin-A, Themophillin
Staphylococcus carnosus	Curvacin, Lacticin-481, Lactocin-S, Pediocin-AcH
Staphylococcus epidermidis	Nisin
Staphylococcus simulans	Nisin
Mycobacterium tuberculosis	Nisin
Yersinia enterocolitica	Thermophillin

[a]Bacteriocins produced by LAB inhibit closely related bacteria belonging to the same genus, other LAB species, Gram positive bacteria, and/or less commonly Gram negative bacteria

TABLE-8: PROBIOTIC / PROBIO-ACTIVE AGENTS – IN VIVO ANTIMICROBIAL ACTIVITY AGAINST PATHOGENS

Susceptible Pathogen	Antimicrobial Agent	Mode of Action	Reference
Antibacterial Activity			
Ristella sp.	LAB species	Antagonistic effect in digestive tract	Ducluzeau *et al.* (1971)
Mycobacterium intracellulare	*L. casei LC9018*	Immunopotentiation effect	Saito *et al.* (1983a)
Mycobacterium fortuitum	Heat-killed *LC9018*	Activated macrophage cidal activity	Saito *et al.* (1987)
Mycobacterium chelonae	Heat-killed *LC9018*	Activated macrophage cidal activity	Saito *et al.* (1987)
Listeria monocytogenes	*L. casei*	Macrophage-mediated host defense	Sato (1984)
	Heat-killed *LC9018*	Augmentation of host response	Nomoto *et al.* (1985)
	Cell wall fractions:	Elevated macrophage cidal activity	Sato *et al.* (1988b)
	(L. casei / L. plantarum /	Elevated macrophage cidal activity	Sato *et al.* (1988b)
	L. acidophilus)	Elevated macrophage cidal activity	Sato *et al.* (1988b)
Pseudomonas aeruginosa	Heat-killed *LC9018*	Induction of activated macrophages	Miake *et al.* (1985)
	Lipoteichoic acid of LC9018	Augmentation of murine resistance	Setoyama *et al.* (1985)
	Heat-killed *LC9018*	Immunostimulant activity	Watanabe & Hotta (1996)
Streptococcus mutans	Water-soluble cell extracts:	Bacterial growth inhibition	Ishihara *et al.* (1985)
	(S. faecium / S. equinus	Bacterial growth inhibition	Ishihara *et al.* (1985)
	L. fermentum / L. salivarius)	Bacterial growth inhibition	Ishihara *et al.* (1985)
Escherichia coli	LAB mixture	Bacterial multiplication-inhibition	Itoh & Freter (1989)
	L. casei	Increased IgA secretion in lumen	Perdigon *et al.* (1991)
Enterotoxigenic *E.coli*	Heat-killed *L.acidophilus*	Protected the mouse challenge	Fourniat *et al.* (1986)
Uropathogenic *E.coli*	*L. fermentum CRL1058*	Inflammatory immune response	Silva de Ruiz *et al.* (1996)
Salmonella enteritidis	*L .acidophilus / B.bifidum*	Increased resistance to lethality	Fichera *et al.* (1987)
Salmonella typhimurium	*L. bulgaricus / S.thermophilus*	Increased defense mechanism	De Simone *et al.* (1988)
	L. casei / L. acidophilus	Prevention of intestinal infection	Perdigon *et al.* (1990)
	L. casei GG	Inhibition of pathogenesis	Hudault *et al.* (1997)
Salmonella dublin	Killed LAB strains	Immunostimulating effect	Shkarupeta *et al.* (1988)
Salmonella typhi	*L. acidophilus*	Bacterial multiplication-inhibition	Rani & Khetarpaul (1998)
Shigella dysenteriae	*L. acidophilus*	Bacterial multiplication-inhibition	Rani & Khetarpaul (1998)
Shigella sonnei	*L. casei / L. acidophilus*	Protective mucosal immunity	Nader de Macias, *et al.* (1992)
Shigella flexneri	*L. acidophilus* S/C supernate[a]	Microbial growth inhibition	Coconnier *et al.* (1997)
Klebsiella pneumoniae	*L. acidophilus* S/C supernate[a]	Microbial growth inhibition	Coconnier *et al.* (1997)
Enterobacter sp.	*L. acidophilus* S/C supernate[a]	Microbial growth inhibition	Coconnier *et al.* (1997)
Bacillus cereus	*L .acidophilus* S/C supernate[a]	Microbial growth inhibition	Coconnier *et al.* (1997)
Helicobacter pylori	*L. salivarius*	Attachment inhibition / IL-8 release	Kabir *et al.* (1997)
Antifungal Activity			
Candida albicans	*(L. acidophilus / L. reuteri /*	Cell-mediated immune modulation	Wagner *et al.* (1997)
	L. casei GG / B. animalis)		
Cryptosporidium parvum	*L. reuteri*	Reversal of immune suppression	Alak *et al.* (1997)
Antiviral Activity			
Herpes Simplex virus type-1	Heat-killed *LC9018*	Induction of peritoneal macrophages	Watanabe & Saito (1986)
	Heat-killed *LC9018*	Production of IFN-γ by macrophage	Watanabe & Yamori(1989)
	Heat-killed *LC9018*	Immunostimulant activity	Watanabe & Hotta (1996)
Murine Cytomegalo virus	Heat-killed *LC9018*	Host-mediated antiviral activity	Ohashi *et al.* (1988)
	Heat-killed *LC9018*	Augmentation of NK cell activity	Ohashi *et al.* (1989)

[a]Spent culture (S/C) supernate; IFN-γ, interferon-gamma; NK cell, Natural Killer cell

TABLE-9: PROBIOTIC LAB – IN VIVO MODULATIONS OF IMMUNE SYSTEM

Effect	LAB Species	Reference
Granulomata induction with PMNLs and monocytes	*L. plantarum / L. brevis*	Bloksma *et al.* (1979)
Induction of NK cell activity in peritoneal exudates	*L. plantarum*	Bloksma *et al.* (1981)
Mitogenic effect on murine lymphocytes	*L. casei*	Shimizu *et al.* (1981)
Induction of cytostatic activity of peritoneal exudate cells	*L. casei LC9018*	Kato *et al.* (1983)
Enhanced humoral antibody production	*L. casei*	Saito *et al.* (1983b)
Delayed type hypersensitivity response	*L. casei*	Saito *et al.* (1983b)
Augmentation of murine NK cell activity	*L. casein LC9018*	Kato *et al.* (1984)
Oxygen radical production by peritoneal MP / Kupffer cells	*L. casein LC9018*	Hashimoto *et al.* (1984)
Oxygen radical production by fixed MP in liver	*L. casein LC9018*	Hashimoto *et al.* (1984)
Migration of MP from blood to reticuloendothelial system	*L. casei*	Sato *et al.* (1983)
Induction of activated MP with tumoricidal activity	*L. casei LC9018*	Kato *et al.* (1985)
Activation of T-lymphocytes to induce cytotoxic cells	*L. casei LC9018*	Kato *et al.* (1985)
Induction of tumor-specific humoral and cellular immunity	*L. casei LC9018*	Yasutake *et al.* (1985)
Production of cytotoxic factors by Kupffer cells	*L. casei LC9018*	Hashimoro *et al.* (1985)
Enhancement of murine hematopoietic response	*L. casei LC9018*	Yokokura *et al.* (1986)
Modulation of myelopoiesis during M-CFC proliferation	*L. casei LC9018*	Nanno *et al.* (1986)
Activation of murine MP	*L. casei / L. bulgaricus*	Perdigon *et al.* (1986)
Increased phagocytic activity of monocytes	*L. casei / L. plantarum*	Zaitseva *et al.* (1986)
Enhanced enzymatic / phagocytic activity of peritoneal MP	*L. acidophilus / S. thermophilus*	Perdigon *et al.* (1987)
Increased phagocytic function of reticuloendothelial system	*L. acidophilus / S. thermophilus*	Perdigon *et al.* (1987)
Activation of liver and peritoneal MP	*L. casei*	Sato *et al.* (1988a)
Increased production of serum-CSF	*L. casei LC9018*	Nanno *et al.* (1988)
Activation of mucosal immunity in intestinal tract	*L. casei / L. acidophilus*	Perdigon *et al.* (1988a)
Humoral immunity / delayed-type hypersensitivity response	*L. casei / L. acidophilus* *L. bulgaricus / S. thermophilus*	Perdigon *et al.* (1988b)
Induction of murine gamma-interferon	*L. casei LC9018*	Suzuki *et al.* (1988)
	L. bulgaricus / S. thermophilus	Pereyra *et al.* (1991)
	L. acidiophilus	Kitazawa *et al.* (1992)
Enhanced proliferation of cytotoxic MP progenitors	*L. casei LC9018*	Nanno *et al.* (1989)
Increased IgA secretion in intestinal lumen	*L. casei*	Perdigon *et al.* (1991)
Priming / triggering of endogenous TNF-alpha	*L. bulgaricus LB51*	Davidkova *et al.* (1992)
Activation of phagocytic / secretory functions of monocytes	*L. bulgaricus LB51*	Popova *et al.* (1993)
Augmentation of murine MP phagocytic activity	*L. acidophilus / B. longum*	Hatcher & Lambrecht (1993)
Potent adjuvant for oral immunization	*L. casei / L. plantarum* *L. fermentum / L. bulgaricus*	Pouwels *et al.* (1996)
Acceleration of granulocyte cell recovery	*L. casei LC9018*	Furuse *et al.* (1997)
Stimulation of IL-1 and TNF-alpha production by MP	*L. acidophilus LA-1*	Rangavajyala *et al.* (1997)
Inhibition of antigen-induced IgE-mediated allergy	*L. casei*	Shida *et al.* (1998)

