CLINICAL MICROBIOLOGY REVIEWS, Oct. 2003, p. 658–672 Vol. 16, No. 4
0893-8512/03/$08.00+0 DOI: 10.1128/CMR.16.4.658–672.2003

Potential Uses of Probiotics in Clinical Practice

Gregor Reid,[1,2,3]* Jana Jass,[1,2] M. Tom Sebulsky,[2] and John K. McCormick[1,2]

Canadian Research and Development Centre for Probiotics, The Lawson Health Research Institute, London, Ontario N6A 4V2,[1] and Departments of Microbiology and Immunology[2] and Surgery,[3] University of Western Ontario, London, Ontario N6A 5C1, Canada

INTRODUCTION

The term probiotic was derived from the Greek, meaning "for life." The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have stated that there is adequate scientific evidence to indicate that there is potential for probiotic foods to provide health benefits and that specific strains are safe for human use (38). An expert panel commissioned by FAO and WHO defined probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host." This is the definition that should be used, and probiotics should not be referred to as biotherapeutic agents (84).

Probiotics represents an expanding research area. A Medline search of the term probiotics illustrates the significant increase in research undertaken in this area during the past 5 years: over 1,000 publications cited, compared to 85 for the previous 25 years. While this demonstrates the potential significance of this emerging field, much still remains to be done to standardize the meaning of the term probiotic and which strains actually fulfill the criteria of true probiotic microorganisms. In addition, although clinical evidence of the tangible benefits of probiotics is mounting, this does not yet reflect the commercial front. Unfortunately, many so-called probiotic products have not been properly identified, documented, manufactured under good manufacturing practices, or proven clinically, yet various companies make claims that lead consumers and caregivers to believe that they are using reliable products. Thus, the establishment of standards and guidelines represents a necessary first step in making sure that probiotic products are indeed legitimate and effective. Such standards and guidelines have recently been generated and will be presented later.

PRESENT STATUS OF PROBIOTICS IN CLINICAL PRACTICE

It is important to first examine the present status of probiotics in clinical practice and the evidence of their effects. In general, probiotics are not a mainstay of clinical practice in North America. For example, an analysis by a high school student of physician practices in a small Canadian city showed that only 31% had any knowledge of probiotics and 24% felt that probiotics had no place in their practices (34). The 31% figure may be much higher in many parts of the Western world, and of those who have knowledge, the accuracy of their information may also be flawed. For example, by definition, yogurt per se is not a probiotic, and many so-called acidophilus products have never been tested and do not fulfill the FAO and

* Corresponding author. Mailing address: Canadian Research and Development Centre for Probiotics, Lawson Health Research Institute, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada. Phone: (519) 646-6100, x65256. Fax: (519) 646-6031. E-mail: gregor@uwo.ca.

WHO criteria for probiotics (37). The fact that 76% of physicians believed that probiotics could have a place in their patient management implies the potential of this approach as well as inadequacies felt by physicians in their current treatment arsenal.

Many health care professionals such as holistic practitioners, naturopaths, chiropractors, and herbalists routinely use products perceived to contain lactobacilli, bifidobacteria, and other possible probiotics. However, depending upon the training center, physicians may not be exposed to programs that discuss and evaluate the advantages and disadvantages of so-called nontraditional, complementary or alternative medicine, within which probiotics is sometimes placed. Governmental agencies such as the Food and Drug Administration are designed to separate and regulate drugs from other substances, which inadvertently makes it very difficult for small health companies to have the resources to seek claims and drug approval status for probiotics. Meanwhile, physicians rightly require that the medicines they prescribe or recommend have been tested, shown to have clinical effects, and be produced in reliable, reproducible product formulations. However, the current research-funding environment has not been conducive to sufficiently adequate testing of many probiotic strains in clinical practice.

Several factors are now leading many physicians to examine probiotics and other alternatives to pharmaceutical remedies. These include the surging levels of multidrug resistance among pathogenic organisms, particularly in hospitals, the increasing demands of consumers for natural substitutes for drugs, and the emergence of scientific and clinical evidence showing the efficacy and effectiveness of some probiotic strains. The FAO and WHO guidelines, albeit several years away from implementation in United Nations member countries, will ensure that reliable, clinically proven probiotics are available. Without such product formulations, physicians have little to offer their patients. Analyses of probiotic strains show that very few are currently available as drugs, foods, or dietary supplements.

EVIDENCE OF PROBIOTIC EFFECTIVENESS

The comprehensive review of the literature by the expert panel of FAO and WHO demonstrated a relatively small number of areas in which probiotics have proven antidisease effects. Examples of these will now be presented here.

Probiotics for Newborns and Children

Intestinal infections in newborn children are common, and in developing countries diarrhea is a prime cause of morbidity and mortality. In the United States, epidemiological estimates indicated that 21 to 37 million diarrheal disease episodes occurred in 16.5 million American children each year (47). Necrotizing enterocolitis is one devastating intestinal disorder that a preterm infant may face within a neonatal intensive care unit. Necrotizing enterocolitis is characterized by abdominal distension, bilious emesis, bloody stools, lethargy, apnea, and bradycardia (20). The disease progresses through an inflammatory cascade with septic shock and intestinal necrosis. Necrotizing enterocolitis has been reported to occur in 10 to 25% of preterm infants (<1,500 g in weight) admitted to the neonatal intensive care unit, and it may involve approximately one third to one half of all very low birth weight infants (47). Of those, approximately half will require surgery. The mortality ranges from 20 to 30%, and of those who survive, approximately 25% experience long-term sequelae, such as short gut syndrome and intestinal obstruction. In some cases, the sequelae result from multisystem organ failure that has damaged the lungs or other organs.

Bacterial colonization or infection of the intestine by pathogens such as *Clostridium*, *Escherichia*, *Klebsiella*, *Salmonella*, *Shigella*, *Campylobacter*, *Pseudomonas*, *Streptococcus*, *Enterococcus*, *Staphylococcus aureus*, and coagulase-negative staphylococci increases the risk of necrotizing enterocolitis. If nonpathogens, such as lactobacilli and bifidobacteria, colonize the intestine, or if breast milk rather than formula is used, the incidence of necrotizing enterocolitis has been reported to fall (73). At the time of this finding in 1990, the authors estimated that in British neonatal units, exclusive formula feeding could account for approximately 500 extra cases of necrotizing enterocolitis and 100 deaths each year. No recent comparison is available, but changes in infant formulas have been made over the past 13 years, so conclusions drawn from that time may or may not be relevant today.

Low-birth-weight premature infants delivered by caesarian section are often ill equipped for life outside the womb. They require intensive care, and for those who are breast fed, the feeding usually only begins several days after the infants are exposed to a plethora of microbes, many of which have pathogenic potential. This indicates that the normal process by which organisms such as lactobacilli are ingested via vaginal birth and propagated by the mother's milk do not take place. As a result, this may allow pathogens to establish within the premature intestine. Furthermore, infants given antibiotics at birth retain an abnormal microbiota 4 weeks later, such is the dramatic impact of these agents (44).

The intestinal microbiota in low-birth-weight premature infants can be dominated by many pathogens such as *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Staphylococcus haemolyticus* (44, 77). In particular, *Clostridium perfringens* has been isolated from 40% of babies with necrotizing enterocolitis, compared with 13% of controls ($P = 0.03$). However, in premature infants given breast milk, lactobacilli and bifidobacteria are present in a more diverse microbiota. For example, in a study of the enteric microbiota of 25 babies with necrotizing enterocolitis compared to 23 matched controls, lactobacilli were less common in the necrotizing enterocolitis babies (12% versus 48%, $P = 0.006$) (13). These findings suggested a correlation between the reduction of lactobacilli and the increased risk of necrotizing enterocolitis.

Other studies indicated that bifidobacteria not only colonized the gut of animals, possibly helping to exclude pathogens; they also reduced endotoxemia and appeared to modulate the inflammatory cascade (20). Perhaps the most impressive indication that probiotics could benefit newborns comes from a human trial with 2.5×10^8 live *Lactobacillus acidophilus* and 2.5×10^8 live *Bifidobacterium infantis* in 1,237 neonates in Colombia. Compared with 1,282 hospitalized patients seen during the previous year, treatment with these strains resulted in a 60% reduction in necrotizing enterocolitis and overall mortality (58). Although historical comparisons are

not an ideal clinical trial design, the results nevertheless are striking and warrant consideration. A subsequent study involved newborn infants with a gestational age of <33 weeks or birth weight of <1,500 g and a standard milk feed supplemented with *Lactobacillus* sp. strain GG in a dose of 6×10^9 CFU once a day until discharge (≈47 days) (30). The study found a reduced rate of necrotizing enterocolitis compared to placebo (1.4% versus 2.7%) but was not statistically significant, suggesting that either the GG strain is not as good as the *L. acidophilus-B. infantis* combination, milk is not an effective delivery system, or probiotics are not as effective as earlier thought (58). A further study of enteral feeding of premature infants with *Lactobacillus* sp. strain GG showed that the organism could be recovered from the stool and was thus delivered and survived passage, even though it did not appear to confer any detectable benefits (90).

Failure of the GG strain to prevent necrotizing enterocolitis does not necessarily indicate a lack of benefit to newborns. A double-blind, randomized, placebo-controlled trial involving 132 participants over a 2-year period showed that daily feeding of two capsules containing 10^{10} *Lactobacillus* sp. strain GG to pregnant mothers who had at least one first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma and after birth to the mother and to the babies for 6 months significantly reduced the incidence of allergic atopic dermatitis (15 of 64 [23%] versus 31 of 68 [46%], $P = 0.0008$) (66). This implies a functional modulation of immunity rather than a specific antipathogen reaction in the gut. This effect has now been shown to remain at 4-year follow up (67).

The immune response within the gastrointestinal tract is a fine balance between the release of proinflammatory (e.g., interleukin-1, -6, and -8 and tumor necrosis factor) and anti-inflammatory (e.g., interleukin-1RA, -4, and -10) cytokines (75a, 135a). In a review on mucosal immunity starting at birth, Walker (146) reported a correlation between a normal gut microbiota and protection against various infections. This is an important observation because it supports the concept of early intestinal colonization with organisms such as lactobacilli and bifidobacteria and possibly subsequent protection from necrotizing enterocolitis and other diseases.

It is estimated that every 15 s a child dies from diarrheal disease somewhere in the world. In a study in 204 undernourished, 6- to 24-month-old children in Peru, once-daily intake of *Lactobacillus rhamnosus* GG 6 days a week for 15 months led to significantly fewer episodes of diarrhea (5.21 versus 6.02 episodes of diarrhea per child per year in the placebo group; $P = 0.028$) (96). However, this type of study is difficult to verify because there is little control over the organisms to which the children are exposed and the compliance in taking the treatment. At the least, probiotics provide a safe and potentially beneficial remedy, especially when delivered in milk, which provides the child with nutrition and a means to overcome adverse effects of fluid loss. Current WHO recommendations state that clinical management of acute diarrhea should include replacement of fluid and electrolytes losses along with nutritional support (150). As such, oral rehydration salts are widely used in diarrheal disease management.

The strongest evidence of a beneficial effect of probiotics has been established with *L. rhamnosus* GG and *B. lactis* BB-12 for prevention and *L. reuteri* SD2222 for treatment (51, 52, 62, 80, 122, 131, 138) of acute diarrhea mainly caused by rotaviruses in children (Table 1). The study designs cited are similar, randomized, double blinded, and placebo controlled. The statistically significant reduction in the duration of diarrhea is consistent and quite convincing, especially for the GG strain used in several of the trials. Note also that unlike many pharmaceutical treatments, there were no significant side effects reported. In a European study, faster hospital discharge was achieved in addition to improvement in clinical outcome (51). Two hundred ninety-one children 1 to 3 months of age were randomly allocated to receive oral rehydration solution plus placebo or 10^{10} *L. rhamnosus* GG. After rehydration in the first 4 to 6 h, patients were offered their usual feedings plus free access to the same solution until diarrhea stopped. Duration of diarrhea was reduced from an average of 3 days to 2.4 days ($P = 0.03$). In a randomized, placebo-controlled study of 40 patients between 6 and 36 months of age hospitalized with acute diarrhea (75% rotavirus), treatment with high doses of *L. reuteri* (assumed to be strain SD2222) (10^{10} to 10^{11} CFU) for up to 5 days resulted in reduction in duration of watery stools (1.6 versus 2.9 days in the placebo group) ($P = 0.07$) (131). In a second prospective, randomized, placebo-controlled trial of children between 6 and 36 months of age admitted for rotavirus-associated diarrhea, three groups received either 10^{10} or 10^7 CFU of *L. reuteri* SD2222 or a matching placebo once a day for up to 5 days. The outcome supported the earlier findings, with a mean duration of watery diarrhea being optimal for patients given the highest dose of lactobacilli (1.5 days versus 1.9 days for the lower dose versus 2.5 days for the placebo group) (132).

