Clinical applications of probiotic agents[1–3]

Jose M Saavedra

ABSTRACT In the past century the beneficial roles of nonpathogenic bacteria in the intestinal lumen were described. In the past decade there has been a dramatic increase in scientific work supporting the concept that there are clinical benefits to ingesting specific nonpathogenic organisms (probiotics). The potential benefits of modifying the intestinal flora composition of certain high-risk groups, eg, premature infants, travelers, and children receiving antibiotics, are emerging in the literature. Studies documenting prophylactic and therapeutic benefits in acute viral gastroenteritis and in atopic disease point not only to the potential applications, but also to the fact that the mechanisms of action of these agents may be due to their interaction with the gut as an immunologic organ. The benefits documented thus far are of varying degree and are most likely dependent on the number of agents, the dose, the dosing patterns, and the characteristics of the host and its underlying luminal microbial environment. Consequently, the safety and specification of a particular probiotic agent and methods of delivery to a particular population for a particular purpose should be carefully documented before making broad recommendations. The cost-benefit assessment of adding probiotics to our diet for prophylactic or therapeutic purposes, as well as better regulation of these agents as commercial products, is also needed. *Am J Clin Nutr* 2001;73(suppl):1147S–51S.

KEY WORDS Probiotics, probiotic agents, intestinal flora, lactose malabsorption, antibiotics, diarrhea, traveler's diarrhea, antibiotic-associated diarrhea, rotavirus, atopic disease, lactobacilli, bifidobacteria

INTESTINAL FLORA AND OUR ENVIRONMENT

The lumen of the intestine is in a continuum with the environment. A substance that is ingested and not absorbed by the gut mucosa will travel through the lumen without traversing any human cell membrane and be returned to the environment. The intestine has evolved into an organ that can propel what we eat through the lumen: our diet is that part of our immediate environment with which we exchange water, minerals, and nutrients via its digestive and absorptive capabilities. In addition, it serves the function of a barrier against those components that may not be of benefit to us, a significant part of which is composed of microorganisms. We live in a heavily contaminated bacterial environment. After the discovery of pathogenic bacteria as a cause of numerous infectious conditions, we began a relentless battle against these pathogens, sterilizing our food supply and arming ourselves with an ever-growing arsenal of antimicrobials, which these organisms eventually learned to resist. An innocent victim of this war is that component of our nonpathogenic bacterial environment that may actually be beneficial to us. As a species, we are clearly outnumbered and a symbiotic relation is not only inevitable but necessary. A human individual has more prokaryotic organisms associated to skin, lung, and gut surfaces than to human eukaryotic cells.

It was after the description of pathogens that we began recognizing the potential benefits of this symbiosis. Later, we clearly established the importance of the intestinal flora in the maintenance of the gut luminal milieu and its effects on intestinal epithelium, mucosal integrity, and vitamin and nutrient metabolism and absorption. Consequently, a logical, albeit simplistic approach to the situations that alter our gut microbial environment (eg, diet and environmental changes and antibiotic use) would be to deliberately increase our association with specific nonpathogenic organisms to counter such an enviromental alteration.

Conceptually, the use of these nonpathogenic probiotic agents constitutes a purposeful attempt to modify the relation with our immediate microbial environment in ways that may benefit our health. We review here several of the clinical applications that have been documented for such agents.

LACTOSE MALABSORPTION

Mammals are born with sufficient lactase activity to use the lactose in the milk from their mothers. There is a reduction of lactase activity in the intestinal brush border of mammals as they age after weaning. The ingestion of dairy products containing lactose leads to signs and symptoms of lactose intolerance, eg, increased abnormal gas, bloating, flatus, abdominal pain, and diarrhea (1). When consumed as a supplement with dairy products or as part of cultured dairy foods, most nonpathogenic bacteria, many of which are used in the fermentation of milk products, including several strains of *Lactobacillus* (eg, *Lactobacillus bulgaricus*) and *Streptococcus thermophilus*, can exert their lactase activity in vivo in the gut lumen, thus facilitating digestion and alleviating intolerance. This has been well shown in both adults and children (2–4).

[1] From the Johns Hopkins University School of Medicine, Balitmore.

[2] Presented at a symposium held at Experimental Biology 2000, in San Diego, April 2000.

[3] Reprints not available. Address correspondence to JM Saavedra, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Brady 320, Baltimore, MD 21287. E-mail: jsaave@jhmi.edu.