PMNL – polymorphonuclear leukocytes; **NK cells** – natural killer cells; **MP** – macrophage; **M-CFC** – macrophage colony forming cells; **CSF** – colony stimulating factor; **TNF** – tumor necrosis factor; **IL** - interleukin

TABLE-10: *IN VITRO* AND *IN VIVO* ANTITUMOR EFFECTS OF *LACTOBACILLUS CASEI* LC9018

Tumor Type (Species)	Effect	Reference
Sarcoma-180 (ICR mice)	Significant prolongation of life span	Kato *et al.*, (1981)
L1210 leukemia (BDF1 mice)	Significant prolongation of life span	Kato *et al.*, (1981)
Sarcoma-180 (ICR mice)	Increased phagocytic function of RES	Kato *et al.*, (1983)
Meth-A sarcoma (BALB/c mice)	Augmentation of NK cell activity	Kato *et al.*, (1984)
K234 tumor (BALB/3T3 mice)	Specific transplantation immunity	Yasutake *et al.*, (1984a)
Meth-A fibrosarcoma (murine)	Suppress free radical / PGE2 production	Hashimoto *et al.*, (1984)
Lewis lung carcinoma (C57BL/6 mice)	Inhibition of pulmonary metastasis	Matsuzaki *et al.*, (1985)
Line-10 hepatoma (Guinea pigs)	Induction of systemic tumor immunity	Matsuzaki *et al.*, (1985)
MBT-2 bladder tumor (murine)	Reduced pulmonary metastasis	Asano *et al.*, (1986)
Lewis lung carcinoma (C57BL/6 mice)	Augmentation of metastasis	Matsuzaki & Yokokura (1987a)
Pretreated tumor cell line (murine)	Release of cytotoxic factors by MP	Hashimoto *et al.*, (1987)
B16 melanoma (C57BL/6 mice)	Alveolar MP cytotoxicity augmented	Matsuzaki *et al.*, (1987b)
Meth A sarcoma (BALB/c mice)	Increased thoracic exudate cells	Matsuzaki *et al.*, (1988a)
Fibrosarcoma (BALB/c mice)	Induction of T-cell mediated immunity	Kato *et al.*, (1988)
B16 melanoma (C57BL/6 mice)	Inhibition of metastasis	Matsuzaki *et al.*, (1988b)
Uterine cervix cancer (clinical trial)	Effective adjuvant chemotherapeutic	Okawa *et al.*, (1989)
Malignant pleurisy (BALB/c mice)	Increase in sclerosing effects on pleura	Tohgo *et al.*, (1989)
Lewis lung carcinoma (C57BL/6 mice)	Augmentation of metastasis	Matsuzaki *et al.*, (1990)
T-cell leukemia cells (human)	Inhibition of protein synthesis / growth	Katayama, (1990)
Lymphoblastic leukemia cells (human)	Inhibition of protein synthesis / growth	Katayama, (1990)
Promonocytic leukemia cells (human)	Inhibition of protein synthesis / growth	Katayama, (1990)
TUR-bladder tumor (clinical trial)	Prevention of recurrence	Aso & Akazan (1992)
Uterine cervix cancer (clinical trial)	Effective adjuvant chemotherapeutic	Okawa *et al.*, (1993)
Colon 26 tumor (BALB/c mice)	Systemic immune response potentiation	Kato *et al.*, (1994)
BBN-induced bladder cancer (rat)	Low malignancy of induced tumors	Tomita *et al.*, (1994)
Yac-1, P815 tumor cells (murine)	Inhibition of viability by cytotoxicity	Fichera and Giese, (1994)
Ehrlich ascites tumor (murine)	Inhibition of viability by cytotoxicity	Fichera and Giese, (1994)
Mammary carcinoma (murine)	Inhibition of viability by cytotoxicity	Fichera and Giese, (1994)
K562, KB tumor cells (human)	Inhibition of viability by cytotoxicity	Fichera and Giese, (1994)
Bladder carcinoma (clinical trial)	Prevention of recurrence	Aso *et al.*, (1995)
Thymic lymphoma (AKR mice)	Inhibition of murine leukemia virus	Watanabe, (1996)
Fibrosarcoma (BALB/c mice)	Cytokine induction in thoracic cavity	Matsuzaki *et al.*, (1996)

TABLE-11: EFFECTS OF *LACTOBACILLUS GG* ON INTESTINAL TRACT – CLINICAL STUDIES

Produces a wide spectrum antimicrobial agent against many enteric pathogens	Silva *et al.* (1987)
Effective prophylaxis in reducing the occurrence of travelers diarrhea	Okasanen *et al.* (1990)
Prevents antibiotic (erythromycin) – associated diarrhea	Siitonen *et al.* (1990)
Effective in shortening the course of acute diarrhea	Isolauri *et al.* (1991)
Decreases glycocholic acid hydrolase activity and tryptic activity	Ling *et al.* (1992)
Survive / temporarily colonize human gastrointestinal tract	Goldin *et al.* (1992)
Enhances circulating antibody secreting cell response in rotaviral diarrhea	Kaila *et al.* (1992)
No adverse effects on nutritionally important short chain fatty acids	Stansbridge *et al.* (1993)
Reverses increased intestinal permeability induced by cow milk	Isolauri *et al.* (1993)
Decreases colonic hydrolytic and reductive enzyme activities	Ling *et al.* (1994)
Provides a nontoxic therapy for endotoxemina and alcohol-induced liver injury[a]	Nanji *et al.* (1994)
Counteracts disturbed microbial balance in the gut	Isolauri *et al.* (1994)
Stimulates rotavirus specific IgA antibody responses and prevents reinfection	Kaila *et al.* (1995)
Elicits an immunostimulating effect on oral rotavirus vaccination	Isolauri *et al.* (1995)
Promotes recovery from actue nonbloody diarrhea	Raza *et al.* (1995)
Effective therapeutic for acute rotaviral gastroenteritis	Majamaa *et al.* (1995)
Effectively colonizes the gut and well tolerated in infants	Sheen *et al.* (1995)
Does not break down intestinal mucus glycoproteins	Biller *et al.* (1995)
Interferes with initiation or progression of DMH-induced intestinal tumorigenesis[a]	Goldin *et al.* (1996)
Promotes IgA immune response in patients with Crohn's disease	Malin *et al.* (1996)
Down regulates anti-CD3 antibody-induced IL-4 production by bovine caseins	Sutas *et al.* (1996)
Reduces urease activity in feces of juvenile chronic arthritis patients	Malin *et al.* (1996)
Generates molecules with suppressive effects on lymphocyte proliferation[b]	Sutas *et al.* (1996)
Elicits antagonistic activity against *Salmonella typhimurium C5* infection[a]	Hudault *et al.* (1997)
Alleviates intestinal inflammation, protects from atopic dermatitis and food allergy	Majamaa & Isolauri (1997)
Shortens duration of watery diarrhea in adjunct to oral rehydration therapy	Shornikova *et al.* (1997)
Potent biotherapeutic against alimentary infection with *Candida albicans*[a]	Wagner *et al.* (1997)
Reduces duration of symptoms and viral excretion in children with mild diarrhea	Guarino *et al.* (1997)
Binds and removes aflatoxin B1, a potent food carcinogen from media[c]	El-Nezami *et al.* (1998)

(a) Data from experimental animal model; (b) in vitro cell culture system; and (c) *in vitro* laboratory media.