In summary, we believe there is sufficient evidence to recommend use of at least one probiotic strain, *L. rhamnosus* GG, in capsule or milk form, to treat acute diarrhea in children, in combination with standard oral rehydration.

HOW PROBIOTICS REDUCE THE DURATION OF DIARRHEA

Several potential mechanisms have been proposed for how lactobacilli reduce the duration of rotavirus diarrhea, but none have been proven and each theory has flaws. The first is competitive blockage of receptor sites (11), in which lactobacilli bind to receptors, thereby preventing adhesion and invasion of the virus. This concept might be plausible if there was evidence for specific receptor competition. In most cases, by the time a probiotic is ingested, the patient will already have had diarrhea for possibly 12 h. By this time, the virus has infected mature enterocytes in the mid- and upper region of the small intestinal villi. The virus and/or its enterotoxin, NSP4 will then have inhibited fluid and electrolyte transport, thereby lowering fluid and glucose absorption. The toxin could have then potentially activated secretory reflexes, causing loss of fluids from secretory epithelia, resulting in diarrhea (74). At best, subsequent competitive exclusion of viruses would only be effective for attachment of progeny, and it is not known whether such inhibition would reduce diarrhea. If lactobacilli somehow competed with the toxin or peptides released from villous endocrine cells, it is feasible that the cascade that leads to diarrhea could be prevented.

The second potential mechanism may be that the immune response is enhanced by lactobacilli, leading to the observed

TABLE 1. Clinical trials involving probiotics that demonstrate reduction of duration of diarrhea in children

Study design	Findings	Reference
Children 1 mo to 3 yr of age with acute-onset diarrhea; double-blind, placebo-controlled trial. Group A, oral rehydration plus placebo; group B, oral rehydration plus *Lactobacillus* GG (at least 10^{10} CFU/250 ml). After rehydration in the first 4 to 6 h, patients were offered their usual feedings plus free access to the same solution until diarrhea stopped.	144 children in group A, 147 in group B. Duration of diarrhea, 71.9 ± 35.8 h in group A vs. 58.3 ± 27.6 h in group B (mean ± SD; $P = 0.03$). In rotavirus-positive children, diarrhea lasted 76.6 ± 41.6 h in group A vs. 56.2 ± 16.9 h in groups B ($P < 0.008$). Diarrhea lasted longer than 7 days in 10.7% of group A vs. 2.7% of group B patients ($P < 0.01$). Hospital stays were significantly shorter in group B than in group A.	51
100 children with diarrhea randomly assigned to receive oral rehydration or oral rehydration followed by administration of lyophilized *Lactobacillus* GG.	Duration of diarrhea was reduced from 6 to 3 days in children receiving *Lactobacillus* GG compared to control; 61 children had proven rotavirus infection.	52
71 well-nourished children between 4 and 45 mo of age suffering from diarrhea received oral rehydration then randomly either *Lactobacillus* GG-fermented milk product, 125 g (10^{10} CFU) twice daily (group 1); *Lactobacillus* GG freeze-dried powder, one dose (10^{10} CFU) twice daily (group 2); or placebo, pasteurized yogurt (group 3), 125 g twice daily for 5 days.	Mean [SD] duration of diarrhea after commencing the therapy was significantly shorter in group 1 (1.4 [0.8] days) and in group 2 (1.4 [0.8] days) than in group 3 (2.4 [1.1] days); $F = 8.70$, $P < 0.001$.	62
After initial oral rehydration, 49 children aged 6 to 35 mo with rotavirus gastroenteritis randomly received either *Lactobacillus* GG, *L. casei* subsp. *rhamnosus* (Lactophilus), or *S. thermophilus* and *L. delbruckii* subsp. *bulgaricus* (Yalacta) twice daily for 5 days.	Mean (SD) duration of diarrhea was 1.8 (0.8) days in children who received *Lactobacillus* GG, 2.8 (1.2) days in those receiving Lactophilus, and 2.6 (1.4) days in those receiving Yalacta ($F = 3.3$, $P = 0.04$). The rotavirus-specific immune responses were different, with *Lactobacillus* GG therapy associated with enhancement of IgA-specific antibody-secreting cells to rotavirus and serum IgA antibody level at convalescent stage.	80
In a double-blind, placebo-controlled trial, 55 infants aged 5–24 mo were randomized to receive a standard infant formula or the same supplemented with *Bifidobacterium bifidum* and *S. thermophilus.*	8 (31%) of the 26 patients who received the control formula and 2 (7%) of 29 who received the supplemented formula developed diarrhea during the course of the study ($P = 0.035$; Fisher's exact test, two-tailed). 10 (39%) controls and 3 (10%) receiving supplement shed rotavirus at some time during the study ($P = 0.025$).	122
40 children (6–36 mo) with acute diarrhea (75% rotavirus) were randomized to receive 10^{10} CFU of either *L. reuteri* or placebo daily for up to 5 days.	Mean (SD) duration of watery diarrhea was 1.7 (1.6) days in the *L. reuteri* group and 2.9 (2.3) days in the placebo group ($P = 0.07$). On the second day of treatment, only 26% of patients receiving *L. reuteri* had watery diarrhea, compared with 81% of those receiving placebo ($P = 0.0005$).	131
81 children (1–36 months) hospitalized for reasons other than diarrhea were enrolled in a double-blind trial and randomly assigned to receive *Lactobacillus* GG ($n = 45$) at 6×10^9 CFU or placebo ($n = 36$) twice daily orally for the duration of their hospital stay.	*Lactobacillus* reduced the risk of nosocomial diarrhea (≥3 loose or watery stools/24h) in comparison with placebo (6.7% vs. 33.3%; relative risk, 0.2; 95% CI, 0.06–0.6). The prevalence of rotavirus infection was similar in both groups (20% vs. 27.8%, respectively; relative risk, 0.72; 95% CI, 0.33–1.56). However, the use of *Lactobacillus* GG compared with placebo significantly reduced the risk of rotavirus gastroenteritis (1 of 45 [2.2%] vs. 6 of 36 [16.7%], respectively; relative risk, 0.13; 95% CI, 0.02–0.79).	138

clinical effect (65). This is supported by the protective effect which local immunoglobulin A (IgA) antibodies appear to confer against rotavirus (148). However, a problem with this theory is that given that diarrhea appears to cease within 1 to 3 days in patients who would otherwise suffer for 4 to 6 days, the lactobacilli would need to trigger the antibody response rapidly so that it interfered with further viral activity. Animal studies do indicate that secretory IgA can be triggered by lactobacillus ingestion (115), but the rate was not determined, nor was the influence on cessation of fluid loss across the secretory cell membranes. Modification of the cytokine profile to one that enhances anti-inflammatory cytokines (23) or attenuation of the virus's and/or toxin's effect on the enteric nervous system might provide rapid cessation of epithelial secretion and diarrhea. Alternatively, stimulation of T cells to produce gamma interferon, leading to potential inhibition of chloride secretion, might also inhibit diarrhea. One aspect of the immunity theory that needs to be clarified is why lactobacilli, which we assume are present in the child' intestine, appear unable to prevent infection, yet those administered orally thereafter help to clear the diarrhea.

A third mechanism could involve a signal(s) from lactobacilli to the host that downregulates the secretory and motility defenses designed to remove perceived noxious substances. Glycosylated intestinal mucins inhibit rotaviruses (151a), and *MUC2* and *MUC3* mRNA expression is increased in response to lactobacillus signaling, protecting cells against pathogenic bacterial adhesion (76). However, direct host cell signaling between lactobacilli and secretory cells has not yet been investigated. Attachment of the virus causes cytokine prostaglandin and nitric oxide release from the enterocytes, both of which could affect motility. The possibility exists that lactobacilli could alter this release (151).

A final theory is that lactobacilli produce substances that inactivate the viral particles. This has been shown in vitro (18), with supernatants from *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 inactivating 10^9 particles of the double-stranded DNA adenovirus and the negative-stranded RNA vesicular stomatitis virus within 10 min. The effect was likely due to acid, but more specific antiviral properties have not been ruled out. Whether or not viral killing activity can inhibit diarrhea remains to be determined.

More detailed mechanistic research is needed to understand how probiotic strains reduce the duration of diarrhea in conjunction with rehydration therapy. Such studies could lead to a better understanding of the dynamics within the intestinal microbiota that is being disrupted and depleted by rapid fecal loss. In doing so, new interventional therapies should be generated to quickly and effectively trigger the cessation of not

TABLE 2. Studies of antibiotic-associated *Clostridium difficile* diarrhea treated with probiotics

Study design	Findings	Reference
Standard antibiotic for 10 days plus either *S. boulardii* (1 g/day for 28 days) or placebo	Significant decrease in recurrences in patients treated with vancomycin (2 g/day) and *S. boulardii* (16.7%) compared with vancomycin and placebo (50%; $P = 0.05$)	137
Standard antibiotic plus *L. rhamnosus* GG in prospective, randomized, placebo-controlled trial	3-week recurrence rate of *C. difficile* infection reduced and improved patient well-being with earlier disappearance of abdominal cramps and diarrhea	101
Prospective double-blind controlled trial of 180 hospitalized patients given antibiotic plus *S. boulardii*	Significantly reduced the rate of diarrhea from 22% in the placebo group to 9.5% in the *S. boulardii* group	136
Prospective, randomized, double-blind, placebo-controlled trial of 302 hospitalized patients on antibiotics who received *Lactobacillus* GG (2×10^{10} CFU/day) or placebo for 14 days	Diarrhea developed in 39 (29.3%) of 133 patients in the GG group and 40 (29.9%) of 134 placebo patients ($P = 0.93$)	143
69 patients over the age of 65 yr prescribed antibiotics were randomized to receive either 113 g of *S. boulardii* twice daily or placebo for as long as they received antibiotics	No evidence that *S. boulardii* altered patients' bowel habits or prevented the appearance of *C. difficile* toxin in the stool	71
Meta-analysis to evaluate efficacy of probiotics in prevention and treatment of diarrhea associated with the use of antibiotics	Odds ratio of 0.39 (95% CI, 0.25 to 0.62; $P < 0.001$) in favor of active treatment over placebo using *S. boulardii* and 0.34 (0.19 to 0.61; $P < 0.01$) for lactobacilli	33

only rotavirus illness but also other gastrointestinal infections that debilitate patients for 2 to 3 days.

ADDITIONAL APPLICATIONS OF ORAL PROBIOTICS

Bacterial Gastroenteritis

In addition to rotavirus infections, many bacterial species can cause intestinal disorders. There is good in vitro evidence that certain probiotic strains can inhibit the growth and adhesion of a range of enteropathogens (12, 24, 25, 48, 59). Such studies are useful in characterizing probiotic organisms but of limited value in terms of predicting the efficacy or proving mechanisms of action. It is feasible that probiotic organisms inhibit or even kill pathogens in the intestinal tract, but verification of this activity has not been obtained in humans. In animal studies, daily intake of *L. rhamnosus* GR-1, *L. fermentum* RC-14 (known to inhibit the growth of *Salmonella* spp.), or *L. rhamnosus* GG led to enhanced secretory IgA production and phagocytic activity and a significant reduction in local and invasive (liver and other organs) infection by salmonellae (115).

A major problem associated with antibiotic use, particularly clindamycin, cephalosporins, and penicillins, is the onset of diarrhea, usually caused by *Clostridium difficile*. This organism is not uncommon in the healthy intestinal tract, but the disruption of the indigenous microbiota by antibiotics may lead to an abnormal elevation of *C. difficile* and subsequent symptoms related to toxin production (7). The acquisition of toxin-producing *C. difficile* is approximately 2,700 cases per 100,000 exposures to antibiotics in the community (8). In an effort to further refine standard antibiotic treatment of *C. difficile* infections, patients have been given standard antibiotic for 10 days plus either the yeast *Saccharomyces boulardii* (1 g/day for 28 days) or placebo. A significant decrease in recurrence was observed in patients treated with high-dose vancomycin (2 g/day) and *S. boulardii* compared with those who received high-dose vancomycin and placebo (16.7% versus 50%, $P = 0.05$) (137) (Table 2).