INTESTINAL FLORA IN INANTS AND PROBIOTICS

Breast-feeding infants protects them from infectious disease. There are multiple mechanisms that can explain such protection. However, given the fact that the cellular and humoral components of human milk may have an effect in modulating the composition of the intestinal flora, a fair amount of attention has been given to the differences in intestinal flora between breast-fed and bottle-fed children. Although there are wide individual and population variations, bifidobacteria generally constitute a significant component of normal intestinal flora in breast-fed infants (5, 6). Some factors in breast milk that may enhance the selective growth of bifidobacteria include the presence of *N*-acetylglucosamine, glucose, lactoferrin, galactose, and fructose, and other not as well-described bifidogenic factors (7–9). Breast-feeding can also affect the occurrence and virulence of colonizing pathogens (10–12). Thus, although the mechanisms have yet to be fully elucidated, it appears that a combination of increased bifidobacterial counts and decreased concentrations of other enterobacteria and luminal host factors may play a role in protecting premature babies and newborns from diarrheal disease.

Modification of the intestinal flora by increasing the predominance of specific nonpathogenic bacteria would seem a reasonable means of attaining a prophylactic or therapeutic effect against enteropathogens. *Lactobacillus* GG can colonize the gut of premature infants, but had little effect on enterobacteria, yeasts, or staphylococci in small observational studies (13, 14). A recent trial documented a reduction of necrotizing enterocolitis in a population of premature newborns given a supplement of *Lactobacillus* GG daily compared with historical control subjects (15). Prospective studies examining this possible application are in progress.

DIARRHEA

Clostridium difficile diarrhea

Several investigators reported the resolution of recurrent *Clostridium difficile* diarrhea with oral supplementation of *Lactobacillus* GG and *Saccharomyces boulardii* in adults (16, 17) and children (18, 19); other *Lactobacillus* strains did not have the same effect (20). Prospective controlled studies are needed to confirm the efficacy of probiotics for this application.

Traveler's diarrhea

Lactobacillus GG was found to be effective in the prevention of traveler's diarrhea in some studies (21) but the effect may not be uniform or consistent, depending on the geographic area or populations studied (22). Other lactobacilli preparations have not produced any significant, positive results (23) and *S. boulardii* may have only a marginal effect (24). The many variables and varied populations involved in these studies do not allow for any generalizations. Although the evidence may suggest a protective effect, the variety of agents used, the difficulties involved with measuring compliance, and the lack of etiologic documentation of diarrhea in several of these studies make forming recommendations or conclusions difficult.

Antibiotic-associated diarrhea

Antibiotics can severely disrupt gut microbial ecology. Ingestion of a probiotic with a prescribed antibiotic can reduce the effect of such microbial alteration and any resulting changes in stool consistency and frequency. Several reports, and more recently a few controlled studies, showed the efficacy of several agents in the management of non–*C. difficile*, antibiotic-associated diarrhea. Agents used included *Enterococcus faecium* SF68 (25), *Lactobacillus* GG (26, 27), *Lactobacillus acidophilus, L. bulgaricus* (28, 29), and *S. boulardii* (30, 31). In general, these agents reduced the changes in bowel habit, decreasing the changes in stool consistency and the duration of loose stools associated with antibiotic use.

Treatment of diarrheal disease in children

The best-established benefit of using probiotic agents has been in the management of acute pediatric diarrheal disease. Several large and well-controlled studies showed a significant decrease in the duration of diarrhea in children who received *Lactobacillus* GG, either as a supplement or in fermented milk, early in the course of the condition (32–34). In a large multicenter trial in which *Lactobacillus* GG was added to an oral rehydration solution and given to children during a diarrheal episode (35), there was also a significant reduction in the duration of illness; similar results were observed with *Lactobacillus reuteri* (36). In 2 controlled trials, *S. boulardii* administration ameliorated the purge during acute diarrheal illness (37, 38). Very few studies have compared the effect of several probiotic agents. In one study, *Lactobacillus* GG was shown to be more effective when compared with *Lactobacillus rhamnosus* or a combination of *S. thermophilus* and *Lactobacillus delbruckii* (39).

In general, depending on the definitions used for diarrhea and duration of illness, the use of probiotics during an episode resulted in a shortened course of illness of 1–3 d, with varying decreases in purge. In addition, the efficacy appears to be greater in diarrhea of viral etiology. The beneficial effect (as it refers to clinical indexes) was shown to be accompanied by a greater immunoglobulin A–antibody secreting response (40) and less rotaviral shedding (34, 39) in children treated with these agents than in children treated with placebo.