TABLE-12a: LAB APPLICATION IN VARIOUS FERMENTED FOODS

Food Product	Origin	LAB Culture
Acidophilus milk	USA	*Lb. acidophilus*
Balao balao	Indonesia	*Lactobacillus sp.*
Breads	Ancient Rome, Egypt	*Lb. brevis, Lb. plantarum, Lb. fermentum, Lc. mesenteroides, S. thermophilus*
Bulgarium buttermilk	Bulgaria	*Lb. delbrueckii* spp. *bulgaricus*
Burong dalag	Southeast Asia	*Lc. mesenteroides, P. pentosaceus, Lb. plantarum*
Butter	Wordwide	*Lc. lactis* spp. *lactis, Lc. lactis* spp. *cremoris, Lc. lactis, Lc mesenteroides.*
Buttermilk	Worldwide	*Lc. lactis* ssp. *lactis*
Cacao	South America	Various LAB
Cheeses	Worldwide	*Lc. lactis* spp. *lactis, Lc. lactis* ssp. *cremoris, Lb. delbrueckii* spp. *bulgaricus, Lb. delbrueckii* ssp. *lactis, Lb. casei, Lb. helveticius, Lb. brevis, Lb. fermentum*
Coffee	Worldwide	Various LAB
Crackers	Europe North America	*Lb. plantarum, Lb. delbrueckii* ssp. *delbruckii, Lb. delbrueckii* ssp. *lactis, Lb. casei, Lb. brevis, Lb. fermentum*
Cucumbers (pickles)	Worldwide	*Lb. plantarum, Lb. brevis, P. pentosaceus*
Cured ham	Europe	*Lb. casei, Lb. plantarum*
Distillery mashes	Europe	*Lb. plantarum, Lb. delbrueckii,* ssp. *delbruckii, Lb. casei, Lb. fermentum*
Dahi	India	*S. thermophilus, Lb. bulgaricus, Lc. diacetylactuis, Leuconostoc* spp.
Dosa	India	*Lc. mesenteroides, E. faecalis, P. pentosaceus*
Feed additives	Europe North America	*Lb. acidophilus, Lb. delbrueckii* ssp. *bulgaricus, Lb. delbrueckii* ssp. *lactis, Lb. reuteri*
Fermented fish	China, Japan	Various LAB
Filmjölk	Sweden	*Lc. lactis, Lc. cremoris, Lc. diacetylactis*
Gari	Africa	*Leuconostoc sp.*
Green olives	Middle East	*Lb. plantarum, Lb. brevis, P. pentosaceus*
Idli	India	*Lc. mesenteroides, E. faecalis, P. pentosaceus*
Izushi	Japan	*Lactobacillus sp.*
Kefir	Caucasus	*Lc. lactis* ssp. *lactis, Lb. delbrueckii* ssp. *bulgaricus*
Kenkey	Africa	*Lactobacillus sp.*
Kimchi	Korea	Various LAB
Kishk	Saudi Arabia	*Lb. brevis, Lb. casei, Lb. plantarum*
Kisra	Middle East	*Lactobacillus sp.*
Koumiss	Mongolia	*Lb. delbrueckii* ssp. *bulgaricus, Lb. delbrueckii* ssp. *lactis*
Lactic acid	Europe	*Lb. delbrueckii* ssp. *delbruckii*
Lactic butter	Europe	*Lc. lactis* ssp. *cremoris*
Lassi	India	*Lactococcus* spp., *Lactobacillus* spp.
Lebanon bologna	Lebanon	*P. acidilactici*
Leben	Middle East	*S. thermophilus, Lb. bulgaricus, Lb. acidophilus, Lc. lactis*

TABLE-12b: LAB APPLICATION IN VARIOUS FERMENTED FOODS

Food Product	Origin	LAB Culture
Magon	Tunisia	*Lactobacillus sp.*
Miso	Japan	*Lactobacillus sp.*
Ogi	Africa	*Lb. plantarum, Lc. lactis* ssp. *lactis*
Oncom	Indonesia	*LAB ssp.*
Pulque	Mexico	*Lb. plantarum, Leuconostoc sp.*
Puto	Portugal	*Lc. mesenteroides, E. faecalis*
Sauerkraut	Europe	*Lb. plantarum, Lb. brevis, Lc. mesenteroides, P. pentosaceus*
Sausages, meats	Europe	*Lb. plantarum, Lb. casei, Lb. curvatus, Lb. reuteri, Lb. sake, P. pentosaceus, P. acidilactii*
Shoyu	Indonesia	*Lactobacillus sp.*
Silage starters	North America	*Lb. plantarum, P. acidilactii*
Sour bread	France, Western Europe	*Lb. sanfrancisco, Lb. brevis*
Sour cream	Worldwide	*Lc. lactis* ssp. *cremoris*
Sour pumpernickel	North Africa	*Lc. mesenteroides*
Soy sauce	Southeast Asia	*Lb. delbrueckii* ssp. *delbruckii*
Sweet dough	France, Western Europe	*Lb. brevis, Lb. plantarum, Lb. fermentus, Lc. lactis* ssp. *cremoris, Lc. mesenteroides* ssp. *cremoris*
Taette	Netherlands	*Lc. lactis* ssp. *lactis* var. *taette*
Tempeh	Indonesia	*Lactobacillus sp.*
Uji	Japan	*Lb. plantarum, L. lactis* ssp. *lactis*
Villi	Finland	*Lc. lactis* ssp. *cremoris, Lc. diacetylactis*
Wine	North America Europe	*Lb. plantarum, Lb. hilgardii, P. damnosus, Lc. mesenteroides, Lc. oenos*
Yakult	Japan	*Lb. casei*
Yogurt	Asia, Balkans	*Lb. delbrueckii* ssp. *bulgaricus, S. thermophilus*

TABLE-13: LAB SUPPLEMENTED FOODS CURRENTLY AVAILABLE IN DIFFERENT MARKETS

Product or Trade Name	Origin	LAB Culture
A B milk products	Denmark	*L. acidophilus, B. bifidum*
Acidophilus bifidus yogurt	Germany	*L. delbrueckii subsp. Bulgaricus, S. thermophilus* *L. acidophilus B. bifidum or B. longum*
B A®	France	*B. longum*
Bifidus milk	Germany	*B. bifidum or B. longum*
Bifidus milk with yogurt flavor	UK	*B. bifidum, B. longum or B. infantis*
Bifidus yogurt	Many countries	*b. bifidum or B. longum*
Bifighurt ®	Germany	*B. bifidum or B. longum*
Bifilakt® or Bifilact ®	USSR	*Lactobacillus sp., Bifidobacterium. sp.*
Biogarde ®	Germany	*L. acidophilus, B. bifidum, S. thermophilus*
Bioghurt ®	Germany	*Similar to Biogarde®*
Biokys®	Czechoslovakia	*B. bifidum, L. acidophilus, P. acidilactici*
Biomild®	Germany	*L. acidophilus, Bifidobacterium. sp.*
Cultura®	Denmark	*L. acidophilus, B. bifidum*
Diphilus milk®	France	*As above*
Mil-Mil®	Japan	*B. bifidum, B. breve, L. acidophilus*
Ofilus®	France	*S. thermophilus, L. acidophilus, B. bifidum or* *Lc. lactis ssp. cremoris, L. acidophilus, B. bifidum*
Progurt®	Chile	*Lactococcus lactis biovar diacetilactis, B. bifidum* *Lactococcus lactis ssp. cremoris, L. acidophilus*
Sweet acidophilus bifidus milk	Japan	*L. acidophilus, B. longum*
Sweet bifidus milk	Japan / Germany	*Bifidobacterium sp.*

Adapted from Tamime et al. (1995)

FIGURES 1 & 2

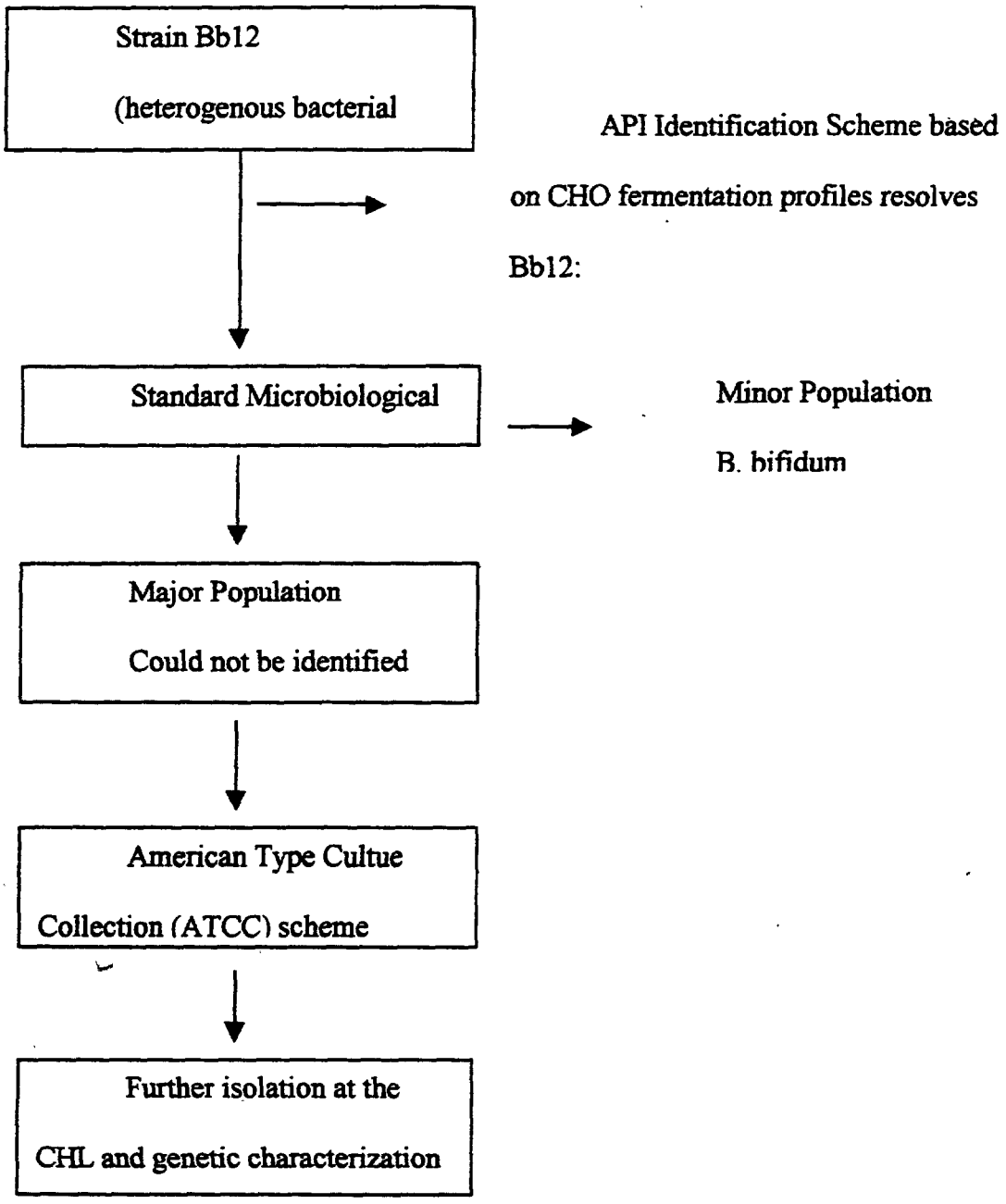

Figure 1. Purification and characterization of strain Bb12.