Other studies have also suggested that probiotics can alleviate the signs and symptoms of *C. difficile* infection (49, 101). Early results from a prospective, randomized, placebo-controlled trial showed that *L. rhamnosus* GG in combination with standard antibiotics reduced the 3-week recurrence rate of *C. difficile* infection and improved patient well-being with earlier disappearance of abdominal cramps and diarrhea (101). Other studies with *S. boulardii* have reduced the incidence of diarrhea in travelers (69). The first human trial of 180 hospitalized patients used a prospective, double-blind controlled design showed that *S. boulardii* given in capsule form concurrently with antibiotics significantly reduced the rate of diarrhea from 22% in the placebo group to 9.5% in the *S. boulardii group* ($P = 0.038$) (136). In a follow-up study of 193 patients, the efficacy of *S. boulardii* has been reported to be 51% (85). The proposed mechanism of action of *S. boulardii* is its release of a 54-kDa protease which digests the *C. difficile* toxin A and B molecules and brush border membrane receptors (21). Unfortunately, no strain numbers have been given to *S. boulardii*; thus, we do not know which ones have been used and which are effective.

However, not all clinical trials have shown effectiveness. In one prospective, randomized, double blind, placebo-controlled trial, 302 hospitalized patients on antibiotics were randomized to receive *Lactobacillus* sp. strain GG (2×10^{10} CFU/day) or placebo for 14 days (143). Diarrhea developed in 39 (29.3%) of 133 patients in the GG group and 40 (29.9%) of 134 placebo patients ($P = 0.93$). Two potential flaws in this study are that only a few *C. difficile* infections were detected upon develop-

ment of diarrhea and that patients self-reported stool consistency. Thus, the findings do not prove or disprove the ability of lactobacilli to treat antibiotic-associated diarrhea. In another study with the yeast probiotic *S. boulardii*, 69 patients over the age of 65 years prescribed antibiotics were randomized to receive either 113 g of *S. boulardii* twice daily or placebo for as long as they received antibiotics (71). There was no evidence that *S. boulardii* altered patients' bowel behavior or prevented the appearance of *C. difficile* toxin in the stool.

A meta-analysis to evaluate the efficacy of probiotics in prevention and treatment of diarrhea associated with the use of antibiotics recently showed an odds ratio of 0.39 (95% confidence interval, 0.25 to 0.62; $P < 0.001$) in favor of active treatment over placebo with *S. boulardii* and 0.34 (0.19 to 0.61; $P < 0.01$) for lactobacilli (33). The authors concluded that *S. boulardii* and lactobacilli have the potential to prevent antibiotic-associated diarrhea, but efficacy remains to be proven. Although such meta-analyses are useful, they become more credible if sufficiently large, similarly planned studies have been undertaken. In this meta-analysis study, there were only nine analyzable trials and four different probiotic strain combinations were used, emphasizing that more studies are required. It should be noted that cases of fungal infections have been reported following *S. boulardii* treatment, albeit in rare instances, usually associated with immunocompromised, catheterized patients (55).

Helicobacter pylori Infections and Complications

Helicobacter pylori is a gram-negative bacterial pathogen responsible for type B gastritis and peptic ulcers and may be a risk factor for gastric cancer. There are some in vitro and animal data to indicate that lactic acid bacteria can inhibit the pathogen's growth and decrease the urease enzyme activity necessary for it to survive in the acidic environment of the stomach (2, 26, 64, 88). In humans, there is also evidence that probiotic strains can suppress infection and lower the risk of recurrences (19, 36, 87). In the first study (19), 120 *H. pylori*-positive patients were randomly assigned to a 7-day triple therapy based on rabeprazole (20 mg twice a day), clarithromycin (250 mg three times a day) and amoxicillin (500 mg three times a day) (RCA group; 60 subjects), or to the same regimen supplemented with a lyophilized and inactivated culture of *Lactobacillus acidophilus*. Eradication of the pathogen occurred in 72% of the antibiotic-treated patients and in 88% of the patients supplemented with live lactobacilli ($P = 0.03$) and 87% given dead organisms ($P = 0.02$). The mechanisms involved are unclear, especially with the dead bacterial preparation, but there is a presumption that the lactobacilli either induced a host response to negatively affect helicobacter survival or inhibited their spread through competitive adhesion to glycolipid receptors.

In the second study (36), 53 patients infected with *H. pylori* were randomized to receive either 180 ml of *L. johnsonii* La1-acidified milk (LC-1) or a placebo twice a day for 3 weeks. All subjects also received clarithromycin (500 mg twice a day) during the last 2 weeks of acidified milk therapy. Esophagogastroduodenoscopy and biopsies were performed along with the urease test and histology. The LC-1 strain decreased *H. pylori* density in the antrum ($P = 0.02$) and the corpus ($P = 0.04$), as well as inflammation and gastritis activity in the antrum ($P = 0.02$ and $P = 0.01$, respectively). The unanswered question is whether the reduction is sufficient for short or long term clinical relief.

Not all efforts with probiotics have been successful, and one study that used yogurt to deliver a proposed probiotic organism failed to confer a beneficial effect (149). However, this study had several flaws; it assessed eradication only by a urea breath test, it used strains that had only inhibited *H. pylori* in vitro and were thus not proven probiotics, and it was only tested in 27 asymptomatic women. Further studies are therefore needed.

Inflammatory Diseases and Bowel Syndromes

Inflammatory bowel diseases such as pouchitis and Crohn's disease may be caused or aggravated by alterations in the gut microbiota (129). Preliminary evidence suggests that a combination of strains (46) rather than a single organism (102) may alleviate symptoms of disease. In the study with the VSL#3 product containing very high doses of four strains of lactobacilli, three strains of bifidobacteria, and one strain of *Streptococcus salivarius* subsp. *thermophilus* (5×10^{11} per g of viable lyophilized bacteria), 40 patients in clinical and endoscopic remission were randomized to receive VSL#3, 6 g/day, or placebo for 9 months (46). Three patients (15%) in the VSL#3 group had relapses within the 9-month follow-up period, compared with 20 (100%) in the placebo group ($P < 0.001$). It was not surprising that the fecal content of lactobacilli, bifidobacteria, and *S. thermophilus* increased significantly from baseline levels in the VSL#3-treated group ($P < 0.01$), given the high numbers of probiotics administered.

The study was very encouraging, and with this product already on the market, it will be interesting to see the outcome of clinical experience. In terms of product manufacturing, however, it is not clear how reproducible the preparations are in terms of the proportions of each strain or the effect each strain has in isolation as well as in combination. Mechanistic studies are needed to determine the product's effect on host cells, mucus, and immune defenses and the long-term impact on intestinal and fecal microbiota and patient outcome.

Based upon some animal experiments and pilot human studies (50), there is a potential therapeutic role for probiotics and prebiotics in patients with inflammatory bowel disease, including children. However, large, double-blinded controlled trials are needed to confirm efficacy and to document dosage and treatment parameters (91), and we must await the results of one such double-blind, placebo-controlled multicenter efficacy study under way to determine whether *L. rhamnosus* GG has any effect in children with Crohn's disease.

The demonstration that abnormal colonic fermentation occurs in some patients with irritable bowel syndrome has led to the suggestion that probiotic therapy might alleviate the condition. A double-blind, placebo-controlled, cross-over, 4-week trial of *L. plantarum* 299V in 12 previously untreated patients with irritable bowel syndrome showed no effect on the breath hydrogen test, gas production rate, or symptoms (126). This finding contradicts an earlier study with the same organism, in which 20 irritable bowel syndrome patients treated with this lactobacillus for 4 weeks reported resolution of their abdomi-

nal pain, compared to 11 patients on placebo ($P = 0.0012$) (93). An improvement in symptoms was noted in 95% of patients in the lactobacillus group versus 15% on placebo ($P < 0.0001$). Whether these positive results were in part due to patient selection or use of probiotics in liquid rather than dried form remains to be determined in a larger patient study. Suffice it to say, the role of probiotics in treating or preventing irritable bowel syndrome has not yet been clearly proven (78).

Cancer

The ability of lactobacilli and bifidobacteria to modify the gut microbiota and reduce the risk of cancer is in part due to their ability to decrease β-glucuronidase and carcinogen levels (57). Cancer recurrences at other sites, such as the urinary bladder, also appear to be reduced by intestinal instillation of probiotics, including *L. casei* Shirota (the strain present in Yakult, a Japanese milk-based drink taken by an estimated 26 million people every day) (5). In vitro studies with *L. rhamnosus* GG and bifidobacteria and an in vivo study with *L. rhamnosus* GG and LC-705 and a *Propionibacterium* sp. showed a decrease in availability of carcinogenic aflatoxin in the lumen (35, 95). Increased activity of glutathione transferase (induced by *Bifidobacterium longum* and lactulose and resistant starch), colonic NADPH-cytochrome P450 reductase, and enhanced removal of O^6-methylguanine from colonic mucosa may also play a role in disease prevention (3, 22). Fermentation products such as butyrate and lactate or a simple decrease in gut transit time could also be important. However, definitive clinical conclusions require efficacy studies in humans.

Mucosal Immunity

Studies with *L. casei* Shirota injected into mice showed a significant increase in natural killer cell activity from mesenteric node cells but not of Peyer's patch cells or spleen cells (83), supporting the concept that some probiotic strains can enhance the innate immune response. Other animal studies clearly indicate that probiotic strains can modify immune parameters (45). Mice were fed daily with 10^9 *L. rhamnosus* (HN001, DR20), *L. acidophilus* (HN017), or *B. lactis* (HN019, DR10), and their immune function was assessed on days 10 and 28. The phagocytic activity of peripheral blood leukocytes and peritoneal macrophages increased significantly compared with the control mice. The proliferative responses of spleen cells to concanavalin A (a T-cell mitogen) and lipopolysaccharide (a B-cell mitogen) were also significantly enhanced in mice given different probiotic strains, and spleen cells produced significantly higher amounts of gamma interferon in response to stimulation with concanavalin A.

Dietary consumption of *B. lactis* HN019 and *L. rhamnosus* HN001 in randomized, placebo-controlled human studies showed measurable enhancement of immune parameters in the elderly (4, 130). The precise mechanisms of action remain to be established in patients, but the ability of *Lactobacillus* strains to activate macrophages (89) and stimulate secretory IgA and neutrophils without release of inflammatory cytokines (42, 65, 115, 133) could be important. It should also be recognized that not all immune effects are necessarily beneficial to the host. As Perdigon and Holgado (100) rightly point out, the major cell wall component of lactobacilli, muramyldipeptide, can be pyrogenic, and some *Lactobacillus* strains can enhance the Th1 proinflammatory pathway and would not be appropriate for mucosal immunity (75). The dosage and duration of therapy must also be considered so as to optimally enhance and not suppress immunity (99).

The use of lactic acid bacteria, such as *L. plantarum* NCIMB 8826, to deliver vaccines is being tested (121). This strain is being tested to deliver nontoxic tetanus toxin fragment C, and three types of constructs induced strong specific immune responses in mice. Future studies of this nature will explore the extent to which lactobacilli can immunize against various pathogens that attack the mucosal surfaces of the mouth, intestine, vagina, and respiratory tract.

Allergy

The composition of the vaginal microbiota has been shown to influence the ultimate asthmatic condition of children. In one Danish study of 2,927 women and their 3,003 infants, maternal vaginal colonization with *Ureaplasma urealyticum* during pregnancy was associated with infant wheezing (odds ratio, 2.0; 95% confidence interval, 1.2 to 3.6), but not with asthma during the fifth year of life, while maternal colonization with staphylococci (odds ratio, 2.2; 95% confidence interval, 1.4 to 3.4) and use of antibiotics in pregnancy (odds ratio, 1.7; 95% confidence interval, 1.1 to 2.6) were associated with asthma (10).