Few negative studies have been reported. *Streptococcus salivarius* ssp., *S. thermophilus*, and *L. delbruckii*, were not efficacious in ameliorating diarrhea in hospitalized children (41) and a commercially available bifidus yogurt and oral preparations of bifidobacteria did eradicate *Campylobacter jejuni* from stools in children with enteritis, although this occurred less rapidly than it did in patients treated with standard erythromycin (42). It is becoming evident that different strains may have different potential benefits.

Most studies of the management of acute diarrhea have been conducted in relatively healthy and stable populations. Further studies are needed in children with protracted diarrhea or other risk factors, including immune deficiencies. Finally, cost-benefit analyses of the effects shown still need to be done, taking into account the agent, the vehicle (eg, supplementation as freeze dried powder and oral rehydration solutions), and the population targeted.

Prevention of diarrheal disease in children

The regular consumption of specific probiotic agents over extended periods of time (weeks to months) was shown to decrease the incidence of acute diarrhea in several well-designed trials. Supplementation of an infant formula with bifidobacteria and *S. thermophilus* resulted in a decreased incidence of diarrheal disease and rotaviral shedding in a population of chronically hospitalized children over 17 mo (43). Other similar stud-

ies in hospitalized children are in progress. In a large, well-controlled study, children in an underprivileged periurban area had a lower incidence of diarrheal disease with the regular administration of a daily dose of *Lactobacillus* GG, 6 d a week for 15 mo (44). The effect was evident only in non-breast-fed infants. Other similar studies in high-risk populations are in progress.

Although few in number, long-term studies involving specific strains of bifidobacteria and lactobacilli, such as the ones mentioned above, suggest the prophylactic potential and safety of such probiotics. For the purpose of long-term prophylactic use, incorporating these agents into the food supply would seem to be a better alternative to daily individual supplementation. Cost-benefit analysis for specific populations and high-risk groups and methods of delivery (eg, infant formula) need to be considered.

BACTERIA COMPARED WITH VIRUSES

The fact that these orally ingested bacterial agents show a prophylactic and therapeutic effect against intestinal viruses suggests that this effect is most likely mediated through the stimulation of gut-associated lymphoid tissue, which results in an increased humoral antigenic response. The increased immunogenicity of rotavirus vaccine when administered with lactobacilli (45) and the clinical observations of decreased rotaviral shedding in populations receiving probiotics, therapeutically or prophylactically, suggests this type of response. The potential immunologic effects induced by these agents are reviewed elsewhere in this issue (46).

These findings of improved antiviral response were also shown in animals (47). Furthermore, passive protection against rotavirus-induced diarrhea in mouse pups born to and nursed by dams fed *Bifidobacteria breve* was also shown (48). This passive protection was associated with increased concentrations of antirotavirus immunoglobulin A in the milk of the dams fed bifidobacteria and immunized orally with rotavirus. These observations suggest numerous potential applications for probiotic use, such as to heighten the immunologic response to vaccines, potentially decreasing the necessary number of boosters, and to further enhance the natural passive protection of breast feeding by maternal ingestion of probiotics.

ATOPIC DISEASE

The traditional approach to food hypersensitivity, of which atopic disease is a manifestation, has been the elimination of potential protein offenders in the diet. This has lead to the use of elimination diets and to the development of increasingly elemental formulations for this purpose. Both approaches are difficult to implement and as costly. Intestinal microflora can contribute to the processing of food antigens in the gut and probiotics could modify the structure of potential antigens and reduce their immunogenicity. Moreover, gut microflora contribute to the generation of a T helper population amenable to oral tolerance induction, as discussed elsewhere in this issue (46). This offers a new therapeutic approach to the management of hypersensitive disorders. The results of the first prospective studies are now available, which show a significant improvement of atopic dermatitis in children (49) and markers of allergic response in children and adults (50, 51) with the use of lactobacilli and bifidobacteria.

