Safety of Probiotics That Contain Lactobacilli or Bifidobacteria

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Lactobacilli and bifidobacteria are extremely rare causes of infection in humans, as are probiotics based on these organisms. This lack of pathogenicity extends across all age groups and to immunocompromised individuals. Strains used for new probiotics should be chosen from the commensal flora of humans and should not carry intrinsic resistance to antibiotics that would prevent treatment of a rare probiotic infection. Vigilance regarding the detection of possible rare cases of infection due to probiotics should be maintained, and isolates should be sent to reference centers for molecular characterization and confirmation.

Because there are published reports of rare infections involving lactobacilli or bifidobacteria, including 2 cases suggested to be associated with probiotic strains, it was considered important to develop a science-driven, evidence-based overview of the safety of lactobacilli and bifidobacteria used as probiotics in foods. A workshop to which recognized experts on probiotics were invited was convened to advise leading manufacturers (Danone, Nestlé, Valio, and Yakult); it took place between September 2000 and the end of January 2001. The objectives of the workshop were (1) to review critically the current scientific and medical literature on probiotic lactobacilli and bifidobacteria, (2) to form a consensus on aspects of the safety of such probiotic bacteria, (3) to advise on research needs to enhance the database on probiotic safety, as appropriate, and (4) to review criteria for evaluation of the safety of new probiotic products.

The workshop panel members had expertise in clinical practice, microbiology, intestinal microecology, pathogenicity, nutrition, toxicology, and public health. Discussion focused on 3 key public health issues: whether probiotic consumption increases the risk of opportunistic infections due to lactobacilli or bifidobacteria, whether such probiotics that are in current use increase the potential for opportunistic infections among immunocompromised people, and whether such probiotics are safe for consumption by young infants and children. Also considered were the possibility of risk assessment and its nature, the criteria needed to guide the screening of new probiotics to determine their safety, and outstanding research needs.

RISK OF PROBIOTIC *LACTOBACILLUS* AND *BIFIDOBACTERIUM* INFECTION IN HEALTHY OR IMMUNOCOMPROMISED PEOPLE

There are many sources of exposure to lactobacilli and bifidobacteria. These sources include probiotics, fermented foodstuffs (e.g., yogurt, cheese, sauerkraut and other fermented vegetables, and olives), as well as the host's own microflora. In many traditional foods, such bacteria play an important role in preventing spoilage and the growth of pathogenic microorganisms [1].

Some probiotic products that contain lactobacilli or bifidobacteria have long histories of safe use—in some cases, for many decades [2]. In healthy humans, lactobacilli are normally present in the oral cavity (10^3–10^4 cfu/g), the ileum (10^3–10^7 cfu/g), and the colon (10^4–10^8 cfu/g), and they are the dominant microorganism in the vagina [3].

Cases of infection due to lactobacilli and bifidobacteria are extremely rare and are estimated to represent 0.05%–0.4% of cases of infective endocarditis and bacteremia [4, 5]. Of interest, increasing consumption of probiotic lactobacilli and bifidobacteria has not led to an increase in such opportunistic infections in consumers. For example, in Finland, where registration of all bacteremia isolates is mandatory, and where it is, in most cases, accompanied by isolate preservation and characterization, the number of infections involving *Lactobacillus* species reported to the National Public Health Institute (Helsinki, Finland) has remained at a constant background level of 10–20 cases per year, representing a mean incidence of 0.2% (range, 0.1%–0.3%) for the years 1995–1999, with no obvious trend. This constant level occurred against the background of a notable increase in the consumption of probiotic products that contain *Lactobacillus rhamnosus* GG and evidence that the incidence of all bacteremias may be increasing, at least in developed countries (e.g., in Finland, there were 103 cases of bacteremia per 100,000 individuals in 1995, 111 cases in 1996, 119 cases in 1997 and 1998, and 127 cases in 1999 [6, 7]).

Most of the rare cases of infection with lactobacilli occur in patients with underlying conditions that are predominantly of a severe nature [4, 5, 8]; most of these patients die within a year of developing infection [8]. Lactobacillemia is a frequent marker of serious or fatal underlying disease [4, 5, 8].