As stated earlier, the application of probiotics to prevent allergic reactions became more prominent with the double-blind, randomized, placebo-controlled trial showing that *L. rhamnosus* GG given to pregnant women for 4 weeks prior to delivery and then to newborn children at high risk of allergy for 6 months caused a significant reduction in early atopic disease (66). Further clinical studies with *L. rhamnosus* GG and *B. lactis* BB-12 appear to have been useful in infants allergic to cow's milk (61, 79). The current understanding suggests that the probiotic organisms reverse increased intestinal permeability, enhance gut-specific IgA responses, promote gut barrier function through restoration of normal microbes, and enhance transforming growth factor beta and interleukin-10 production as well as cytokines that promote production of IgE antibodies (63, 66). The role of T-helper 1 (Th1) enhancement and T-helper 2 (Th2) reduction remains to be proven.

WOMEN'S REPRODUCTIVE AND BLADDER HEALTH

Although research on probiotics for the female urogenital tract has been ongoing for over 20 years, only recently have others recognized that probiotic applications go beyond consumption of foods. Evidence from a 64-patient randomized, placebo-controlled trial (114) indicates that daily oral intake of 10^9 to 10^{10} *L. rhamnosus* GR-1 and *L. fermentum* RC-14 leads to transfer of the organisms from the rectum to the vagina as well as an overall depletion of coliforms and yeasts in the vagina (109, 112, 115). In these studies of over 100 reportedly healthy women, a significant number presented with an abnormal vaginal microbiota indicative of bacterial vaginosis. This agrees with the findings of others and shows clearly that the vaginal microbiota is often abnormal during the menstrual

cycle and postmenopause even when the subject is asymptomatic (68, 125, 142).

Questions have been raised as to how abnormal is defined, given that subjects are not ill. The vaginal microbiota is often in a state of flux, as shown by Nugent score analysis, culture, and molecular tracking (17, 29, 32, 144). The Nugent score (94) is determined by microscopic analysis of vaginal cells collected from the vagina. When the field of view is dominated by lactobacillus morphotypes, the score is low (0 to 3), and when it is dominated solely by gram-negative rods (indicative of anaerobes like *Gardnerella vaginalis* or uropathogens like *Escherichia coli*) or gram-positive cocci like group B streptococci or enterococci, the score is high (8 to 10). Intermediate values indicate the presence of pathogens and lactobacilli in a sort of transition state. The factors that contribute to the transition from asymptomatic to symptomatic infection or a return to one that is healthy remain to be determined.

Nevertheless, the incidence of urinary tract infection, bacterial vaginosis, and yeast vaginitis, estimated to affect one billion women each year (the rate for urinary tract infection alone is 0.5 cases per person per year), means that the likelihood of infection is high. Indeed, the presence of pathogens dominating the vagina increases severalfold the likelihood that a woman will develop a symptomatic infection. In short, an abnormal microbiota may indeed lead to a symptomatic vaginal or bladder infection.

The concept of restoring the *Lactobacillus* content of the vaginal microbiota as a barrier to prevent infection was first conceived by Canadian urologist Andrew Bruce in the early 1970s. Extensive research since then has shown that certain *Lactobacillus* strains are able to colonize the vagina following vaginal suppository use (18) and reduce the risk of urinary tract infection, yeast vaginitis (108), and bacterial vaginosis (112). Strain selection at that time, and even recently, has been based upon in vitro tests and source of the strains (17, 111, 116, 144), but human studies provide the definitive answer to whether or not strains can function as probiotics.

A study on the prevention of urinary tract infection entailed once-weekly vaginal administration of a suppository containing 10^9 *L. rhamnosus* GR-1 and *L. fermentum* B-54 for 1 year and comparing the rate of urinary tract infection occurrence with that in the previous year in 25 women (108). There was a significant reduction in urinary tract infection during lactobacillus use (from an average of six episodes per year to 1.6 episodes per year [30%]; $P < 0.0001$). No side effects were reported. This compares favorably with two studies that used daily antibiotic therapy to prevent urinary tract infection. The first was a randomized study of 64 patients with a history of recurrent urinary tract infections given trimethoprim (100 mg) at night for 1 year or methenamine hippurate (1,000 mg) every 12 h or asked to cleanse the perineum (especially the periurethral area) twice daily with povidone-iodine solution (15). In this study, the urinary tract infection recurrence rates fell from 6 in the previous year to 2.1, 2.0, and 2.2, respectively, for the three groups.

The second study was an 18-year assessment of 219 women given one of three nitrofurantoin regimens daily for 1 year (14). The mean incidence of urinary tract infection fell from 6.9 per year to 1.3 per year. Notably, 14% of patients were allergic to the antibiotic, and 40% reported at least one adverse side effect, with nausea, gastrointestinal, genitourinary, and skin effects being the most common. In addition, 25.6% of 43 patients taking 50 mg of microcrystalline nitrofurantoin stopped prematurely as a result of an adverse event. Even the use of another antibiotic, cefaclor, 250 mg daily, in 37 patients resulted in an average of 2.4 breakthrough infections per year; 12.8% reported a side effect, and 7.7% stopped taking the drug (16). In short, *Lactobacillus* therapy taken once per week with no side effects resulted in as low a rate of urinary tract infection as several daily antibiotic regimens with numerous side effects. While a larger phase three trial has not been performed with *Lactobacillus* strains GR-1 and B-54, the phase two findings are worthy of note.

In terms of preventing or treating bacterial vaginosis, recent studies have shown that daily ingestion of capsules containing *L. rhamnosus* GR-1 and *L. fermentum* RC-14 by 19 women with a bacterial vaginosis microbiota resulted in a normal microbiota (by Nugent scoring) in 81% of cases, compared to 50% in women given placebo ($P = 0.001$) (109, 112, 114). This is not yet sufficient evidence to use oral probiotics for symptomatic bacterial vaginosis treatment, but it does illustrate the potential to reduce the incidence of recurrent bacterial vaginosis that is common after antibiotic treatment (54, 72). Another study, the results of which are only available on a web site, show that *L. crispatus* CTV05 given vaginally after metronidazole treatment for bacterial vaginosis resulted in a clinical cure at 30 days in subjects colonized by lactobacilli (70%) compared to noncolonized (47%) patients receiving placebo ($P < 0.001$). Further studies with vaginal *Lactobacillus* treatment of bacterial vaginosis that are more likely to deliver lactobacilli in higher numbers and more quickly than oral ingestion are warranted. Indeed, certain *Lactobacillus* strains, including *L. crispatus* CTV05, *L. rhamnosus* GR-1, and *L. fermentum* RC-14, are able to remain in the vagina for several months after insertion (18, 118).

The prevention or resolution of bacterial vaginosis is particularly important in women at risk of human immunodeficiency virus (HIV) infection. Studies have shown that women with bacterial vaginosis (no lactobacilli) are at significantly increased risk of HIV (127). Studies of 94 prostitutes in Madagascar showed bacterial vaginosis prevalence of 85% (9); a study in Malawi showed an odds ratio of 3.0 (95% confidence interval, 2.4 to 3.8) for an association between bacterial vaginosis and HIV (139); another Malawi study of 1,196 HIV-seronegative women showed that bacterial vaginosis was significantly associated with antenatal HIV seroconversion (adjusted odds ratio = 3.7) and postnatal HIV seroconversion (adjusted rate ratio = 2.3) (140); a cross-sectional study of 144 female commercial sex workers in Chiang Mai, Thailand, found a significant association between bacterial vaginosis and seropositivity for HIV (odds ratio, 2.7; 95% confidence interval, I0.3 to 5.0) (27); and a study of 4,718 women in Uganda showed an adjusted odds ratio of HIV-1 infection and bacterial vaginosis of 2.08 (95% confidence interval, 1.48 to 2.94) (127).

The depletion of lactobacilli and the risk of HIV was further illustrated in a study of 657 HIV-1-seronegative women in Kenya, where only 26% were colonized with *Lactobacillus* species, and the absence of these organisms was associated with an increased risk of acquiring HIV-1 infection (hazard ratio, 2.0; 95% confidence interval, 1.2 to 3.5) (82). The authors con-

cluded that treatment of bacterial vaginosis and promotion of vaginal lactobacilli may reduce a woman's risk of acquiring HIV-1, gonorrhea, and trichomoniasis.

Having shown that certain *Lactobacillus* strains can colonize the vagina, this raises the questions of whether and how probiotics can reduce the risk of HIV infection (56). As stated earlier, supernatants from the strains *L. rhamnosus* GR-1 and *L. fermentum* RC-14 can inactivate viruses within minutes. It is presumed that a simple acidification of the vagina could affect HIV, but whether other mechanisms such as blocking receptor binding of the virus to $CD4^+$ cells are at work remains to be investigated. Given the failure of current management and interventional steps to halt the AIDS epidemic, use of oral or vaginal lactobacilli appears worthy of consideration, especially given that they can be delivered relatively inexpensively to large numbers of people on the African continent, where drug supplies are often inaccessible or financially prohibitive.

Another potential application is for pregnant women to reduce the risk of bacterial vaginosis infection associated with infant mortality and preterm labor. Oral probiotics would be particularly useful in this case, as they can be administered safely during pregnancy (66). For reasons not yet known, some lactobacilli, such as *L. rhamnosus* GG and *L. acidophilus* NCFM, appear to be not well suited for the urogenital tract (18, 106), while products on the market such as the vaginal suppository Fermalac, comprising *L. rhamnosus* and other strains (Rosell, Montreal, Canada), have no peer-reviewed studies proving eradication of bacterial vaginosis. Thus, for clinical practice at present, there are few clinically proven, commercially available options to antibiotic and antifungal therapy for urogenital infections and antiviral drugs for HIV spread.

PROBIOTICS FOR SURGICAL INFECTIONS

Legend has it that fermented milks were used to help the healing of wounds and to fight infection before antiseptics and antibiotics were available. Nevertheless, the application of viable lactic acid bacteria to an infected wound would represent a paradigm shift in current surgical practice. In a series of animal studies, *L. fermentum* RC-14 and proteins produced by this organisms were shown to prevent severe *Staphyloccus aureus* surgical implant infection (40). Although this does not prove human efficacy, the concept illustrates a different approach to wound infection management. Given the emergence of vancomycin-resistant strains of multidrug-resistant *S. aureus*, which cause major clinical problems within hospital settings, the application of probiotics or protein-derived products to wounds is worthy of further investigation.

Application of probiotics for surgical patients is not necessarily limited to skin and wounds. In the first of three intriguing studies, *L. plantarum* 299 given with enteral fiber nutrition decreased the rate of postoperative infections in liver transplant patients at very high risk of infection, organ rejection, and death (105). These patients are immunosuppressed and malnourished, and the infecting organisms often originate in the patient's own intestine, spreading through translocation. Endotoxemia from gut pathogen overgrowth also leads to further complications. The lactobacilli in that study were administered 24 h after surgery four times a day for 6 weeks. Only 13% of patients developed infections, compared to 34 to 48% in controls, with a drop in enterococcal infections being notable. Interestingly, the drop in infections occurred for pneumonia, sepsis, and cholangitis as well as others. In a second paper by Rayes et al. (104), patients undergoing major abdominal surgery (such as resection of the liver, stomach, or pancreas) benefited from *L. plantarum* 299 in terms of fewer infections, fewer antibiotics prescribed, shorter hospital stay, and lower incidence of other complications. In the third randomized, double-blind study, patients with severe acute pancreatitis who received freeze-dried lactobacilli with oat fiber for 1 week had significantly fewer episodes (4.5% versus 30%) of infection and pancreatic abscesses (98).

Rather than use antibiotics in an attempt to "decontaminate" the intestine prior to surgery, these studies indicate that there is merit to administering probiotic organisms to reduce the risk of complications. Further studies are needed on other probiotic strains with different food additives and to determine whether some patients should not be given this therapy because of elevated risk of *Lactobacillus*-associated bacteremia (103).

GUIDELINES FOR THE EVALUATION OF PROBIOTICS

In May 2002, a joint working group of the FAO and the WHO drafted new guidelines for the evaluation of probiotics in food (37). FAO and WHO and the countries they represent requested guidelines and recommendations for the criteria and methodologies required to identify and define probiotics and establish the minimum requirements needed to accurately substantiate health claims. Although the FAO and WHO reports focused on foods, many of the recommendations, including the definition of probiotics, were endorsed at a May 2002 meeting of the International Scientific Association for Probiotics and Prebiotics. Based on these guidelines (outlined in Fig. 1), several important criteria and standards must be introduced to ensure that physicians know that the products which they prescribe or recommend are of suitable quality and reliability. In brief, these guidelines address the following points.