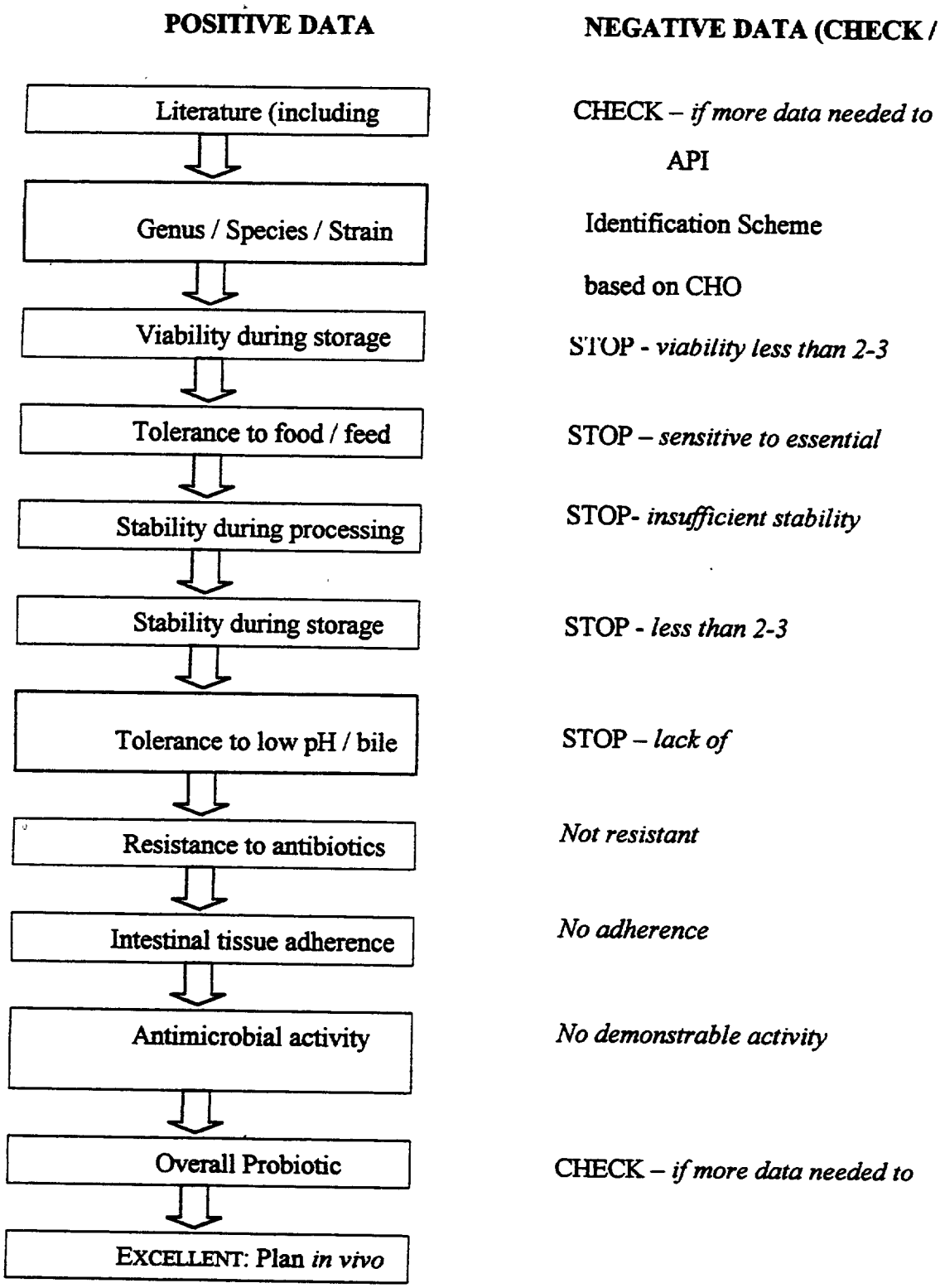

FIGURE-2: Flow Diagram for *in vitro* selection of Strains for Probiotic Use

Adapted from Havenaar *et al.*, (1992)

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SUMMARY STATEMENT

EXPERT PANEL REGARDING THE "GENERAL RECOGNITION" OF BIFIDOBACTERIUM, LACTOBACILLUS AND STREPTOCOCCUS THERMOPHILUS FOR USE IN INFANT FORMULA

Members of the Expert Panel were requested by Nestlé U.S.A. to independently review the safety of two genera and one species of bacteria for addition to infant formula. The training and experience of members of the Expert Panel is provided in Attachment 1.

To assist in this review, original scientific articles were provided to the Panel. A list of these articles is contained in Attachment 2.

Following review of the above articles and of other information considered relevant by individual members of the Expert Panel, a meeting was held in Washington, D.C. on April 18-19, 1996 at the law offices of King & Spalding, 1730 Pennsylvania Avenue, N.W. The focus throughout the discussions was on the safety for use in infants and children of formulas containing *Bifidobacterium, Lactobacillus* and *Streptococcus thermophilus*. Scientific literature was critiqued and discussion ensued regarding *Bifidobacterium, Lactobacillus*, and *Streptococcus thermophilus* and the following issues were addressed:

a) history and taxonomy;

b) prior use in foods;

c) infant's and young child's evolving intestinal microflora;

d) potential growth and nutritional consequences for infants and young children; and

e) adverse effects; in the areas of allergenic potential, virulence and toxicity.

The Expert Panel has come to a consensus on the following points, whereas:

I. Addition to infant formula of bacterial strains of two genera, namely, *Bifidobacterium* and *Lactobacillus*, and strains of one species, *Streptococcus thermophilus* result in a "new" infant formula for the purpose of improving the overall wholesomeness of food for infants and children. Such formulas are not "new" in Europe and Asia where they have been used for decades.

II. The use of these ingredients in infant formula was not contemplated when the "functionality" categories delineated in 21 C.F.R. § 170.3 were developed; nevertheless, arguments can be made that the following categories may apply: synergist, antimicrobial agent, enzyme, and nutrient supplement.

III. The literature and evolving science support the rationale for adding these organisms to infant formula in light of:

a) the ontogeny of infant intestinal microflora;

b) the developmental expression of intestinal carbohydrate enzyme activity in the infant and child, and

c) the potential for the prevention of common infectious diarrheal agents.

IV. Similarly, the literature and evolving science support the value of these uses of *Bifidobacterium, Lactobacillus,* and *Streptococcus thermophilus* in infant formula. Although at this time definitive conclusions about their efficacy for specific indications cannot be reached, assessments of the safety and GRAS status of these organisms can be appropriately made.

V. Concerning the safety and GRAS status of *Bifidobacterium, Lactobacillus,* and *Streptococcus thermophilus* for addition to infant formula:

Bifidobacterium: organisms of this genus, with the exception of *B. dentium*, may be viewed as safe and as GRAS on the basis of available scientific data. *B. dentium* is an identified agent of dental carries and, accordingly, should not be used in infant formula. Note was taken of the recent study of Moore, W.E.C. and Moore, L.H., Appl. Environ. Microbiol. 1995; 61:3202-3207, in which certain *Bifidobacterium* species were identified as being associated with populations at risk of colonic cancer. The panel's consensus was that neither the data presented nor the scientific issues posed by this publication are of sufficient strength to warrant concern.

Lactobacillus: No reservations concerning the safety or GRAS status of members of this genus were raised by the panel. In reaching this conclusion, the Panel considered the position taken by the European Union sponsored workshop organized by the *Lactobacillus* Industrial Platform (LABIP)

concerning the role of *L. rhamnosus*, *L. casei* (*L. paracasei*), and *L. plantarum* in opportunistic infections in compromised hosts (Adams, M.R., Martue, P., Int. J. Food Microbiol. 1995; 27:263-264).

Streptococcus thermophilus: no reservations were raised about the safety or GRAS status of this species.

VI. Unlike the case with normal newborns and infants, insufficient information is available to assess the use and safety of such infant formulas fed to severely immunocompromised infants and young children (e.g., those suffering from AIDS or SCIDS and those receiving chemotherapy or radiation such that entry into the systemic compartment might reasonably occur). Information from studies on the risks or possible benefits of such preparations of foods in these few groups is too limited at this time to draw definitive conclusions and, thus, any such use should be under medical supervision.

CONCLUSIONS

Assuming that current good manufacturing practice, including appropriate stability assessment, is followed in the production of the contemplated finished infant formulas:

In our opinion, the addition of *Bifidobacterium* (with the exception of *B. dentium*), *Lactobacillus*, and *Streptococcus thermophilus* to infant formula is safe;

Moreover, in our opinion, experts qualified by adequate training and experience, would generally recognize on the basis of the available scientific information ("scientific procedures") *Bifidobacterium* (with the exception of

B. dentium), *Lactobacillus*, and *Streptococcus thermophilus* as safe for addition to infant formula.

Each of the foregoing conclusions incorporates the limitation noted in paragraph VI above.