CLINICAL SAFETY AND TOLERANCE OF PROBIOTICS

Fermented dairy and cereal products have been consumed for centuries. Over the past few years, young children have exponentially increased their consumption of fermented milks (yogurts) with no record of apparent adverse events. A recent review identified 143 human clinical trials using multiple probiotic agents between 1961 and 1998, involving >7500 subjects with no adverse events reported (52). Nevertheless, it is important to establish the safety of long-term probiotic consumption by the general public and by high-risk groups if specific recommendations and indications are to be made. Few studies have closely followed large populations for long periods of time and monitored adverse events (45, 53). Intakes of 10^6–10^9 colony-forming units daily of bifidobacteria and lactobacilli for ≤1 y resulted in no observed adverse effects. In addition, children receiving bifidobacteria not only tolerated the agent well from a gastrointestinal point of view, but generally experienced less-frequent and less-hard bowel movements and a decreased frequency of diaper rash (54). Although only very rare instances of bacteremia from lactobacilli have been reported, most such studies have not documented the occurrence by products or agents currently in the food supply. In addition, it is impossible in most such cases to determine whether this was not lactobacilli bacteremia from translocation of the individual's own native colonic flora, which frequently contains certain components of lactobacilli. In summary, most lactobacilli and bifidobacteria used in the food industry, as well as the better-studied lactobacilli and bifidobacteria reported in clinical trials, appear to be safe for the general adult and pediatric populations.

With regard to doses, the use of multiple probiotic strains and the many doses and dosing patterns result in making generalizations difficult. The range of doses reported is quite broad. It is generally considered that doses between 10^6 and 10^9 colony-forming units daily are required and most clinical trials use doses within these ranges. It is important that the specific strains and dosing patterns are clearly reported and that there is adequate quality control to ensure the possibility of ultimate recommendations of a specific agent for a specific purpose.

Finally, contributing to the difficulties of recommending probiotic agents for clinical application is the lack of regulation and standardization. Many over-the-counter probiotics now widely available in health food stores are neither reliable nor effective as remedies (55). They are sold under the general umbrella of probiotics, with disguised or sometimes overt claims. Difficulties related to the claims that can be made for a food component, eg, a probiotic in fermented milk, in terms of disease prevention or amelioration, compound the problem. The general category of these functional foods or food components has established the need to revise these claims processes and definitions. Sensible regulation of products and claims, and responsible actions by the industry, are critical to standardizing and establishing uses and recommendations for specific probiotic strains.

CONCLUSIONS

Probiotics are clearly established as an adjuvant in the management of lactose malabsorption and acute diarrhea, particularly acute infant diarrhea viral etiology. Probiotics may also have a prophylactic effect in terms of decreasing the incidence of illness when taken regularly, the effect of which appears to

be greater in high-risk populations (eg, children who are hospitalized, non-breast-fed, or living in underprivileged conditions). The documented therapeutic effects include decreases in duration (shorter episodes) or severity (diminution of purging) of illness. Most of these effects were statistically significant but relatively small and documented in relatively healthy populations. It is possible that these effects might be more evident and of greater clinical relevance in the high-risk populations, including infants and children with malnutrition, severe malabsorption, or protracted diarrhea. Several strains have also been proven to ameliorate the course of antibiotic-associated diarrhea.

Probiotic agents appear promising for the management of *C. difficile* colitis, atopic disease, necrotizing enterocolitis, and other gut conditions, such as inflammatory bowel disease. A significant number of observations point to an immunomodulatory effect as the mechanism of action of these agents. Most of the agents currently being studied and in use appear to be safe, with no apparent adverse effects noted in the thousands of subjects reported or those individuals consuming fermented milk products.

Specific agents with proven efficacy and specific doses of such agents need to be studied in double-blind, controlled trials before specific claims are made because not all probiotic agents behave similarly. Future studies involving the comparisons between agents and doses, cost-benefit analyses, and efforts to determine the exact mechanisms by which these agents yield their effects need to be conducted.