Strain Identification

The first consideration is to identify and characterize the organism to the genus and species level with internationally accepted methods, such as DND-DNA hybridization and sequencing of DNA encoding 16S rRNA (147). For strain typing, pulsed-field gel electrophoresis is the gold standard, but randomly amplified polymorphic DNA can also be used (43). Determination of the presence of extrachromosomal genetic elements such as plasmids can also contribute to strain typing and characterization (117). Once strains have been identified, their nomenclature must be corroborated by reference to the *Approved Lists of Bacterial Names* (135) or updated lists also cited in this journal. This is important, for example, to exclude the term *Lactobacillus sporogenes*, a species that is not recognized but which is used by a number of companies to describe the organisms contained within their products (123).

The second consideration for particular strains that are being targeted for probiotic use is to have a clear and consistent strain designation, such as *L. rhamnosus* GG. This will allow

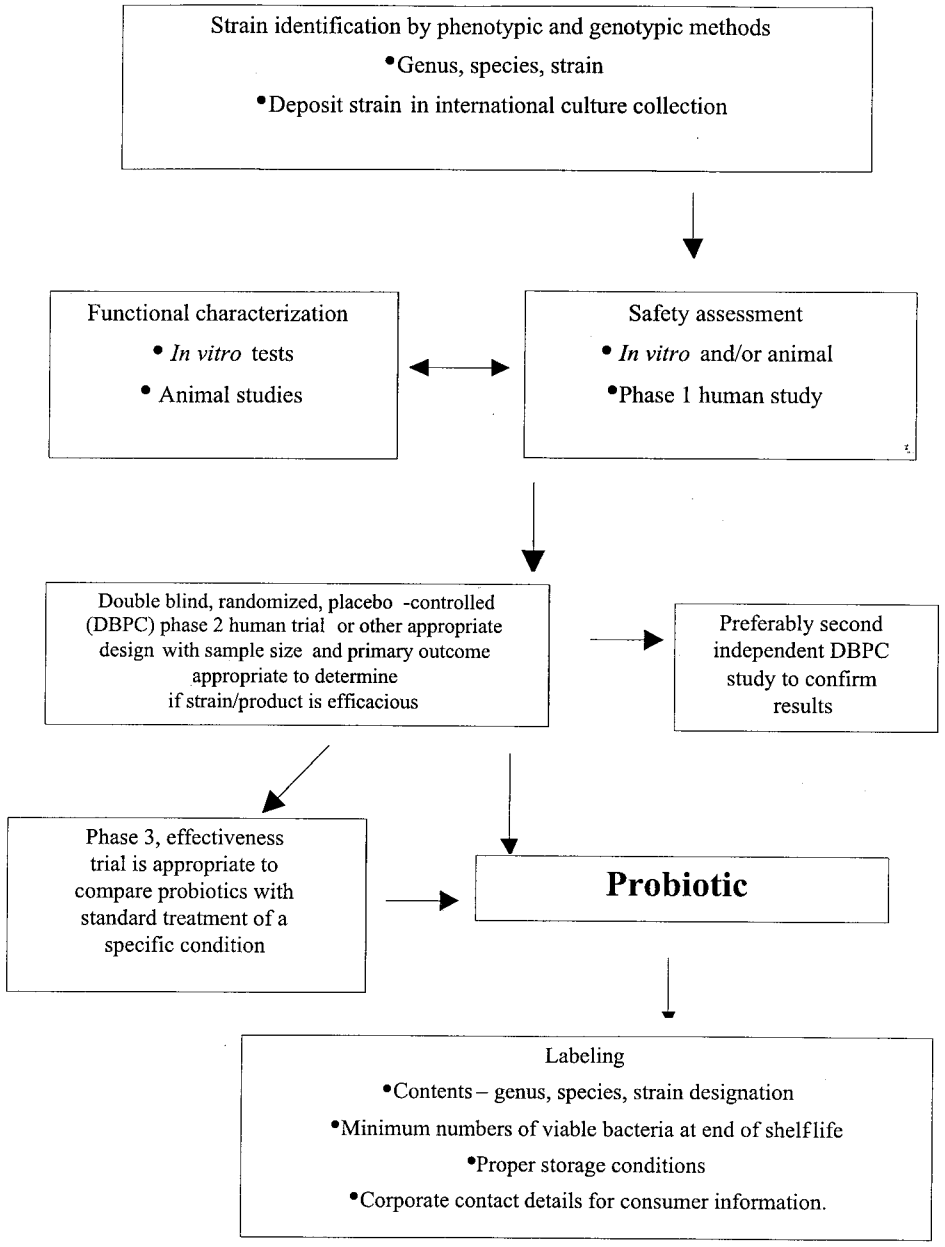

FIG. 1. FAO and WHO guidelines for probiotics in food.

physicians and consumers to track publications associated with that strain and to verify that the strain has indeed been shown to have probiotic benefits. An important example of why the failure to name strains leads to confusion is a publication which cited so-called probiotic yogurts as being responsible for side effects in patients (134). In fact, 12 of the 16 strains or products mentioned did not fit the definition of true probiotics (107). Likewise, claiming benefits on web sites or labels for strains that are either not in the product or have never been proven for that product formulation (60, 119, 152) is tantamount to the misrepresentation of facts. This is not to say that such products are necessarily inferior or unreliable; it's just that the producers need to prove their merit.

In Vitro and In Vivo Experiments

In the early 1980s, when a few groups were discovering probiotic strains of lactobacilli and developing the field, in vitro assays provided useful selection systems. Thus, characteristics such as adhesion to cells, production of bacteriocins, acids, and hydrogen peroxide, and the ability to inhibit adhesion of pathogens were deemed to be important to confer probiotic effects (6, 48, 97, 111, 116, 119). These methods still have their place in characterizing strains, but they are insufficient on their own to define a probiotic organism. Simply put, the expression of such factors in vivo and verification that they comprise key mechanisms of action is needed before they can adequately

predict the function of probiotic microorganisms in the human body. In vitro tests, such as bile salts resistance, can correlate with gastric survival in vivo (28), and spermicide resistance (86) can help vaginal probiotics survive in users of these products, making these two tests exceptions.

Of the other tests that require validation of in vivo performance, many still have merit in assessing and characterizing organisms as well as investigating potential mechanisms of action. For example, research that showed that lactobacilli could adhere to intestinal cells and signal mucus production which prevented pathogen adhesion (76) was extremely valuable and provided a new appreciation for function in the gut. Studies may also provide insight into how lactobacilli interfere with *E. coli* pathogenesis, including analysis of lipoteichoic acid antagonism of the inflammatory effect of lipopolysaccharide through competition with soluble CD14 (lipopolysaccharide binding protein) (145). This important concept shows how probiotic bacteria may directly produce benefits for the mammalian host.

Safety

Probiotics are viable organisms, and therefore it is feasible that they could infect the host. Historical data indicates that probiotic lactobacilli and bifidobacteria administered in food and in capsular form are safe for human use (1, 92, 107). Their occurrence as normal commensals of the mammalian microbiota and their established safe use in diverse food and supplement products worldwide support this conclusion. Nevertheless, side effects have been reported, including rare systemic infections. There is a need to be careful when administering live bacteria to immunocompromised subjects and those with intestinal bleeding (77, 81, 103). Care must also be taken to ensure that excessive immune stimulation is not induced in individuals who are susceptible to the development of arthritis or other complications.

The issue of safety becomes more complicated if one considers organisms such as *Enterococcus* spp. as probiotics (39). These bacteria are present in relatively high numbers in the intestine and are often included in so-called probiotic cocktails, particularly in animal feed. However, enterococci have emerged as an important cause of nosocomial infections, and isolates are increasingly vancomycin resistant (41, 53). The same is true of *Saccharomyces boulardii*, an organism used widely as a probiotic yet one which has been associated with episodes of fungemia (55).

A case has been made that *Enterococcus* and perhaps also *S. boulardii* not be referred to as probiotic for human use (38), but the onus should be on the producer that this and any other organism contemplated for human use not be a significant risk. For example, *E. coli* would not be regarded as a prime probiotic candidate because of so many of its strains are pathogenic, yet *E. coli* 83972 has been used quite effectively to prevent bladder infections in spinal cord-injured patients (31). That protocol was a prospective, nonrandomized, pilot clinical trial on 44 patients with spinal cord injury who had neurogenic bladder and had frequent episodes of symptomatic urinary tract infection. The bladders of the patients were inoculated with *E. coli* 83972, and among the 30 who became colonized with this organism there was a 63-fold reduction in the rate of symptomatic urinary tract infection versus the baseline prestudy period (mean, 0.06 versus 3.77 episodes of symptomatic urinary tract infections/patient-year, $P < 0.001$). In this example, safety did not appear to be an issue, and indeed the benefits outweighed the risks as far as could be determined. A more subtle form of safety may involve minimizing the transfer of drug resistance genes.

In order to establish safety guidelines for probiotic organisms, recognizing that many are Generally Recognized as Safe, the FAO and WHO recommended that probiotic strains be characterized at a minimum with a series of tests, including antibiotic resistance patterns, metabolic activities, toxin production, hemolytic activity, infectivity in immunocompromised animal models, side effects in humans, and adverse incidents in consumers (37). One possible scheme for testing toxin production has been recommended by the European Union Scientific Committee on Animal Nutrition (124). Given the rare incidence of side effects with *Lactobacillus* probiotics, large monitoring studies might prove useful. To date, there have been no reports of adverse overdose events caused by probiotics.

Phase 2 Clinical Studies

Phase 2 clinical studies assess the efficacy of a product against a placebo. The outcome for the individual should be a statistically and biologically significant improvement in condition, symptoms, signs, well-being, or quality of life, reduced risk of disease or longer time period to the next occurrence, or faster recovery from illness. More clinical evidence of this type is needed to gain credibility among the broader medical community. These need to provide a physician with the name of the strain, its product formulation, and the specific use for which it has been shown to be effective. Strains *L. rhamnosus* GG and *L. reuteri* SD2222 have accumulated some good clinical data, but trying to correlate these with the product formulations for sale in the United States is not simple. The GG strain is produced in liquid form in Finland, while in the United States the product is in capsules. The SD2222 studies are even less clear in that the strain number is rarely stated in published studies and is not on the label of the product sold in the United States.

Phase 3 Clinical Studies

Phase 3 studies assess the effectiveness of a product in comparison with a standard therapy for a particular disease. In general, randomized, blinded studies will provide the answer, assuming that sample size has been properly calculated and outcomes realistically predicted. Such studies should include quality-of-life measurement tools and consider risk-benefit ratios. For example, a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor product (statin) may reduce low-density lipoprotein cholesterol by 45% but may also cause rhabdomyolysis, kidney damage, or death (http://www.fda.gov/cder/drug/infopage/baycol/baycol-qa.htm). On the other hand, if animal studies that show that 10^4 *L. reuteri* CRL1098 per day for 7 days prevent hypercholesterolemia and produce a 17% increase in the ratio of high-density lipoprotein to low-density lipoprotein (141) could be repeated in humans, would this provide a clinically significant treatment option without side

effects? In other words, phase 3 studies require careful planning and an evaluation of multiple endpoints before probiotics should be discarded from the health care armamentarium. The performance of more phase 3 studies on probiotic strains is required to determine fully their place, if any, in the treatment and prevention of more serious clinical conditions and whether or not this approach can replace or complement pharmaceutical use.

Health Claims and Labeling

For the most part, only general health claims are currently allowed on foods containing probiotics. In time, this could change for probiotics shown to be superior to placebo in certain situations. Specific health claims are allowed on drug-approved probiotics that have gone through phase 3 clinical studies. Specific claims and labeling are required to better inform the user of the true benefits of the product. For example, statements such as "reduces the incidence and severity of rotavirus diarrhea in infants" would be more informative than "improves gut health." Just so, enforcing the removal of claims made by certain companies on their web site, unless peer-reviewed studies verify the claims, will greatly improve consumer confidence (119). Recommendations are presently under review by Codex, the governing body on claims for foods, and hopefully changes to guidelines and standards will be forthcoming.