Douglas L. Archer, Ph.D.

W. Gary Flamm, Ph.D.

Benjamin D. Gold, M.D.

Dallas G. Hoover, Ph.D.

John B. Luchansky, Ph.D.

Dwayne C. Savage, Ph.D.

Myron Winnick, M.D.

April 19, 1996

GR

GRAS Exemption Claim and Exemption Notification for the Use in Infant Formula of *Bifidobacterium, Lactobacillus* and *Streptococcus thermophilus*

Submitted by Nestlé USA

June 9, 2000

Volume II
(Original)

DUPLICATE

KING & SPALDING

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June 9, 2000

Office of Premarket Approval
HFS-215
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Exemption Claim and Exemption Notification for the Use in Infant Formula of *Bifidobacterium, Lactobacillus* and *Streptococcus thermophilus*

Dear Sir or Madam:

Attached please find an original plus two copies of a GRAS Exemption Claim and a GRAS Exemption Notification for the use of three "probiotics" in infant formula (and other food for infants and children). The request is supported not only by extensive clinical studies and investigation reported in the scientific literature but also by the comprehensive report of a panel of qualified and distinguished experts. Moreover, the two genera and one species of lactic acid bacteria that are the subject of the claim and notification have been and continue to be broadly used in the food supply worldwide. As the expert panel has observed, the addition of the three lactic acid bacteria will improve the overall wholesomeness of formulas. We look forward to the office's review of the claim and notification and are prepared to address any questions or concerns that may arise.

Sincerely,

Fred H. Degnan

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HFS-215
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Exemption Claim and Exemption Notification for the Use in Infant Formula of *Bifidobacterium, Lactobacillus* and *Streptococcus thermophilus*

Dear Sir or Madam:

Attached please find an original plus two copies of a GRAS Exemption Claim and a GRAS Exemption Notification for the use of three "probiotics" in infant formula (and other food for infants and children). The request is supported not only by extensive clinical studies and investigation reported in the scientific literature but also by the comprehensive report of a panel of qualified and distinguished experts. Moreover, the two genera and one species of lactic acid bacteria that are the subject of the claim and notification have been and continue to be broadly used in the food supply worldwide. As the expert panel has observed, the addition of the three lactic acid bacteria will improve the overall wholesomeness of formulas. We look forward to the office's review of the claim and notification and are prepared to address any questions or concerns that may arise.

Sincerely,

Fred H. Degnan

000702

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12th ANNUAL MEETING OF THE NORTH AMERICAN SOCIETY FOR PEDIATRIC GASTROENTEROLOGY AND NUTRITION

Orlando, Florida

A. IMPORTANT

The principal author affirms that the material herein:

1) will not have been previously published or presented at any national or international meeting before June 1, 1998 (except for Fellow Research Award applicants cut-off date is January 1, 1998).

2) studies involving human subjects conform to the principles of the Declaration of Helsinki of the World Medical Association (Clinical Research 1966; 14:103) and meet all of the requirements governing informed consent of the country in which it was performed. If human subjects were exposed to risk not required by their medical needs, the study was approved by an appropriate committee or, if no such committee was available and informed consent was needed, it was obtained in accordance with the principles enunciated in "The Institutional Guide to DHEW Policy on Protection of Human Subjects" or

3) any animal studies conform with the "Guiding Principles in the Care and Use of Animals" of the American Physiological Society, and

4) that potential conflicts of interest will be disclosed at time of presentation of abstract. All potential conflicts of interest should be detailed in a separate letter to the NASPGN Secretary-Treasurer. Such information will not be available to the abstract review committee and will not prejudice review of the abstract.

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E. NAME OF RESPONSIBLE AUTHOR (Please type)

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LONG TERM CONSUMPTION OF INFANT FORMULAS WITH LIVE PROBIOTIC BACTERIA: SAFETY AND TOLERANCE.

A Abi-Hanna, N Moore, R Yolken, J Saavedra. Division of GI and Nutrition Johns Hopkins University, School of Medicine, Baltimore, MD.

Safety and tolerance of chronic consumption of feedings with live probiotics in infants has not been well documented. We carried out a prospective double blind study in healthy free-living infants attending day care centers. 131 infants were randomized to receive standard milk based formula (Nestlé, USA) with: Bifidobacteria (B), 10^8 CFU/g and S. thermophilus (ST), 10^7 CFU/g (High supplement -HS); or B, 10^7 CFU/g and ST, 10^7 CFU/g (Low supplement - LS); or formula with no supplement (placebo - P). Only exclusion criteria were breast feeding and history of milk allergy. Standardized weight and length were obtained monthly. Data on formula intake, GI tolerance, and general health indicators were collected by weekly phone call and visits to day care centers. **Results:** 119 infants (39 on HS, 40 on LS and 40 on P) were enrolled at 6.8 ± 2.5 mo. of age and followed for a total of 68.1 subject-years. 12 subjects consuming formula for < 2 weeks were excluded from analysis. Average consumption at 3 and 12 mo. of age provided 104 and 45.3 Kcal/kg/d respectively. There were no differences in age, gender, formula consumption, or length of study between groups. 9 infants were withdrawn by their parents for perceived effects related to the formula (6 for diarrhea and/or vomiting, 1 constipation, 1 otitis media, 1 rash). constipation, 1 otitis media, 1 rash).

	HS	LS	P
Age at enrollment (months ± SD)	7.1 ± 2.7	6.4 ± 3.6	6.7 ± 2.4
Wt/age at enrollment Ψ (± SD)	0.5 ± 0.9	0.5 ± 0.8	0.5 ± 0.8
Wt/age at end of study Ψ (± SD)	-0.3 ± 0.8	0.4 ± 0.9	-0.4 ± 0.9
Ht/age at enrollment Ψ (± SD)	-0.08 ± 0.7	0.1 ± 0.8	-0.1 ± 0.6
Ht/age at end of study Ψ (± SD)	0 ± 0.7	0.2 ± 0.7	0 ± 0.6
Health care visits *	0.56	2.36	2.91
Vomiting episodes *	0.02	0.02	0.03
"Diarrhea episodes" * §	1.16	1.87	1.96
Episodes of discomfort with stool * §	2.1	2.4	2.1
Day care days missed due to illness *	3.25	3.37	3.1

*Ψ Z scores by NCHS tables, * per 100 subject days; § as perceived by parent.* No statistical differences were found in these or other health indicators. **Conclusions:** Long term consumption of these live probiotic containing products by infants, as their sole formula, was well tolerated, safe, and resulted in adequate growth.

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NA- Not applicable

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000816 - 000838	Gasser, F.	Safety of lactic acid bateria and their occurrence in human clinical infections	1994	Bulletin Institut Pasteur	Volume 92, pgs 45-67
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NA- Not applicable

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June 14, 2000

Linda Kahl, Ph.D.
Office of Premarket Approval
HFS-215
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Re: GRAS Exemption Claim and GRAS Exemption Notification for the Use in Infant Formula of *Bifidobacterium, Lactobacillus* and *Streptococcus thermophilus*

Dear Dr. Kahl:

Thank you for your call this afternoon concerning the above Claim and Exemption filed by Nestlé on June 9, 2000. Please be assured that Nestlé's claim is based on the conclusion that the uses in infant formula of *Bifidobacterium, Lactobacillus and Streptococcus thermophilus* at levels consistent with good manufacturing practice are exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act ("the Act") because Nestlé has determined that the uses are GRAS within the meaning of Section 201(s) of the Act. Please do not hesitate to call if you have further questions or desires for additional clarification. Again, thank you for your courtesies in this matter.

Sincerely,

Fred H. Degnan

000931

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DIRECT DIAL:

August 7, 2000

Paulette M. Gaynor
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
1110 Vermont Avenue, N.W.
Washington, DC 20201

Dear Dr. Gaynor:

Thank you for your patience in letting me take a week to respond to your request for the figures and tables which should have accompanied the two unpublished manuscripts behind Tab E of our GRAS submission. I apologize for the fact that these inadvertently were not included in our original submission. The delay in getting the copies to you resulted from, as I explained, vacation schedules. I hope that neither our inadvertence nor the ensuing delay from the time of your request has caused you too much inconvenience. If you have any questions or concerns, please do not hesitate to contact me.

Best regards,

Fred H. Degnan

000933

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November 14, 2000

Linda Kahl, Ph.D.
Office of Premarket Approval
HFS-215
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204

Dear Dr. Kahl:

Thank you for taking the time on November 1, 2000 to share with Roger Clemens and me your thoughts and questions, and the thoughts and questions of Paulette Gaynor and Robert Merker, concerning the GRAS notification submitted on behalf of Nestlé for *Lactobacillus, Bifidobacterium and S. thermophilus*.

Your inquiries focused on two general areas of concern: "conditions of use" and "identity." With regard to the former, you requested clarification as to whether the intended use envisioned by the notification was for "starter" or "follow-on" formula or both. Let me clarify that Nestlé wishes the notification to be limited to "follow-on" formulas. As you know, "starter" formulas are for use throughout the entire 12 months of infancy, while "follow-on" formulas are expressly labeled and promoted for use by infants 4 months or older.

Also with regard to intended use, you questioned whether the notification applied to soy-based as well as milk-based products. Let me clarify on this point as well that the notification pertains *only* to milk-based formulas.