Immunocompromised patients generally are more vulnerable to infection with pathogens and have a higher incidence of opportunistic infections. However, there is no published evidence that consumption of probiotics that contain lactobacilli or bifidobacteria increases the risk of opportunistic infection among such individuals. In addition, 2 clinical studies have been conducted to assess the safety of probiotics in small groups of specific immunocompromised patients (e.g., patients with HIV infection), and the findings of these studies support the safety of probiotics consumed by such groups [9, 10].

Several attempts have been made to evaluate the factors that might predispose severely ill patients to infections with lactobacilli or bifidobacteria [8, 11]. In some cases, invasive procedures that involve the gastrointestinal tract (which has large commensal populations of lactobacilli or bifidobacteria) and other organs, together with chronic immunosuppressive and antibiotic therapy, were proposed to contribute to an increased risk [12]. However, statistical analysis has not been used for the most part, and, when it has been used, too few cases have been studied to permit the development of general recommendations.

To our knowledge, there is no published medical guidance regarding hospital patients' consumption of probiotics or other products that contain viable lactobacilli or bifidobacteria. Although guidance has been issued for probiotic yeast preparations, it is not warranted for probiotic lactobacilli or bifidobacteria in food on the basis of current evidence.

CONSUMPTION OF PROBIOTICS BY INFANTS AND YOUNG CHILDREN

Existing probiotic lactobacilli and bifidobacteria are suitable for infants and children. Several studies have shown that products that contain lactobacilli and bifidobacteria are well tolerated in this age group [10, 13–15]. Strains added to food products for infants generally are restricted to producers of L-lactic acid (see also the Contraindications and Precautions section below) [16, 17]. There are also specific compositional (e.g., electrolyte loading and nutrient content) legal requirements for food products intended for infants and young children [18, 19].

CONTRAINDICATIONS AND PRECAUTIONS

Recently, there have been several documented cases of fungemia associated with a *Saccharomyces cerevisiae* (*Saccharomyces boulardii*) probiotic, for which suspensions were prepared at the patient's bedside. Investigation of these cases indicated that infection was due to contamination of indwelling catheters [20]. The authors of the study recommended that probiotics in powdered form, such as *S. cerevisiae* probiotics, should be prepared under hygienic conditions to prevent line contamination. There appear to be no recorded cases of indwelling line–associated lactobacillemia due to probiotics.

Overgrowth of commensal lactobacilli can be a feature of patients with short bowel syndrome and is frequently associated with D-lactic acidosis [21]. Antibiotic therapy and dietary carbohydrates appear to be the most important predisposing factors, although ingested D-lactate–producing bacteria, coupled to antibiotic use, has been implicated once [22]. Consumption of strains that produce L-lactate exclusively is not likely to present a problem for such patients and may be useful in their treatment [23].

DETECTION AND IDENTIFICATION OF *LACTOBACILLUS* AND *BIFIDOBACTERIUM* SPECIES

For many clinical isolates of lactobacilli, the accuracy of identification—in particular, identification to the species level—is a subject of doubt because of the choice of inappropriate or

insufficient identification methods. Much of this is the result of their infrequency as putative pathogens and the subsequent lack of familiarity with these isolates among laboratory workers. In addition, contamination of samples by the patients' commensal flora is always a risk.

Identification is a critical step in assessment of the safety of potential novel probiotic strains [24]. In the clinical setting, identification is performed for 2 main reasons: (1) to have a definitive identification to the species level for diagnostic purposes, and (2) for epidemiological purposes. Identification should be verified by molecular studies because physiological characterization alone—for example, by fermentation profile—is insufficient to achieve reliable identification [25]. Strain characterization and typing may include certain phenotypic characteristics, but it ideally should be based on molecular approaches (e.g., amplified fragment–length polymorphism analysis). In the majority of cases, this level of identification and characterization is beyond the capabilities of hospital laboratories. It is advisable to send isolates recovered from these rare clinical cases to an appropriate national reference center.