THE WAY FORWARD

The use of probiotics in general clinical practice is not far away, given that products such as VSL#3 are already being used. Molecular tools will continue to be used to understand and manipulate lactic acid bacteria (70) with a view to producing vaccines and new and improved probiotic products. The critical step in wider application will be to make products available that are safe and clinically proven in a specific formulation easily accessible to physicians and consumers. Efforts are needed to advance the scientific knowledge of probiotics and determine their mechanisms of action, as well as describe when and why they fail in certain situations. Various processing advances, such as microencapsulation and bacterial coating and addition of prebiotic compounds used as growth factors by probiotic organisms, will provide a means to optimize the delivery and survival of strains at the site of action.

ACKNOWLEDGMENTS

The Canadian Research and Development Centre for Probiotics was made possible by a grant from the Ontario Research and Development Challenge Fund. Support from the Natural Sciences and Engineering Research Council is gratefully acknowledged.

G. Reid declares that he owns intellectual property rights associated with *Lactobacillus* strains GR-1 and RC-14.

REFERENCES

1. **Adams, M. R., and P. Marteau.** 1995. On the safety of lactic acid bacteria from food. Int. J. Food Microbiol. **27:**263–264.
2. **Aiba, Y., N. Suzuki, A. M. Kabir, A. Takagi, and Y. Koga.** 1998. Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic murine model. Am. J. Gastroenterol. **93:**2097–2101.
3. **Arimochi, H., T. Kinouchi, K. Kataoka, T. Kuwahara, and Y. Ohnishi.** 1997. Effect of intestinal bacteria on formation of azoxymethane-induced aberrant crypt foci in the rat colon. Biochem. Biophys. Res. Commun. **238:**753–757.
4. **Arunachalam, K., H. S. Gill, and R. K. Chandra.** 2000. Enhancement of natural immune function by dietary consumption of *Bifidobacterium lactis* (HN019). Eur. J. Clin. Nutr. **54:**263–267.
5. **Aso, Y., H. Akaza, T. Kotake, T. Tsukamoto, K. Imai, and S. Naito.** 1995. Preventive effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. The BLP Study Group. Eur. Urol. **27:**104–109.
6. **Barbes, C., and S. Boris.** 1999. Potential role of lactobacilli as prophylactic agents against genital pathogens. AIDS Patient Care STDs **13:**747–751.
7. **Bartlett, J. G.** 2002. Clinical practice. Antibiotic-associated diarrhea. N. Engl. J. Med. **346:**334–339.
8. **Beaugerie, L., A. Flahault, F. Barbut, P. Atlan, V. Lalande, P. Cousin, M. Cadilhac, and J. C. Petit.** 2003. Antibiotic-associated diarrhoea and *Clostridium difficile* in the community. Aliment Pharmacol. Ther. **17:**905–912.
9. **Behets, F., J. Andriamiadana, D. Rasamilalao, N. Ratsimbazafy, D. Randrianasolo, G. Dallabetta, and M. Cohen.** 2001. Sexually transmitted infections and associated socio-demographic and behavioural factors in women seeking primary care suggest Madagascar's vulnerability to rapid HIV spread. Trop. Med. Int. Health **6:**202–211.
10. **Benn, C. S., P. Thorsen, J. S. Jensen, B. B. Kjaer, H. Bisgaard, M. Andersen, K. Rostgaard, B. Bjorksten, and M. Melbye.** 2002. Maternal vaginal microflora during pregnancy and the risk of asthma hospitalization and use of antiasthma medication in early childhood. J. Allergy Clin. Immunol. **110:**72–77.
11. **Bernet, M. F., D. Brassart, J. R. Neeser, and A. L. Servin.** 1994. *Lactobacillus acidophilus* LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut **35:**483–489.
12. **Bernet-Camard, M. F., V. Lievin, D. Brassart, J. R. Neeser, A. L. Servin, and S. Hudault.** 1997. The human *Lactobacillus acidophilus* strain LA1 secretes a nonbacteriocin antibacterial substance(s) active in vitro and in vivo. Appl. Environ. Microbiol. **63:**2747–2753.
13. **Blakey, J. L., L. Lubitz, N. T. Campbell, G. L. Gillam, R. F. Bishop, and G. L. Barnes.** 1985. Enteric colonization in sporadic neonatal necrotizing enterocolitis. J. Pediatr. Gastroenterol. Nutr. **4:**591–595.
14. **Brumfitt, W., and J. M. Hamilton-Miller.** 1998. Efficacy and safety profile of long-term nitrofurantoin in urinary infections: 18 years experience. J. Antimicrob. Chemother. **42:**363–371.
15. **Brumfitt, W., J. M. Hamilton-Miller, R. A. Gargan, J. Cooper, and G. W. Smith.** 1983. Long-term prophylaxis of urinary infections in women: comparative trial of trimethoprim, methenamine hippurate and topical povidone-iodine. J. Urol. **130:**1110–1114.
16. **Brumfitt, W., J. M. Hamilton-Miller, S. Walker, and D. Roberts.** 1992. Cefaclor as a prophylactic agent for recurrent urinary infections: a comparative trial with macrocrystalline nitrofurantoin. Drugs Exp. Clin. Res. **18:**239–244.
17. **Burton, J. P., P. A. Cadieux, and G. Reid.** 2003. Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. Appl. Environ. Microbiol. **69:**97–101.
18. **Cadieux, P., J. Burton, G. Gardiner, I. Braunstein, A. W. Bruce, C. Y. Kang, and G. Reid.** 2002. *Lactobacillus* strains and vaginal ecology. JAMA **287:** 1940–1941.
19. **Canducci, F., A. Armuzzi, F. Cremonini, G. Cammarota, F. Bartolozzi, P. Pola, G. Gasbarrini, and A. Gasbarrini.** 2000. A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. Aliment Pharmacol. Ther. **14:**1625–1629.
20. **Caplan, M. S., and T. Jilling.** 2000. Neonatal necrotizing enterocolitis: possible role of probiotic supplementation. J. Pediatr. Gastroenterol. Nutr. **30**(Suppl. 2)**:**S18–S22.
21. **Castagliuolo, I., M. F. Riegler, L. Valenick, J. T. LaMont, and C. Pothoulakis.** 1999. *Saccharomyces boulardii* protease inhibits the effects of *Clostridium difficile* toxins A and B in human colonic mucosa. Infect. Immun. **67:**302–307.
22. **Challa, A., D. R. Rao, C. B. Chawan, and L. Shackelford.** 1997. *Bifidobacterium longum* and lactulose suppress azoxymethane-induced colonic aberrant crypt foci in rats. Carcinogenesis **18:**517–521.
23. **Christensen, H. R., H. Frokiaer, and J. J. Pestka.** 2002. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J. Immunol. **168:**171–178.
24. **Coconnier, M. H., M. F. Bernet, S. Kerneis, G. Chauviere, J. Fourniat, and A. L. Servin.** 1993. Inhibition of adhesion of enteroinvasive pathogens to human intestinal Caco-2 cells by *Lactobacillus acidophilus* strain LB decreases bacterial invasion. FEMS Microbiol. Lett. **110:**299–305.
25. **Coconnier, M. H., V. Lievin, M. F. Bernet-Camard, S. Hudault, and A. L. Servin.** 1997. Antibacterial effect of the adhering human *Lactobacillus acidophilus* strain LB. Antimicrob. Agents Chemother. **41:**1046–1052.
26. **Coconnier, M. H., V. Lievin, E. Hemery, and A. L. Servin.** 1998. Antagonistic activity against *Helicobacter* infection in vitro and in vivo by the human *Lactobacillus acidophilus* strain LB. Appl. Environ. Microbiol. **64:** 4573–4580.