With regard to "identity" issues, you inquired whether the notification pertained to the genera identified as a whole or whether it was more focused on particular species and strains. Let me clarify here, too, that the scope of the petition in light of your inquiry is on *Bifidobacterium* Bb12 and *S. thermophilus* Th4. The *Bifidobacterium* is of human origin; the origin of the *S. thermophilus* is cheese (mozzarella). The two Hopkins

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studies relied upon in our submission involved the feeding of these specific organisms to healthy as well as ill and immunocompromised infants. The remaining studies, information, and narrative discussion provided in our notification, along with the expert panel reports, further substantiate the position that the organisms, for the use Nestlé intends, are GRAS.

You also inquired as to whether the Company had any specifications for the level of "resuscitated organisms." Based on the Hopkins studies, the Company intends to specify within the range of 10^6 to 10^8 cfu of each organism per gram of finished formula as the target for each batch.

I would like to emphasize our appreciation for the opportunity to discuss your questions in the constructive forum our conference call provided. I hope you considered the call to be as productive a session as we did.

Please call if any questions or concerns remain.

Sincerely,

Fred H. Degnan

cc: Roger A. Clemens, Ph.D.
Melanie Fairchild-Dzanis

AM

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September 10,2001

RECEIVED
OCT 2001
OFFICE OF
FOOD ADDITIVE SAFETY

Paulette M. Gaynor, Ph.D.
Office of Food Additive Safety
HFS-215
Center for Food Safety and Applied Nutrition
Food and Drug Administration
1110 Vermont Avenue, N.W.
Washington, DC 20201

Dear Dr. Gaynor:

I am submitting this letter and the attachments to it to memorialize our conference call held this past August 23 between representatives from the Office of Food Additive Safety, CFSAN and representatives of Nestlé USA. The call was held to discuss the Company's pending GRAS notification for the use in infant formula of *Bifidobacterium* and *Streptococcus Thermophilus*. Attending the call on behalf of Nestlé were Dr. Jose Saavedra, Medical Director, Nestlé USA, Dr. Roger Clemens, a consultant and expert in probiotics and nutrition, and me. The attached memorandum prepared by Dr. Clemens provides a general breakdown as to where responsive information to some of your general areas of inquiry can be found in the notification. The bulk of our call was spent on three relatively specific issues (aspiration pneumonia, intussusception, and considerations related to potential excessive doses of probiotics). The attached summary of our responses on those points has been prepared by Dr. Saavedra.

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I believe the foregoing and the attachments amply cover and reflect the substance of our call. Thank you and Dr. Kahl, sincerely, for scheduling the call and helping make it, in our view, a candid, constructive exchange.

Sincerely,

Fred H. Degnan

FDA Questions Re: Probiotic Notification

General Questions:

1. Are the two microorganisms well characterized?

   See submission, Volume 1, Tab A, pages 53-55 (Bifidobacterium), page 78 (Streptococcus).

2. What is the history of use of these two microorganisms in infant formula? Volume of use, estimates of number of subjects exposed, period of time of use in market.

   See submission, Volume 1, page 55-57 (Food Applications), and meeting notes from 1996 (notes not included). This type of infant formula has been produced (more than 40,000 lbs/year), marketed and accepted in Europe since 1991. In 1995-97, probiotic containing follow-on formula or "growing up milk" were introduced in Europe, Philippines, and China. Ten years of commercial distribution and more than 50 clinical studies among nearly 3,000 subjects have failed to present a single adverse event when infants and children consume this type of product for periods up to 18 months.

3. Are the two microorganisms susceptible to antimicrobial agents? Antibiotic sensitivity chart(s).

   See submission, Volume 1, Tab **A,** pages 57-59 (Bifidobacterium; virulence), pages 81-82 (Streptococcus; virulence), Volume 3, Tab F, Probiotics and Antibiotic Resistance. None of these organisms has demonstrated a broad-spectrum resistance to antibiotics. In addition, none of these organisms has demonstrated a broad antibiotic resistance upon the acute or chronic therapeutic or subtherapeutic doses of antibiotics.

4. What consideration has been given to concerns about gene transfer? Gene uptake of DNA? Gene recombination forming new pathogens? Information on capability for genetic material and plasmid transfer, comparison with other lactobacilli, etc.

5. Has a level of exposure to infants been shown to be safe for these two organisms?

   See submission Volume 1, Table 1 (Therapeutic Clinical Trials with Probiotics Fed Infants and Children, 1963-2000) and Table 2 (Prophylactic Clinical Trials with Probiotics Fed Infants and Children, 1963-2000). None of the feeding doses of $10^7 - 10^{11}$ cfu / day presented any adverse effects.

6. What are the accepted levels of these microorganisms in human milk banks?

   None of the microorganisms under discussion is naturally found in breastmilk, therefore, is not found in breastmilk available through the Human Milk Banking Association of North America.

August 10,2001
Created by **Roger** Clemens

Questions concerning the interaction between the two microorganisms and (1) the infant gut, and **(2)** other microorganisms in the infant gut.

7. How will the two microorganisms affect the environment of the infant's gut?

See submission, volume 1, pages 14-16 (Notes from March 2, 2000 meeting with Center Officials; volume 2, Tab D

8. What assurance is provided that there will be no transfer of genes or plasmids in the infant gut?

See submission, volume 1, pages 18-19 (Notes from March 2, 2000 meeting with Center Officials)

Questions regarding the effects of the two microorganisms on infant health.

9. Does the consumption of formula containing the two microorganisms affect an infant's metabolism?

See submission, volume 1, pages 20-21 (Notes from March 2, 2000 meeting with Center Officials)

10. What metabolic endpoints are produced by the two microorganisms and what are the effects of these endpoints on the health of infants?

See submission, volume 1, pages 22-24 (Notes from March 2, 2000 meeting with Center Officials)

11. Would consumption of the two microorganisms lead to greater incidence of infectious disease in infants?

See submission, Volume 1, Tab A, pages 57-59 (Bifidobacterium; Potential Adverse Effects), pages **8**1-83 (Streptococcus, Potential Adverse Effects). None of the known clinical studies and extensive experience indicate an increased morbidity of any kind associated with the consumption of an infant formula or growing up milk among infants and children.

12. What is the risk of aspiration-induced pneumonia?

There is not any known evidence that Bifidobacterium or *S. thermophilus* causes pneumonia. None of the clinical studies and practical commercial experience indicated any adverse effects among infants and children, including aspiration and possible upper respiratory infections.

13. Does consumption of infant formula containing the two microorganisms modulate (by increasing or by decreasing) the systemic immune system of infants?

See submission, Volume 1, pages 16-18 (Notes from March 2, 2000, meeting with Center Officials); Tab **A,** pages 6**1-63** (Bifidobacterium; Immunomodulatory Effects), **and** pages 84-85 (Streptococcus, Immunomodulatory Effects).

14. Would consumption of the two microorganisms lead to (1) adverse changes in normal physiological development of the immune system of the infant gut? (2) modulation of developing immune system? (3) stimulation of intestinal lymphoid?

    See submission, Volume 1, pages 19-20 (Notes from March 2, 2000, meeting with Center Officials), Volume 1, Tab **A;** Volume 2, Tab D; Volume 3, Tab E. None of the clinical studies, even among high-risk infants (atopic disease, Crohn's disease, chronic diarrheal disease), suggest probiotics may present untoward immunological effects in infants and children.

Possible relation between probiotic containing formulations or foods and aspiration pneumonia in infants with gastro esophageal (GE) reflux.

Aspiration pneumonia is an infection of the lung parenchyma (tissue) that results from the presence of oral, gastric or foreign material in the lower airway. Typically, aspiration of these materials will induce an inflammatory reaction in the airway depending on the location, the volume aspirated and the nature of these foreign substances. Material can be aspirated - directly, when a individual eats or drinks, and the swallowing mechanisms are not adequately coordinated to protect the airway, or - indirectly, when esophageal or gastric contents reflux back to the mouth or pharynx and can then be aspirated into the airway, again, when adequate swallowing mechanisms don't protect from entry of these in to the airway (larynx, trachea or bronchi).

When this aspiration occurs in infants, the most common effects are in the upper airway, and are reactive and inflammatory consequences (stridor, cough and wheezing from laryngeal and tracheal irritation , most commonly chemically induced, and typically they are not infectious). Gross aspiration of large amounts of oral secretions, with normal flora, or of refluxed materials occurs in significantly compromised infants or individuals, including tracheo esophageal fistulas, laryngeal, clefts, unconscious subjects, and infants and children with severe swallowing dysfunction due to CNS involvement, cerebral palsy, etc.

GE reflux occurs in essentially in all infants, and practically all normal infants will regurgitate ("spit up") gastric contents, in varying amounts and frequency. Most infants of course, do not develop aspiration pneumonia secondary to reflux. Thus, normal infants have mechanisms to protect them from aspiration and its complications. However, some infants and children can develop "pathologic gastro esophageal reflux". This is generally defined as GE reflux which leads to poor growth (from not taking and maintaining caloric intake), esophagitis (from gastric acid irritation of the distal –lower- esophagus), and respiratory symptoms, sometimes from aspiration of refluxed gastric and or esophageal contents. These respiratory symptoms, as mentioned above, typically present as inflammatory reactions of the airway, due to gastric acid contents irritating the upper airway and causing inflammatory reactivity. Aspiration pneumonia, in this context, and in the absence of the anatomic and neurologic risk factors mentioned above are not a common or significant complication, even in the majority of infants who "spit up".