REFERENCES

1. Saavedra JM. Carbohydrate malabsorption. In: Brandt LJ, ed. Clinical practice of gastroenterology. Philadelphia: Current Medicine Publishers, 1999:1312–8.
2. Kolars JC, Levitt MD, Aouji M, Savaiano DA. Yogurt—an autodigesting source of lactose. N Engl J Med 1984;310:1–3.
3. Montes RG, Saavedra JM, Bayless TM, Perman JA. Effect of milks inoculated with *Lactobacillus acidophilus* or a yogurt starter culture in lactose-maldigesting children. J Dairy Sci 1995;78:1657–64.
4. Shermak MA, Saavedra JM, Jackson TL, Huang SS, Bayless TM, Perman JA. Effect of yogurt on symptoms and hydrogen production in lactose-malabsorbing children. Am J Clin Nutr 1995;62:1003–6.
5. Braun, OH. Effects of consumption of human milk and other formulas on intestinal bacterial flora in infants. In: Lebenthal E, ed. Textbook of gastroenterology and nutrition in infancy. New York: Raven Press, 1989:247–51.
6. Yoshita M, Fujita K, Sakata H. Development of the normal intestinal flora and its clinical significance in infants and children. Bifido Microflora 1991;10:11–27.
7. McKellar RC, Moddler HW. Metabolism of fructoligosaccharides by *Bifidobacterium* sp. Appl Microbiol Biotechnol 1989;31:537–41.
8. Gauhe AP, Gyorgy PA, Hoover, JR A, et al. Bifidus factor. Preparations obtained from human milk. Arch Biochem 1954;48:214–24.
9. Drasar BS, Roberts AK. Control of the large bowel microflora. In: Hill MJ, Marsh PD, eds. Human microbial ecology. Boca Raton, FL: CRC Press, 1990:87–111.
10. Steinwender G, Schimpl G, Sixl B, et al. Effect of early nutritional deprivation and diet on translocation of bacteria from the gastrointestinal tract in the newborn rat. Pediatr Res 1995;39:415–20.
11. Maxson RT, Jackson RJ, Smith SD. The protective role of enteral IgA supplementation in neonatal gut origin sepsis. J Pediatr Surg 1995;176:231–3.
12. Mata LJ, Urrutia JJ. Intestinal colonization of breastfed children in rural area of low socio-economic level. Ann N Y Acad Sci 1971;176:93–109.
13. Millar MR, Bacon C, Smith SL, Walker V, Hall, MA. Enteral feeding of premature infants with *Lactobacillus* GG. Arch Dis Child 1993;69:483–7.
14. Reuman PD, Duckworth DH, Smith KL, Bagan R, Buccarelli RL, Ayoub EM. Lack of effect of *Lactobacillus* on gastrointestinal bacterial colonization in premature infants. Pediatr Infect Dis 1986;5:663–8.
15. Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium* infantis to neonates in an intensive care unit. Int J Infect Dis 1999;3:197–202.
16. Gorbach SL, Chang TW, Goldin B. Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. Lancet 1987;2:1519.
17. McFarland LV, Suwawicz CM, Greenberg RN, et al. A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. JAMA 1994;271:1913–8.
18. Biller JA, Katz AJ, Flores AD, Buie TM, Gorbach SL. Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J Pediatr Gastroenterol Nutr 1995;21:224–6.
19. Buts JP, Corthier G, Delmee M. *Saccharomyces boulardii* for *Clostridium difficile*-associated enteropathies in infants. J Pediatr Gastroenterol Nutr 1993;16:419–25.
20. Aronsson LT, Barany P, Nord CE. *Clostridium difficile* associated diarrhoea in uremic patients. Eur J Clin Microbiol 1987;6:352–6.
21. Hilton E, Kolakowski P, Singer C, Smith M. Efficacy of *Lactobacillus* GG as a diarrheal preventative in travelers. J Travel Med 1997;4:41–3.
22. Oksanen PJ, Salminen S, Szxelin M, et al. Prevention of traveler's diarrhoea by *Lactobacillus* GG. Ann Med 1990;22:53–6.
23. Pozo-Olano JD, Warram JG Jr, Gomez RG, Cavazos MG. Effect of a lactobacilli preparation on travelers diarrhea: a randomized double blind clinical trial. Gastroenterology 1978;74:829–30.
24. Kollaritsch H, Holst H, Grobara P, Wiederman G. Prophylaxe der Reisediarrhoe mit *Saccharomyces boulardii*. (Prophylaxis of traveler's diarrhea with *Saccharomyces boulardii*.) Fortschr Med 1993; 111:153–6 (in German).
25. Wunderlich PD, Braun L, Fumagalli I, D-Apuzzo V, Heim F, Karly M, et al. Double-blind report on the efficacy of lactic acid-producing *Enterococcus* SF68 in the prevention of antibiotic-associated diarrhoea and in the treatment of acute diarrhoea. J Int Med Res 1989;17:333–8.
26. Siitonen S, Vapaatalo J, Salminen S, et al. Effect of *Lactobacillus* GG yogurt in prevention of antibiotic-associated diarrhoea. Ann Med 1990;22:57–9.
27. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. J Pediatr 1999;135:564–8.
28. Gotz V, Romankiewica JA, Moss J, Murray HW. Prophylaxis against ampicillin-associated diarrhea with a *Lactobacillus* preparation. Am J Hosp Pharm 1979;36:754–7.
29. Silva M, Jacobus N, Deneke C, Gorback S. Antimicrobial substance from a human *Lactobacillus* strain. Antimicrob Agents Chemother 1987;31:1231–3.
30. Surawicz CM, Elmer GW, Speelman P, McFarland LV, Chinn J, van Belle G. Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardii:* a prospective study. Gastroenterology 1989;96: 981–8.
31. McFarland LV, Surqwica CM, Greenberg RN, et al. Prevention of β-lactam-associated diarrhea by *Saccharomyces boulardii* compared to placebo. Am J Gastroenterol 1995;90:439–48.
32. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula, T. A human *Lactobacillus* strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics 1991;88: 90–7.
33. Raza S, Graham SM, Allen SJ, Sultana S, Cuevas L, Hart CA. *Lactobacillus* GG promotes recovery from acute nonbloody diarrhea in Pakistan. Pediatr Infect Dis J 1995;14:107–11.