APPROACHES TO EXAMINING POTENTIAL RISKS

Lactobacilli and bifidobacteria are ubiquitous in the diet and in the healthy large intestine soon after birth. A classical risk assessment approach, similar to that used for pathogens [26], is not possible or warranted. Some studies of lactobacilli have attempted to define virulence factors. Such classical approaches, although useful for known pathogens, are inherently flawed when applied to normal commensals, lactobacilli, or bifidobacteria. In the case of the risk assessment approach for pathogens, pathogenicity is demonstrated and is normally a consequence of several properties, including colonization factors and virulence factors, acting in concert. Frequently, such factors as adhesion are considered to be virulence factors when pathogens are studied. However, mucosal adhesion and other colonization factors are essential features of most commensals. For example, there is a distinct mucosal-associated flora in the gastrointestinal tract. There is little value in screening organisms of low clinical significance and with no proven virulence determinants for such characteristics as potential virulence factors, particularly in the absence of gastrointestinal commensals as comparative controls.

There is no evidence that ingested probiotic lactobacilli or bifidobacteria pose any risk of infection greater than that associated with commensal strains. In quantitative terms, the existing data suggest that the risk of bacteremia, which is the most commonly reported of these infections, is <1 case per million individuals. It is virtually impossible to propose a risk of death because of the common association of infections involving lactobacilli with fatal underlying conditions or the presence of polymicrobial infections. However, the risk is unequivocally in the "negligible" range. There have been 180 cases of lactobacillemia reported during the past 30 years. However, there have been only 69 cases of infective endocarditis attributed to lactobacilli reported during the same period.

Two cases have been reported in which the lactobacillus that was isolated was indistinguishable from the probiotic strains recently consumed by the patient [27, 28]. The case of infection due to a strain of *L. rhamnosus* that was similar to the *L. rhamnosus* GG strain was observed in a 74-year-old woman with non–insulin-dependent diabetes [27]. The woman had a liver abscess that was associated with right-side basal pneumonia and right-side pleural empyema. No obvious cause of the liver abscess was found, and an aspirate of the hepatic abscess showed the presence of an *L. rhamnosus* microorganism. The woman reported having a daily intake of ∼0.5 L of dairy drinks containing *L. rhamnosus* GG during the 4 months before her symptoms developed. The clinical strain was compared with different *L. rhamnosus* strains, and it appeared to be indistinguishable from the GG strain. Another case of infection due to *L. rhamnosus* was recently reported elsewhere [28]. It developed in a 67-year-old man who had mild mitral valve regurgitation, had undergone removal of carious teeth, and had received as prophylaxis 3 g of amoxicillin 1 h before the procedure was performed. This man consumed probiotic capsules that contained a mixture of *L. rhamnosus, Lactobacillus acidophilus,* and *Streptococcus faecalis.* Because he found the capsules too large to swallow, he was in the habit of emptying their contents into his mouth and chewing and then swallowing them with milk. A few days after the dental extraction was performed, the patient developed endocarditis, and *L. rhamnosus* was isolated from several blood cultures. Further analysis showed that one of the organisms cultured from the probiotic capsule was indistinguishable from that isolated from blood culture (on the basis of the appearance of the culture, the sensitivity pattern, and the findings of an API50 test and pyrolysis mass spectrometry).

PROPOSED CRITERIA FOR SCREENING OF THE SAFETY OF NEW PROBIOTICS

In the development of new probiotics, species ideally should be selected from fecal flora commensals of healthy human volunteers who have not ingested products for ≥1 month or from among probiotic lactic acid bacteria that have a long history of safe use in food products. Not all of these strains are known to be of human origin, and, therefore, this is not a prerequisite for safety. Furthermore, because some lactobacilli or bifidobacteria may just transit through the gastrointestinal tract, "real" human commensals may be difficult to define. Although

data from studies of humans provide information on the safety of probiotics used in existing products, we should not rely solely on such studies for the safety screening of new probiotic products.