Nevertheless, aspiration of enough foreign materials will occur in certain circumstances, as mentioned above. When this happens, the most common reaction is a "chemical pneumonitis", again from acid contents irritating lung tissue. Infection often occurs secondarily, and is usually caused by pathogenic flora (organisms normally found in the oral and respiratory tract) which can thrive in this environment. Most commonly these organisms are a mixed flora, mostly gram negatives, including *Klebsiella,* anaerobes such as *Bacteroides,* and gram positive organisms such as *Staphylococci.* These organisms are pathogens that can also cause infectious pneumonia that is unrelated to aspirative problems, as many other infectious pathogens of the airway.

We are not aware of any report of pneumonia due to *Bifidobacteria* or *Streptococcus thermophilus*, or other well described probiotic agents, nor related to foods (like fermented milk products). These agents have not been associated with pneumonia, occurring as a spontaneous infection, and are not included in extensive lists of respiratory pathogens. They also have not been associated to pneumonia due to direct aspiration while swallowing (as when eating foods with probiotics), nor by aspiration or regurgitated or vomited contents (following ingestion of probiotic containing foods, including yogurts or other fermented milk products). It is important to note that pneumonia can occur, spontaneously and from aspiration due to these organisms, which are normally found in the intestine *(Staphylococci, Klebsiella, Bacteroides*, etc), however, pneumonia has not been described with *Bifidobacteria*, despite this being the most common type of organism in the intestine of many infants. These probiotic organisms simply don't have this pathogenic capability.

In summary, there is no actual nor theoretical evidence to link probiotics, specially *bifidobacteria* to any potential for aspiration pneumonia in general, nor in infants with GE reflux.

- GE reflux in extremely common in normal infants. Respiratory complications occur in a significant minority of these.
- When respiratory problems occur, upper airway, reactive (bronchospasm), and non infectious inflammatory respiratory complications are the most common.
- Aspiration pneumonia is extremely unusual in the absence of significant risk factors (anatomical, neurologic, etc), and when it occurs it is most

common a chemical pneumonitis, which can become secondarily infected.

- When infection does occur, the organisms associated to these events have never been those ingested in probiotic containing foods, nor from the probiotic species which already are present in the intestine of children.
- In all the studies including adults, children and infants, ingesting probiotic organisms pneumonia associated to bifidobacteria have not been described. Thus, probiotic foods would have no more reason to be of any concern than any other foods or formulations, even in populations with the risk factors mentioned above.

Possible relationship between ingestion of probiotics and intussusception.

Intussusception occurs when a portion of the intestinal tract is telescoped into a segment just distal to it. It's among the most common causes of intestinal obstruction in infants; it is more common in male infants and is rare in neonates. Occasionally, affected infants resolve spontaneously, but most require medical or surgical intervention. The cause of intussusception is unknown. Some conditions that have been associated as contributory or possible triggers include viral infections, otitis media, gastroenteritis, and upper respiratory infections. In a small number of patients, recognizable "lead points" can be identified. These masses of protrusions into the intestinal lumen may stimulate intestinal peristalsis in an attempt to extrude the mass, thus causing an intussusception. Lead points are more common in very young and older patients. Examples of these lead points are an inverted appendiceal stump, Meckel diverticulum, an intestinal polyp, duplication, or lymphosarcoma. Other lesions include metastatic tumors, hemangioma, foreign bodies, parasitic infection, and fecoliths; and they can occur after cancer chemotherapy. Lead points are more common in very young and older patients. Recently, an increased risk for development of intussusception has been associated with the administration of rotaviral vaccine. It has been postulated that swollen Peyer patches in the ileum may act as a lead pont, stimulate intestinal peristalsis in an attempt to extrude the mass, thus causing an intussusception. This could be the mechanism in viral intestinal infections or rotavirus vaccine administration.

Given the generalities mentioned above, there is very little reason, if any at all, for any concerns that probiotic agents could increase the chances for intussusception infants and children.

- We are not aware of any association of ingestion of probiotic agents and intussusception in subjects of any agent consuming large amounts of probiotic supplemented or fermented foods.
- Intussusception has not been reported in any controlled or uncontrolled studies we are aware of, even in "high risk populations" such as infants and children in developing country situations where the exposure to large numbers of pathogens which would lead to increase in Peyer patch number or size (which could act as potential lead points) could heighten the risk.
- There are also theoretical reasons to suspect that the decrease in acute episodes of gastroenteritis, and more recently, of respiratory infections demonstrated in infant and children populations consuming probiotics may actually decrease the chances of development of intussusception.
- Additionally, it has become more evident from recent research that probiotics in general modulate the response of gut associated lymphoid tissue, rather than simply increasing lymphoid or inflammatory responses, and actually decreasing inflammatory reactions such as those associated to allergic food sensitization.
- Finally, many infants already have large amounts of bifidobacteria in the gut, in many situations significantly more than could ever be provided by any fermented product or probiotic formulations (see dose issue below), and breastfeeding infants (with large bifidobacterial populations in their intestine) are not more prone to intussusception (if anything they may be protected from it).

Considerations related to potentially "excessive doses of probiotics" in supplemented formulations or as supplements.

Most probiotic supplementation and intake doses have been determined by attempts to deliver doses "close" to what is already present in the large intestine and by technical and practical constraints. In fermented milk products for example, the maximal bacterial mass in the product will be limited by the substrate available for their metabolism in the milk, as well as the decrease in pH in the fermented milk product, which limits the growth of the organisms to maximum levels. Thus, products containing more than 10^6 to 10^8 are rarely feasible or available. Most yogurts or probiotic supplements, including capsules with lyophilized organisms contain below $10^6 - 10^8$ organisms per serving.

In the normal intestine (which can have close to 500 species) bacterial counts can reach up to $10^{12} - 10^{14}$ organisms per ml.

It is clear that significant "doses" of probiotics can be delivered for consumption, but these will rarely exceed the levels of some species already resident in the colon. Breast fed infants, for example, have amounts of bifidobacteria significantly above many formula fed infants. Supplemented formulas can increase bifidobacteria levels in children with low levels (such as those who are formula fed) but would not be able to deliver levels higher than what most breast feeding babies already have.

In addition, it should be noted that any increments in supplementation, to be significant in the context of ranges of intestinal organisms, need to be measured in logarithmic increases. As an example, to deliver one more log of probiotic bacteria using a particular product (such as a yogurt serving, or formula serving or capsule) this means increasing the dose by 10 times. Thus, if that probiotic product contains 10^7 organisms, one would have to take 10 servings (10 yogurt cups or 10 capsules) to increase intake by only one log. Consequently "overdosing" of these agents, with currently available products is not practically possible

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November 13, 2001

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FOOD ADDITIVE SAFETY

Paulette M. Gaynor, Ph.D.
Office of Food Additive Safety
HFS-215
Center for Food Safety and Applied Nutrition
Food and Drug Administration
1110 Vermont Avenue, N.W.
Washington, DC 20201

Re: GRAS Notification No, 49, *Bifidobacterium* and *Streptococcus Thermophilus*

Dear Dr. Gaynor:

Attached please find Roger Clemens' responses to the tentative questions posited last February concerning aspects of the manufacturing process for the cultures at issue as well as the antibiotic resistance profile for *S. Thermophilus*. Upon your review of these materials, please let me know if there is any additional information we can provide.

Sincerely,

Fred H. Degnan

001053

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1. Explain whether the manufacturing process includes washing steps or centrifugation to remove residual media components prior to lyophilization.

In summary, prime ampoules of the lactic acid cultures are aseptically prepared following microbiological GLP for production of commercial ampules. All media ingredients for commercial production with these ampules are food grade quality and sterilized in the fermenters, Cultures are grown under specified conditions and then centrifuged to concentrate the culture and to reduce the presence of residual fermentation media components. The culture concentrate is then lyophilized.

The general manufacturing steps for the probiotic cultures were described by Naidu, Bidlack and Clemens.[1] Specific steps based on those used by Chr. Hansen (Copenhagen, Denmark), the lactic acid bacteria supplier for Nestlé, are outlined below.

Prime Ampule Procedures:

Isolated lactic acid cultures are grown in two (2) liters of sterile media. The culutres are monitored for growth rate and lactic acid bacteria population density.

[1] Naidu AS, WR Bidlack, RA Clemens, Probiotic spectra of lactic acid bacteria (LAB), Crit Rev Food Sci Nutr 1999;38(1):12-126

When the desired population density is achieved, the culture is aseptically transferred to five (5) ml vials and rapidly heat-sealed under partial vacuum. These vials are then frozen and stored in liquid nitrogen. Twenty vials are evaluated €or quality and genetic punty. The quality tests include assessment of possible contaminants such as coliforms, *Bacillus cereus,* yeast and mold, and the presence of other lactics. Once the lot of prime ampules are evaluated and meet release criteria, they are accepted for production ampule preparation.

Production Ampule Procedures:

Production ampules are produced from prime ampules. The prime ampules are used to inoculate 1-2 liters of sterile media. This fermentation in this media is monitored and stopped at the optimum point (e.g., pH, concentration, viability) for harvesting. The ferment (media plus culture) is then transferred to 10 ml vials and frozen in liquid nitrogen. Similar to the primary ampules, the production ampules are tested for culture purity and genetic stability. When a statistical sampling of these vials and their contents meets release criteria, the contents are then used to produce the pre-inoculum culture for commercial production.