27. **Cohen, C. R., A. Duerr, N. Pruithithada, S. Rugpao, S. Hillier, P. Garcia, and K. Nelson.** 1995. Bacterial vaginosis and HIV seroprevalence among female commercial sex workers in Chiang Mai, Thailand. AIDS **9:**1093–1097.
28. **Conway, P. L., S. L. Gorbach, and B. R. Goldin.** 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J. Dairy Sci. **70:**1–12.
29. **Culhane, J. F., V. Rauh, K. F. McCollum, V. K. Hogan, K. Agnew, and P. D. Wadhwa.** 2001. Maternal stress is associated with bacterial vaginosis in human pregnancy. Matern. Child Health J. **5:**127–134.
30. **Dani, C., R. Biadaioli, G. Bertini, E. Martelli, and F. F. Rubaltelli.** 2002. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol. Neonate **82:**103–108.
31. **Darouiche, R. O., W. H. Donovan, M. Del Terzo, J. I. Thornby, D. C. Rudy, and R. A. Hull.** 2001. Pilot trial of bacterial interference for preventing urinary tract infection. Urology **58:**339–344.
32. **Delaney, M. L., and A. B. Onderdonk.** 2001. Nugent score related to vaginal culture in pregnant women. Obstet. Gynecol. **98:**79–84.
33. **D'Souza, A. L., C. Rajkumar, J. Cooke, and C. J. Bulpitt.** 2002. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. Br. Med. J. **324:**1361.
34. **Edmunds, L.** 2001. The underuse of probiotics by family physicians. Can. Med. Assoc. J. **164:**1577.
35. **El-Nezami, H., H. Mykkanen, P. Kankaanpaa, S. Salminen, and J. Ahokas.** 2000. Ability of *Lactobacillus* and *Propionibacterium* strains to remove aflatoxin B, from the chicken duodenum. J. Food Prot. **63:**549–552.
36. **Felley, C. P., I. Corthesy-Theulaz, J. L. Rivero, P. Sipponen, M. Kaufmann, P. Bauerfeind, P. H. Wiesel, D. Brassart, A. Pfeifer, A. L. Blum, and P. Michetti.** 2001. Favourable effect of an acidified milk (LC-1) on *Helicobacter pylori* gastritis in man. Eur. J. Gastroenterol. Hepatol. **13:**25–29.
37. **Food and Agriculture Organization of the United Nations and World Health Organization.** 2002, posting date. Guidelines for the evaluation of probiotics in food. Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report. (Online.)
38. **Food and Agriculture Organization of the United Nations and World Health Organization.** 2001, posting date. Regulatory and clinical aspects of dairy probiotics. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report. Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report. (Online.)
39. **Franz, C. M., W. H. Holzapfel, and M. E. Stiles.** 1999. Enterococci at the crossroads of food safety? Int. J. Food Microbiol. **47:**1–24.
40. **Gan, B. S., J. Kim, G. Reid, P. Cadieux, and J. C. Howard.** 2002. *Lactobacillus fermentum* RC-14 inhibits *Staphylococcus aureus* infection of surgical implants in rats. J. Infect. Dis. **185:**1369–1372.
41. **Gardiner, D., S. Murphey, E. Ossman, and D. Jungkind.** 2002. Prevalence and acquisition of vancomycin-resistant enterococci in a medical intensive care unit. Infect. Control Hosp. Epidemiol. **23:**466–468.
42. **Gardiner, G., C. Heinemann, M. Baroja, A. Bruce, D. Beuerman, J. Madrenas, and G. Reid.** 2002. Oral administration of the probiotic combination *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 for human intestinal applications. Int. Dairy J. **12:**191–196.
43. **Gardiner, G. E., C. Heinemann, A. W. Bruce, D. Beuerman, and G. Reid.** 2002. Persistence of *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA. Clin. Diagn. Lab. Immunol. **9:**92–96.
44. **Gewolb, I. H., R. S. Schwalbe, V. L. Taciak, T. S. Harrison, and P. Panigrahi.** 1999. Stool microflora in extremely low birthweight infants. Arch. Dis. Child. Fetal Neonatal **80:**F167–F173.
45. **Gill, H. S., K. J. Rutherfurd, J. Prasad, and P. K. Gopal.** 2000. Enhancement of natural and acquired immunity by *Lactobacillus rhamnosus (HN001), Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019). Br J. Nutr. **83:**167–176.
46. **Gionchetti, P., F. Rizzello, A. Venturi, P. Brigidi, D. Matteuzzi, G. Bazzocchi, G. Poggioli, M. Miglioli, and M. Campieri.** 2000. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology **119:**305–309.
47. **Glass, R. I., J. F. Lew, R. E. Gangarosa, C. W. LeBaron, and M. S. Ho.** 1991. Estimates of morbidity and mortality rates for diarrheal diseases in American children. J. Pediatr. **118:**S27–33.
48. **Gopal, P. K., J. Prasad, J. Smart, and H. S. Gill.** 2001. In vitro adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an enterotoxigenic *Escherichia coli*. Int. J. Food Microbiol. **67:**207–216.
49. **Gorbach, S. L.** 2000. Probiotics and gastrointestinal health. Am. J. Gastroenterol. **95:**S2–4.
50. **Guandalini, S.** 2002. Use of *Lactobacillus*-GG in paediatric Crohns disease. Dig. Liver Dis. **34**(Suppl. 2)**:**S63–S65.
51. **Guandalini, S., L. Pensabene, M. A. Zikri, J. A. Dias, L. G. Casali, H. Hoekstra, S. Kolacek, K. Massar, D. Micetic-Turk, A. Papadopoulou, J. S. de Sousa, B. Sandhu, H. Szajewska, and Z. Weizman.** 2000. *Lactobacillus* sp. strain GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. J. Pediatr. Gastroenterol. Nutr. **30:**54–60.
52. **Guarino, A., R. B. Canani, M. I. Spagnuolo, F. Albano, and L. Di Benedetto.** 1997. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. J. Pediatr. Gastroenterol. Nutr. **25:**516–519.
53. **Hamer, D. H., and C. J. Gill.** 2002. From the farm to the kitchen table: the negative impact of antimicrobial use in animals on humans. Nutr. Rev. **60:**261–264.
54. **Hay, P.** 2000. Recurrent bacterial vaginosis. Curr. Infect. Dis. Rep. **2:**506–512.
55. **Hennequin, C., C. Kauffmann-Lacroix, A. Jobert, J. P. Viard, C. Ricour, J. L. Jacquemin, and P. Berche.** 2000. Possible role of catheters in *Saccharomyces boulardii* fungemia. Eur. J. Clin. Microbiol. Infect. Dis. **19:**16–20.
56. **Hillier, S. L.** 1998. The vaginal microbial ecosystem and resistance to HIV. AIDS Res. Hum. Retroviruses **14**(Suppl. 1)**:**S17–S21.
57. **Hosoda, M., H. Hashimoto, F. He, H. Morita, and A. Hosono.** 1996. Effect of administration of milk fermented with *Lactobacillus acidophilus* LA-2 on fecal mutagenicity and microflora in the human intestine. J. Dairy Sci. **79:**745–749.
58. **Hoyos, A. B.** 1999. 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. **3:**197–202.
59. **Hudault, S., V. Lievin, M. F. Bernet-Camard, and A. L. Servin.** 1997. Antagonistic activity exerted in vitro and in vivo by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection. Appl. Environ. Microbiol. **63:**513–518.
60. **Hughes, V. L., and S. L. Hillier.** 1990. Microbiologic characteristics of *Lactobacillus* products used for colonization of the vagina. Obstet. Gynecol. **75:**244–248.
61. **Isolauri, E., T. Arvola, Y. Sutas, E. Moilanen, and S. Salminen.** 2000. Probiotics in the management of atopic eczema. Clin. Exp. Allergy **30:**1604–1610.
62. **Isolauri, E., M. Juntunen, T. Rautanen, P. Sillanaukee, and T. Koivula.** 1991. A human *Lactobacillus* strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics **88:**90–97.
63. **Isolauri, E., Y. Sutas, P. Kankaanpaa, H. Arvilommi, and S. Salminen.** 2001. Probiotics: effects on immunity. Am. J. Clin. Nutr. **73:**444S–450S.
64. **Kabir, A. M., Y. Aiba, A. Takagi, S. Kamiya, T. Miwa, and Y. Koga.** 1997. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. Gut **41:**49–55.
65. **Kaila, M., E. Isolauri, E. Soppi, E. Virtanen, S. Laine, and H. Arvilommi.** 1992. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. Pediatr. Res. **32:**141–144.
66. **Kalliomaki, M., S. Salminen, H. Arvilommi, P. Kero, P. Koskinen, and E. Isolauri.** 2001. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet **357:**1076–1079.
67. **Kalliomaki, M., S. Salminen, T. Poussa, H. Arvilommi, and E. Isolauri.** 2003. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet **361:**1869–1871.
68. **Keane, F. E., C. A. Ison, and D. Taylor-Robinson.** 1997. A longitudinal study of the vaginal flora over a menstrual cycle. Int. J. STDs AIDS **8:**489–494.
69. **Kollaritsch, H., H. Holst, P. Grobara, and G. Wiedermann.** 1993. Prevention of travelers diarrhea with *Saccharomyces boulardii.* Results of a placebo controlled double-blind study. Fortschr. Med. **111:**152–156.
70. **Kullen, M. J., and T. R. Klaenhammer.** 1999. Identification of the pH-inducible, proton-translocating F1F0-ATPase (*atpBEFHAGDC*) operon of *Lactobacillus acidophilus* by differential display: gene structure, cloning and characterization. Mol Microbiol. **33:**1152–1161.
71. **Lewis, S. J., L. F. Potts, and R. E. Barry.** 1998. The lack of therapeutic effect of *Saccharomyces boulardii* in the prevention of antibiotic-related diarrhoea in elderly patients. J. Infect. **36:**171–174.
72. **Livengood, C. H., 3rd, D. E. Soper, K. L. Sheehan, D. E. Fenner, M. G. Martens, A. L. Nelson, M. Ismail, J. M. Thorp, M. Lappin, B. J. Long, T. Blackwelder, R. L. Sweet, and S. Sagov.** 1999. Comparison of once-daily and twice-daily dosing of 0.75% metronidazole gel in the treatment of bacterial vaginosis. Sex. Transm. Dis. **26:**137–142.
73. **Lucas, A., and T. J. Cole.** 1990. Breast milk and neonatal necrotising enterocolitis. Lancet **336:**1519–1523.
74. **Lundgren, O., and L. Svensson.** 2001. Pathogenesis of rotavirus diarrhea. Microbes Infect. **3:**1145–1156.
75. **Maassen, C. B., C. van Holten-Neelen, F. Balk, M. J. den Bak-Glashouwer, R. J. Leer, J. D. Laman, W. J. Boersma, and E. Claassen.** 2000. Strain-dependent induction of cytokine profiles in the gut by orally administered *Lactobacillus* strains. Vaccine **18:**2613–2623.

75a. **MacDermott, R. P.** 1996. Alterations of the mucosal immune system in inflammatory bowel disease. J. Gastroenterol. **31:**907–916.

76. **Mack, D. R., S. Michail, S. Wei, L. McDougall, and M. A. Hollingsworth.**

1999. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. Am. J. Physiol. **276:**G941–G950.
77. **Mackie, R. I., A. Sghir, and H. R. Gaskins.** 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. Am. J. Clin. Nutr. **69:**1035S–1045S.
78. **Madden, J. A., and J. O. Hunter.** 2002. A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics. Br. J. Nutr. **88**(Suppl. 1)**:**S67–S72.
79. **Majamaa, H., and E. Isolauri.** 1996. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. J. Allergy Clin. Immunol. **97:**985–990.
80. **Majamaa, H., E. Isolauri, M. Saxelin, and T. Vesikari.** 1995. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. J. Pediatr. Gastroenterol. Nutr. **20:**333–338.
81. **Marteau, P. R.** 2002. Probiotics in clinical conditions. Clin. Rev. Allergy Immunol. **22:**255–273.
82. **Martin, H. L., B. A. Richardson, P. M. Nyange, L. Lavreys, S. L. Hillier, B. Chohan, K. Mandaliya, J. O. Ndinya-Achola, J. Bwayo, and J. Kreiss.** 1999. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J. Infect. Dis. **180:**1863–1868.
83. **Matsuzaki, T., and J. Chin.** 2000. Modulating immune responses with probiotic bacteria. Immunol. Cell Biol. **78:**67–73.
84. **McFarland, L. V., and G. W. Elmer.** 1995. Biotherapeutic agents: past, present and future. Microecol. Ther. **23:**46–73.
85. **McFarland, L. V., C. M. Surawicz, R. N. Greenberg, G. W. Elmer, K. A. Moyer, S. A. Melcher, K. E. Bowen, and J. L. Cox.** 1995. Prevention of beta-lactam-associated diarrhea by *Saccharomyces boulardii* compared with placebo. Am. J. Gastroenterol. **90:**439–448.
86. **McGroarty, J. A., G. Reid, and A. W. Bruce.** 1994. The influence of non-oxynol-9-containing spermicides on urogenital infection. J. Urol. **152:**831–833.
87. **Michetti, P., G. Dorta, P. H. Wiesel, D. Brassart, E. Verdu, M. Herranz, C. Felley, N. Porta, M. Rouvet, A. L. Blum, and I. Corthesy-Theulaz.** 1999. Effect of whey-based culture supernatant of *Lactobacillus acidophilus* (*johnsonii*) La1 on *Helicobacter pylori* infection in humans. Digestion **60:**203–209.
88. **Midolo, P. D., J. R. Lambert, R. Hull, F. Luo, and M. L. Grayson.** 1995. In vitro inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. J. Appl. Bacteriol. **79:**475–479.
89. **Miettinen, M., A. Lehtonen, I. Julkunen, and S. Matikainen.** 2000. Lactobacilli and streptococci activate NF-kappa B and STAT signaling pathways in human macrophages. J. Immunol. **164:**3733–3740.
90. **Millar, M. R., C. Bacon, S. L. Smith, V. Walker, and M. A. Hall.** 1993. Enteral feeding of premature infants with *Lactobacillus* sp. strain GG. Arch. Dis. Child. **69:**483–487.
91. **Mitsuyama, K., A. Toyonaga, and M. Sata.** 2002. Intestinal microflora as a therapeutic target in inflammatory bowel disease. J. Gastroenterol. **37**(Suppl. 14)**:**73–77.
92. **Naidu, A. S., W. R. Bidlack, and R. A. Clemens.** 1999. Probiotic spectra of lactic acid bacteria (LAB). Crit. Rev. Food Sci. Nutr. **39:**13–126.
93. **Niedzielin, K., H. Kordecki, and B. Birkenfeld.** 2001. A controlled, double-blind, randomized study on the efficacy of *Lactobacillus plantarum* 299V in patients with irritable bowel syndrome. Eur. J. Gastroenterol. Hepatol. **13:**1143–1147.
94. **Nugent, R. P., M. A. Krohn, and S. L. Hillier.** 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J. Clin. Microbiol. **29:**297–301.
95. **Oatley, J. T., M. D. Rarick, G. E. Ji, and J. E. Linz.** 2000. Binding of aflatoxin B1 to bifidobacteria in vitro. J. Food Prot. **63:**1133–1136.
96. **Oberhelman, R. A., R. H. Gilman, P. Sheen, D. N. Taylor, R. E. Black, L. Cabrera, A. G. Lescano, R. Meza, and G. Madico.** 1999. A placebo-controlled trial of *Lactobacillus* sp. strain GG to prevent diarrhea in undernourished Peruvian children. J. Pediatr. **134:**15–20.
97. **Ocana, V. S., A. A. Pesce de Ruiz Holgado, and M. E. Nader-Macias.** 1999. Selection of vaginal H_2O_2-generating *Lactobacillus* species for probiotic use. Curr. Microbiol. **38:**279–284.
98. **Olah, A., T. Belagyi, A. Issekutz, M. E. Gamal, and S. Bengmark.** 2002. Randomized clinical trial of specific *Lactobacillus* and fibre supplement to early enteral nutrition in patients with acute pancreatitis. Br. J. Surg. **89:**1103–1107.
99. **Perdigon, G., S. Alvarez, and A. Pesce de Ruiz Holgado.** 1991. Immunoadjuvant activity of oral *Lactobacillus casei*: influence of dose on the secretory immune response and protective capacity in intestinal infections. J. Dairy Res. **58:**485–496.
100. **Perdigon, G., and A. P. d. R. Holgado.** 2000. Mechanisms involved in the immunostimulation by lactic acid bacteria., p. 213–233. *In* R. Fuller and G. Perdigon (ed.), Probiotics. Kluwer Academic Publishers, Amsterdam, The Netherlands.
101. **Pochapin, M.** 2000. The effect of probiotics on *Clostridium difficile* diarrhea. Am. J. Gastroenterol. **95:**S11–S13.
102. **Prantera, C., M. L. Scribano, G. Falasco, A. Andreoli, and C. Luzi.** 2002. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohns disease: a randomised controlled trial with *Lactobacillus* sp. strain GG. Gut **51:**405–409.
103. **Rautio, M., H. Jousimies-Somer, H. Kauma, I. Pietarinen, M. Saxelin, S. Tynkkynen, and M. Koskela.** 1999. Liver abscess due to a *Lactobacillus rhamnosus* strain indistinguishable from *L. rhamnosus* strain GG. Clin. Infect. Dis. **28:**1159–1160.
104. **Rayes, N., S. Hansen, D. Seehofer, A. R. Muller, S. Serke, S. Bengmark, and P. Neuhaus.** 2002. Early enteral supply of fiber and lactobacilli versus conventional nutrition: a controlled trial in patients with major abdominal surgery. Nutrition **18:**609–615.
105. **Rayes, N., D. Seehofer, S. Hansen, K. Boucsein, A. R. Muller, S. Serke, S. Bengmark, and P. Neuhaus.** 2002. Early enteral supply of *Lactobacillus* and fiber versus selective bowel decontamination: a controlled trial in liver transplant recipients. Transplantation **74:**123–127.
106. **Reid, G.** 2000. In vitro analysis of a dairy strain of *Lactobacillus acidophilus* NCFMTM as a possible probiotic for the urogenital tract. Int. Dairy J. **10:**415–419.
107. **Reid, G.** 2002. Safety of *Lactobacillus* strains as probiotic agents. Clin. Infect. Dis. **35:**349–350.
108. **Reid, G., A, W. Bruce, and M. Taylor.** 1995. Instillation of *Lactobacillus* and stimulation of indigenous organisms to prevent recurrence of urinary tract infections. Microecol. Ther. **23:**32–45.
109. **Reid, G., D. Beuerman, C. Heinemann, and A. W. Bruce.** 2001. Probiotic Lactobacillus dose required to restore and maintain a normal vaginal flora. FEMS Immuno. Med. Microbiol. **32:**37–41.
110. Reference deleted.
111. **Reid, G., and A. W. Bruce.** 2001. Selection of *Lactobacillus* strains for urogenital probiotic applications. J. Infect. Dis. **183**(Suppl. 1)**:**S77–S80.
112. **Reid, G., A. W. Bruce, N. Fraser, C. Heinemann, J. Owen, and B. Henning.** 2001. Oral probiotics can resolve urogenital infections. FEMS Immunol. Med. Microbiol. **30:**49–52.
113. Reference deleted.
114. **Reid, G., D. Charbonneau, J. Erb, B. Kochanowski, D. Beuerman, R. Poehner, and A. W. Bruce.** 2003. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. FEMS Immunol. Med. Microbiol. **35:**131–134.
115. **Reid, G., D. Charbonneau, S. Gonzalez, G. Gardiner, J. Erb, and A. W. Bruce.** 2002. Ability of *Lactobacillus* GR-1 and RC-14 to stimulate host defences and reduce gut translocation and infectivity of *Salmonella typhimurium*. Nutraceut. Food **7:**168–173.
116. **Reid, G., R. L. Cook, and A. W. Bruce.** 1987. Examination of strains of lactobacilli for properties that may influence bacterial interference in the urinary tract. J. Urol. **138:**330–335.
117. **Reid, G., J. A. McGroarty, L. Tomeczek, and A. W. Bruce.** 1996. Identification and plasmid profiles of *Lactobacillus* species from the vagina of 100 healthy women. FEMS Immunol. Med. Microbiol. **15:**23–26.
118. **Reid, G., K. Millsap, and A. W. Bruce.** 1994. Implantation of *Lactobacillus casei* var *rhamnosus* into vagina. Lancet **344:**1229.
119. **Reid, G., C. Zalai, and G. Gardiner.** 2001. Urogenital lactobacilli probiotics, reliability and regulatory issues. J. Dairy Sci. **84:**E164–E169.
120. Reference deleted.
121. **Reveneau, N., M. C. Geoffroy, C. Locht, P. Chagnaud, and A. Mercenier.** 2002. Comparison of the immune responses induced by local immunizations with recombinant *Lactobacillus plantarum* producing tetanus toxin fragment C in different cellular locations. Vaccine **20:**1769–1777.
122. **Saavedra, J. M., N. A. Bauman, I. Oung, J. A. Perman, and R. H. Yolken.** 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet **344:**1046–1049.
123. **Sanders, M. E., L. Morelli, and S. Bush.** 2001. "*Lactobacillus sporogenes*" is not a *Lactobacillus* probiotic. ASM News **67:**385–386.
124. **Scientific Committee on Animal Nutrition.** 2001. Report of the Scientific Committee on Animal Nutrition on the criteria for assessing the safety of microorganisms resistant to antibiotics of human clinical and veterinary importance. European Commission Health and Consumer Protection Directorate-General.
125. **Schwebke, J. R., C. M. Richey, and H. L. Weiss.** 1999. Correlation of behaviors with microbiological changes in vaginal flora. J. Infect. Dis. **180:**1632–1636.
126. **Sen, S., M. M. Mullan, T. J. Parker, J. T. Woolner, S. A. Tarry, and J. O. Hunter.** 2002. Effect of *Lactobacillus plantarum* 299v on colonic fermentation and symptoms of irritable bowel syndrome. Dig. Dis. Sci. **47:**2615–2620.
127. **Sewankambo, N., R. H. Gray, M. J. Wawer, L. Paxton, D. McNaim, F. Wabwire-Mangen, D. Serwadda, C. Li, N. Kiwanuka, S. L. Hillier, L. Rabe, C. A. Gaydos, T. C. Quinn, and J. Konde-Lule.** 1997. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. Lancet **350:**546–550.
128. Reference deleted.