Conventional safety evaluation approaches, such as those for toxicology testing proposed by the Organization for Economic Cooperation and Development in 1995 [29], are appropriate as a first step in the evaluation of new probiotics. If a product is entirely novel (e.g., the microorganisms that it contains are not present in traditional diets), then more data may be required [30]. Thus, a useful starting point could be a 90-day conventional rat-feeding study. However, there is a view that animal models are of limited value in such microbial risk assessment [31]. There is no firm consensus for such models, even between the authors of the present study. Despite this lack of firm consensus, the 90-day rat-feeding model has the advantage of being consistent with published recommendations for safety evaluation [29, 30]. Such feeding studies should be designed to pay special attention to the structure and function of the organs of the digestive system, and hematologic analysis should be extended to test for any translocating organisms.

Some of the safety evaluation models proposed in the literature appear to be of little or no value [32]. Bypassing the normal route of administration (e.g., by using intravenous administration) may produce data that are difficult to interpret, as are data produced in models under extreme conditions. Recently, changes have been proposed in the protocols for LD_{50} (median lethal dose) tests, and several authorities have become more critical of the need for such tests. Assays for which the end point is based solely on the death of laboratory animals are questionable from scientific and ethical points of view.

Where animal models may serve the most useful function in the evaluation of the safety of new probiotics is in immunocompromised hosts. Immunodeficient gnotobiotic mice have been used to assess the safety of probiotic bacteria [33] as well as the potential to protect against *Candida* infection [34]. In experiments assessing safety, some *Lactobacillus* probiotics were associated with death among dogs <4 weeks of age [33]. Testing the use of new probiotics among immunocompromised neonatal mice may be a sensible precaution.

Many markers of the activity of gut bacteria have been studied [35]. Some such markers recently have been advocated [36] for the screening of probiotics. These markers include formation of biogenic amines, azoreductase, nitroreductase, β-glucuronidase activity, induction of thrombins, dissolution of thrombi by various hydrolases, aggregation of thrombocytes, adhesion to fibrinogen or fibronectin, mucin degradation, and hemolysis. Transferable antibiotic resistance was a further consideration (see the Antibiotic Resistance section below). Some such measurements in vitro may have potential value but are not necessarily good predictors of activity in vivo. The relevance of many of these markers requires further study, and, in particular, desired outcomes should be proposed. Furthermore, the activities considered to be markers are present among components of the normal gastrointestinal flora. Evaluation is further complicated by the fact that net changes in various enzymatic activities in different parts of the gastrointestinal tract are not solely dependent on the ability of ingested bacteria (in foods or as probiotics) to undertake these reactions.

The safety of probiotics should be confirmed in studies of humans. Although many research tools based on animal models or in vitro techniques are available, data from studies of humans are preferred whenever possible. In addition to self-reporting of symptoms and noninvasive measurements, such as measurements of body weight or blood pressure, the parameters of hematologic analysis and of serum/plasma chemical analysis detailed by Wolf et al. [9] can provide useful information on the functioning of the immune and hematopoietic systems and, also, on the integrity of several internal organs, such as the kidneys and the liver. Clinically normal ranges for these parameters are known, and changes may indicate probiotic-induced effects.

ANTIBIOTIC RESISTANCE

Many strains of lactobacilli are naturally resistant to vancomycin. It is accepted that antibiotic nonsusceptibility/resistance is not, in itself, a hazard unless it renders the probiotic untreatable in rare cases of infection or unless it can be transferred to potential pathogens for which resistance could have therapeutic consequences. The vancomycin resistance genes of *Lactobacillus* species appear to be chromosomally located and are not easily transferable to other genera [37]. Vancomycin would not be used for the treatment of a case of lactobacillemia. When used as probiotics, selected strains should be susceptible to ≥2 major antibiotics. It currently is difficult to interpret studies of gene transfer in vivo, and the methods involved need to be further developed. The focus should be on transfer to *Enterococcus* species and *Staphylococcus aureus,* for which there are potential clinical consequences, rather than on homologous gene transfer.