All work on ampules is performed under a Class 1 microbiological hood. The most stringent controls are associated with each step in order to minimize the possibility of microbiological contamination and to maintain culture purity.

001055

Inoculum

The production ampule is used to prepare the production inoculum. Using aseptic technique and sterile media, the inoculum is incubated under specifiad conditions to optimize growth and yield, and then cooled to 4°C.

This cooled pre-inoculum material is transferred under aseptic conditions to a sterilized 50-500 liter fermentation tank. Following the prescribed time under standardized growth conditions, the tank contents are cooled and tested for possible contamination. Once the contents pass quality control standards, the material is used to inoculate the commercial fermentation tanks, The transfer of inoculum to the fermentation tanks is performed using a pre-sterilized pumping system.

Fermentation

All of the raw ingredients are food grade and must meet specified compositional and microbiological standards, The sterilized media in the fermenters is tested for *B. cereus*, coliforms, Enterococcus, and *Staphylococcus aureus*. After the fermentation is completed, the ferment is once again tested for *B. cereus*,

- coliforms, Enterococcus, *S. aureus* as well as the concentration of cultured lactic acid bacteria via total plate count (TPC).

Once the ferment meets quality control release standards and achieves the desired lactic acid bacteria population, it is centrifuged to concentrate and prepare the culture for lyophilization and to reduce the concentration of the growth media. Again, the concentrated culture is tested for the presence of *B. cereus*, coliforms, Enterococcus, *S. aureus* and assessed for total count of lactic acid bacteria. Additional microbiological assessment of the finished lyophilized product includes fecal streptococci, yeast / mold; aerobic sporeformers, *Listeria, Salmonella* as well as the possible presence of othm microbiological contaminants previously indicated.

2. Present information exists regarding the antibiotic resistance profile for *S. thermophilus*.

S. thermophilus and *L. bulgaricus* are required bacterial cultures for yogurt marketed in the United States (21 CFR 131.200) and have been an essential component of yogurts for decades. This statute does not specify the strain of *S. thermophilus* nor does it indicate any standard or requirement to evaluate potential antibiotic resistance of yogurt cultures. While many investigators have reported antibiotic resistances of lactic acid bacteria, only one publication since 1980

reports on antibiotic resistance and sensitivity of *S. thermophilus*. (Sozzi T and MB Smiley, Antibiotic resistances of yogurt starter cultures *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, Appl Environ. Micro 1980; 40(5):862-865).

Sozzi and Smiley evaluated the potential antibiotic resistance of 15 strains of *S. thermophilus* relative to 35 antimicrobial agents. These strains were obtained from yogurt manufacturers located in Europe and the USA. The antibiotic resistance pattern from,this study is shown in the attached table. All cultures were sensitive to ampicillin (0.5 μg), aureomycin (10 μg), bacitracin (5U), carbenicillin (50μg), cephaloridine (30 μg), cephalothin (30 μg), chloromycetin (5 μg), clindamycin (2 μg), dicloxacillin (1 μg). doxycycline (30 μg), erythromycin (2 μg), Mandelamine (3 μg), methicillin (5 μg), oleandomycin (10 μg), penicillin G (0.5 U), rifampin (5 μg), Temrnycin (10 μg), and tetracycline (1 μg). None was resistant to penicillin, tetracycline, or erythromycin. In this study, most strains of *S. thermophilus* were naturally resistant to neomycin, polymyxin B, and streptomycin. More recent studies, however, suggest some unspecified strains of *S. thermophilus* are penicillin-resistant.[2,3] None of the antibiotic resistance assessment studies included the Th4 strain of *S. thermophilus*. Since *S.*

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2. Chirica LC, Guray T, Gurakan GC, Bozoglu TF, Characterization of extracellular beta-lactamases from penicillin G-resistant cells of Streptococcus thermophilus, J Food Prot 1998; 61(7):896-898
3. Korycha-Dahl M, Richardson T, Bradley RL Jr, Use of microbial beta-lactamase to destroy penicillin added to milk, J Dairy Sci 1985; 68(8):1910-1916

thermophilus has a long history of food usage in many countries, including the United States, and since that none of the studied strains of this organism demonstrated a resistance to a broad spectrum of antibiotics, but rather presented a sensitivity to many common antimicrobial agents, the continued use of *S. thermophilus*, including the Th4 strain used by Nestlé, in the food supply does not present an adverse public health risk.

1. Explain whether the manufacturing process includes washing steps or centrifugation to remove residual media components prior to lyophilization.

In summary, prime ampoules of the lactic acid cultures are aseptically prepared following microbiological GLP for production of commercial ampules. All media ingredients for commercial production with these ampules are food grade quality and sterilized in the fermenters. Cultures are grown under specified conditions and then centrifuged to concentrate the culture and to reduce the presence of residual fermentation media components. The culture concentrate is then lyophilized.

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All work on ampules is performed under a Class I microbiological hood. The most stringent controls are associated with each step in order to minimize the possibility of microbiological contamination and to 'maintain culture purity,

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S. thermophilus and *L. bulgaricus* are required bacterial cultures for yogurt marketed in the United States (21 CFR 131.200) and have been an essential component of yogurts for decades. This statute does not specify the strain of *S. thermophilus* nor does it indicate any standard or requirement to evaluate potential antibiotic resistance of yogurt cultures. While many investigators have reported antibiotic resistances of lactic acid bacteria, only one publication since 1980

reports on antibiotic resistance and sensitivity of *S. thermophilus*. (Sozzi T and MB Smiley, Antibiotic resistances of yogurt starter cultures *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, Appl Environ Micro 1980; 40(5):862-865).

Sozzi and Smiley evaluated the potential antibiotic resistance of 15 strains of *S. thermophilus* relative to 35 antimicrobial agents. These strains were obtained from yogurt manufacturers located in Europe and the USA. The antibiotic resistance pattern from this study is shown in the attached table. All cultures were sensitive to ampicillin (0.5 μg), aureomycin (10 μg), bacitracin (5U), carbenicillin (50μg), cephaloridine (30 μg), cephalothin (30 μg), chloromycetin (5 μg), clindamycin (2 μg), dicloxacillin (1 μg). doxycycline (30 μg), erythromycin (2 μg), Mandelamine (3 μg), methicillin (5 μg), oleandomycin (10 pg), penicillin G (0.5 U), rifampin (5 μg), Terramycin (10 μg), and tetracycline (1 μg). None was resistant to penicillin, tetracycline, or erythromycin. In this study, most strains of *S. thermophilus* were naturally resistant to neomycin, polymyxin B, and streptomycin, More recent studies, however, suggest some unspecified strains of *S. thermophilus* are penicillin-resistant.[2,3] None of the antibiotic resistance assessment studies included the Th4 strain of *S. thermophilus*. Since *S.*

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2. Chirica LC, Guray T, Gurakan GC, Bozoglu TF, Characterization of extracellular beta-lactanlases from penicillin G-resistant cells of Streptococcus thermophilus, J Food Prot 1998; 61(7):896-898
3. Korycha-Dahl M, Richardson T, Bradley RL Jr, Use of microbial beta-lactamase to destroy penicillin added to milk, J Dairy Sci 1985; 68(8):1910-1916

thermophilus has a long history of food usage in many countries, including the United States, and since that none of the studied strains of this organism demonstrated a resistance to a broad spectrum of antibiotics, but rather presented a sensitivity to many common antimicrobial agents, the continued use of *S. thermophilus*, including the Th4 strain used by Nestlé, in the food supply does not present an adverse public health risk.

1 **Table 1. Antibiotic Resistance[4] Patterns for strains** of *S. thermophilus*

Antibiotic	Conc	Bacterial Strains														
		YS-1	YS-2	YS-3	YS-4	YS-5	YS-6	YS-7	YS-8	YS-9	YS-10	YS-11	YS-12	YS-14	YS-15	YS-16
Cloxacillin'	1 µg					R										
Colimycin	10 µg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Dihydrostreptomycin[5]	10 µg	R	R	R	R	R	R	R	R	R	R		R	R		R
Doxycycline	10 µg															
Furadantin'	50 µg		R	R		R	R									
	300 µg															
Gentamicin	10 µg	R	R	R	R	R	R			R	R	R	R	R		R
Kanamycin	5 µg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	30 µg	R	R		R	R	R	R	R	R	R		R	R		R
Lincomycin[5]	2 µg															
Mandelamine	2 µg															
Mycostatin	100 µg	R	R	R		R	R	R		R	R	R	R	R	R	R
Nalidixic acid	30 µg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Neomycin'	5 µg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	30 µg	R	R	R	R	R	R	R	R	R	R	R	R	R		R
Novobiocin'	10 µg	R						R								
	30 µg	R														
Oleandomycin	5 µg		R													
Oxacillin'	1 µg							R								
Polymyxin B[5]	50 µg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sulfamethoxazole-trimethoprim	300 µg	R	R	R		R	R	R		R	R		R	R	R	R
Streptomycin	2 µg	R	R	R	R	R	R	R	R	R	R		R	R	R	R
	10 µg	R	R	R	R	R	R	R		R	R		R	R		R
Sulfadiazine	300 µg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sulfamethoxypyridazine	300 µg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

2

[4] Resistance is denoted by R; unless so indicated, strains were sensitive.

[5] Antibiotic reported by GW Reinhold and MS Reddy [Sensitivity or resistance dairy starter and associated microorganisms to selected antibiotics, J. Milk Food Tech 1974;37:517-521]

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