34. Guarino A, Canani RB, Spagnuolo MI, Albano F, Benedetto L. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. J Pediatr Gastroenterol Nutr 1997;25:516–9.
35. Guandalini S, Pensabene L, Zikri MA, et al. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. J Pediatr Gastroenterol Nutr 2000;30:54–60.
36. Shornikova AV, Casas IA, Isolarui E, Mykkanen H, Vesikari T. *Lactobacillus reuteri* as a therapeutic agent in acute diarrhea in young children. J Pediatr Gastroenterol Nutr 1997;24:399–404.
37. Chapoy P. Treatment of acute infantile-diarrhea: controlled trial of *Saccharomyces boulardii*. Ann Pediatr 1985;32:561–3.
38. Cetina-Sauri G, Basto GS. Evaluacion terapeutica de *Saccharomyces boulardii* en ninos con diarrhea aguda. (Therapeutic evaluation of *Saccharomyces boulardii* in children with acute diarrhea.) Tribuna Med 1989;56:111–5 (in Spanish).
39. Majamaa H, Isolarui E, Saxelin M, Vesikari T. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. J Pediatr Gastroenterol Nutr 1995;20:333–8.
40. Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. Pediatr Res 1992;32:141–4.
41. Pearce JL, Hamilton JR. Controlled trial of orally administered lactobacilli in acute infantile diarrhea. J Pediatr 1974;84:261–2.
42. Hotta M, Sato Y, Iwata S, et al. Clinical effect on *Bifidobacterium* preparations on pediatric intractable diarrhea. Keio J Med 1987;36:298–314.
43. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 1994;344:1046–9.
44. Oberhelmean RA, Gilman RH, Sheen P, et al. A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. J Pediatr 1999;134:15–20.
45. Isolauri E, Joensuu J, Suomalainen H, Luomala M, Veikari T. Improved immunogenicity of oral DxRRV reassortant rotavirus vaccine by *Lactobacilluus casei* GG. Vaccine 1995;13:310–2.
46. Isolauri E. Probiotics in human disease. Am J Clin Nutr 2001;73(suppl):1142S–6S.
47. Duffy LC, Zielezny MA, Riepenhoff-Talty M, et al. Effectiveness of *Bifidobacterium* in mediating the clinical course of murine rotavirus diarrhea. Pediatr Res 1994;36:690–5.
48. Yasui H, Kiyoshima J, Ushihama H. Passive protection against rotavirus-induced diarrhea of mouse pups born to and nursed by dams fed *Bifidobacterium breve* YIT4064. J Infect Dis 1995;172:403–9.
49. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol 1997;99:179–86.
50. Isolauri E, Arvola T, Sutas Y, Salminen S. Probiotics in the management of atopic eczema. Clin Exp Allergy 2000;30:1604–10.
51. Pelto L, Isolauri E, Lilius EM, Nuutila J, Salminen S. Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. Clin Exp Allergy 1998;28:1474–9.
52. Naidu AS, Bidlack WR, Clemens RA. Probiotic spectra of lactic acid bacteria (LAB). Crit Rev Food Sci Nutr 1999;39:13–126.
53. Abi-Hanna A, Moore N, Yolken R, Saavedra JM. Long-term consumption of infant formulas with live probiotic bacteria: safety and tolerance. J Pediatr Gastroenterol Nutr 1998;7:A84 (abstr).
54. Saavedra JM, Abi-Hanna A, Moore N, Yolken R. Effect of long term consumption of infant formulas with Bifidobacteria and *S. thermophilus* on stool patterns and diaper rash in infants. J Pediatr Gastroenterol Nutr 1998;27:A82 (abstr).
55. Hamilton-Miller JM, Shah S, Smith CT. "Probiotic" remedies are not what they seem. BMJ 1996;312:55–6.