129. **Shanahan, F.** 2000. Immunology. Therapeutic manipulation of gut flora. Science **289:**1311–1312.
130. **Sheih, Y. H., B. L. Chiang, L. H. Wang, C. K. Liao, and H. S. Gill.** 2001. Systemic immunity-enhancing effects in healthy subjects following dietary consumption of the lactic acid bacterium *Lactobacillus rhamnosus* HN001. J. Am. Coll. Nutr. **20:**149–156.
131. **Shornikova, A. V., I. A. Casas, E. Isolauri, H. Mykkanen, and T. Vesikari.** 1997. *Lactobacillus reuteri* as a therapeutic agent in acute diarrhea in young children. J. Pediatr. Gastroenterol. Nutr. **24:**399–404.
132. **Shornikova, A. V., I. A. Casas, H. Mykkanen, E. Salo, and T. Vesikari.** 1997. Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. Pediatr. Infect. Dis. J. **16:**1103–1107.
133. **Shu, Q., and H. Gill.** 2002. Immune protection mediated by the probiotic *Lactobacillus rhamnosus* HN001 (DR20) against *Escherichia coli* O157:H7 infection in mice. FEMS Immunol. Med. Microbiol. **34:**59.
134. **Sipsas, N. V., D. I. Zonios, and T. Kordossis.** 2002. Safety of *Lactobacillus* strains used as probiotic agents. Clin. Infect. Dis. **34:**1283–1285.
135. **Skerman, V. B. D., V. McGowan, and P. H. A. Sneath.** 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. **30:**225–420.
135a. **Souza, D. G., R. Guabiraba, V. Pinho, A. Bristow, S. Poole, and M. M. Teixeira.** 2003. IL-1-driven endogenous IL-10 production protects against the systemic and local acute inflammatory response following intestinal reperfusion injury. J. Immunol. **170:**4759–4766.
136. **Surawicz, C. M., G. W. Elmer, P. Speelman, L. V. McFarland, J. Chinn, and G. van Belle.** 1989. Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardii*: a prospective study. Gastroenterology **96:**981–988.
137. **Surawicz, C. M., L. V. McFarland, R. N. Greenberg, M. Rubin, R. Fekety, M. E. Mulligan, R. J. Garcia, S. Brandmarker, K. Bowen, D. Borjal, and G. W. Elmer.** 2000. The search for a better treatment for recurrent *Clostridium difficile* disease: use of high-dose vancomycin combined with *Saccharomyces boulardii*. Clin. Infect. Dis. **31:**1012–1017.
138. **Szajewska, H., M. Kotowska, J. Z. Mrukowicz, M. Armanska, and W. Mikolajczyk.** 2001. Efficacy of *Lactobacillus* sp. strain GG in prevention of nosocomial diarrhea in infants. J. Pediatr. **138:**361–365.
139. **Taha, T. E., R. H. Gray, N. I. Kumwenda, D. R. Hoover, L. A. Mtimavalye, G. N. Liomba, J. D. Chiphangwi, G. A. Dallabetta, and P. G. Miotti.** 1999. HIV infection and disturbances of vaginal flora during pregnancy. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **20:**52–59.
140. **Taha, T. E., D. R. Hoover, G. A. Dallabetta, N. I. Kumwenda, L. A. Mtimavalye, L. P. Yang, G. N. Liomba, R. L. Broadhead, J. D. Chiphangwi, and P. G. Miotti.** 1998. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. AIDS **12:**1699–1706.
141. **Taranto, M. P., M. Medici, G. Perdigon, A. P. Ruiz Holgado, and G. F. Valdez.** 2000. Effect of *Lactobacillus reuteri* on the prevention of hypercholesterolemia in mice. J. Dairy Sci. **83:**401–403.
142. **Taylor-Robinson, D., M. McCaffrey, J. Pitkin, and R. F. Lamont.** 2002. Bacterial vaginosis in climacteric and menopausal women. Int. J. STDs AIDS **13:**449–452.
143. **Thomas, M. R., S. C. Litin, D. R. Osmon, A. P. Corr, A. L. Weaver, and C. M. Lohse.** 2001. Lack of effect of *Lactobacillus* sp. strain GG on antibiotic-associated diarrhea: a randomized, placebo-controlled trial. Mayo Clin. Proc. **76:**883–889.
144. **Vasquez, A., T. Jakobsson, S. Ahrne, U. Forsum, and G. Molin.** 2002. Vaginal *Lactobacillus* flora of healthy Swedish women. J. Clin. Microbiol. **40:**2746–2749.
145. **Vidal, K., A. Donnet-Hughes, and D. Granato.** 2002. Lipoteichoic acids from *Lactobacillus johnsonii* strain La1 and *Lactobacillus acidophilus* strain La10 antagonize the responsiveness of human intestinal epithelial HT29 cells to lipopolysaccharide and gram-negative bacteria. Infect. Immun. **70:**2057–2064.
146. **Walker, W. A.** 2000. Role of nutrients and bacterial colonization in the development of intestinal host defense. J. Pediatr. Gastroenterol. Nutr. **30**(Suppl. 2)**:**S2–S7.
147. **Wang, J., C. Jenkins, R. I. Webb, and J. A. Fuerst.** 2002. Isolation of Gemmata-like and Isosphaera-like planctomycete bacteria from soil and freshwater. Appl. Environ. Microbiol. **68:**417–422.
148. **Ward, L. A., B. I. Rosen, L. Yuan, and L. J. Saif.** 1996. Pathogenesis of an attenuated and a virulent strain of group A human rotavirus in neonatal gnotobiotic pigs. J. Gen. Virol. **77:**1431–1441.
149. **Wendakoon, C. N., A. B. Thomson, and L. Ozimek.** 2002. Lack of therapeutic effect of a specially designed yogurt for the eradication of *Helicobacter pylori* infection. Digestion **65:**16–20.
150. **World Health Organization.** 1995. The treatment of diarrhoea — a manual for physicians and other senior health workers. World Health Organization/CDR/95.3. World Health Organization, Geneva, Switzerland.
151. **Xu, J., and W. Verstraete.** 2001. Evaluation of nitric oxide production by lactobacilli. Appl. Microbiol. Biotechnol. **56:**504–507.
151a. **Yolken, R. H., C. Ojeh, I. A. Khatri, U. Sajjan, and J. F. Forstner.** 1994. Intestinal mucins inhibit rotavirus replication in an oligosaccharide-dependent manner. J. Infect. Dis. **169:**1002–1006.
152. **Zhong, W., K. Millsap, H. Bialkowska-Hobrzanska, and G. Reid.** 1998. Differentiation of *Lactobacillus* species by molecular typing. Appl. Environ. Microbiol. **64:**2418–2423.