RESEARCH NEEDS

Continuing research needs can be divided into 4 broad categories. First, there needs to be a better understanding of the host and microbial factors that play a role in *Lactobacillus* infections, including the mechanisms of translocation, bloodstream survival, and infectivity. Second, there needs to be further proof of the efficacy of probiotics for the treatment and/or prevention of diseases, ailments, and infections. Advocacy of such use of probiotics must be based on evidence against

stringent criteria of best-practice investigative methods. Third, we need to understand the mechanism of action of probiotics in those circumstances for which the efficacy of probiotics is proven. Finally, on the basis of the first 2 criteria, research is needed into the development of improved probiotics for particular targeted use as therapeutics or therapeutic adjuncts, including the development of probiotics as vaccine delivery vehicles.

It is also important to establish optimal strain identification, molecular characterization methods, and optimal standard operating procedures, to ensure commonality of approach. Full characteristics and appropriate molecular profiles of currently used probiotics generated by standard operating procedures should be undertaken. Such methods should currently include 16S ribosome sequence analysis for speciation and genomic DNA fragment analysis for strain differentiation (e.g., by fluorescent amplified fragment–length polymorphism analysis or PFGE profile [with reference strain on the same gel]). There may increasingly be a role for new technologies for identification (e.g., Matrix-assisted laser desorption/ionization time-of-flight cell surface mass spectral profiles) that are independent of any sophisticated bacterial cell manipulation or nucleic acid extraction procedures. Such methods should, by necessity, be applied to clinical isolates when there is suspicion of probiotic strain involvement. These databases of probiotic strain characteristics should be made freely available to researchers and should include comparable data on commensal nonprobiotic strains of the species used as probiotics. Such information and methodological guidance would help shed light on the etiology of these rare lactobacilli or bifidobacteria infections.

CONCLUSIONS

Continued vigilance in identifying, typing, and cataloguing all bacteria associated with bacteremia is necessary. Inappropriate methods are still frequently applied for the identification of species and strains of lactobacilli and bifidobacteria, and harmonization is needed. Ongoing investigations of the molecular biology of lactobacilli and bifidobacteria, including genome sequencing of various strains, will eventually yield data useful for identifying interstrain differences at the molecular level.

Responsible probiotics manufacturers have put in place procedures for surveillance of adverse events caused by existing strains and for screening of new strains. The safety criterion used for such products is at least "as safe as" that used for appropriate traditional reference food products. Current evidence suggests that the risk of infection with probiotic lactobacilli or bifidobacteria is similar to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers, including immunocompromised hosts.

References

1. Hammes WP, Tichaczek PS. The potential of lactic acid bacteria for the production of safe and wholesome food. Z Lebensm Unters Forsch **1994**; 198:193–201.
2. Shortt C. The probiotic century: historical and current perspectives, Trends in Food Science and Technology **1999**; 10:411–7.
3. Hill GB, Eschenbach DA, Holmes KK. Bacteriology of the vagina. Scand J Urol Nephrol Suppl **1984**; 86:23–39.
4. Gasser F. Safety of lactic-acid bacteria and their occurrence in human clinical infections. Bulletin de L'Institut Pasteur **1994**; 92:45–67.
5. Saxelin M, Chuang NH, Chassy B, et al. Lactobacilli and bacteremia in southern Finland, 1989–1992. Clin Infect Dis **1996**; 22:564–6.
6. Salminen MK, Tynkkynen S, Rautelin H, et al. *Lactobacillus* bacteremia during a rapid increase in probiotic use of *Lactobacillus rhamnosus* GG in Finland. Clin Infect Dis **2002**; 35:1155–60.
7. Statistics Finland: Finland in figures. Helsinki: Library of Statistics, **2000**
8. Husni RN, Gordon SM, Washington JA, Longworth DL. *Lactobacillus* bacteremia and endocarditis: review of 45 cases. Clin Infect Dis **1997**; 25:1048–55.
9. Wolf BW, Wheeler KB, Ataya, DG, Garleb KA. Safety and tolerance of *Lactobacillus reuteri* supplementation to a population infected with the human immunodeficiency virus. Food Chem Toxicol **1998**; 36: 1085–94.
10. Cunningham-Rundles S, Ahrné S, Bengmark S, et al. Probiotics and immune response. Am J Gastroenterol **2000**; 95:S22–5.
11. Patel R, Cockerill FR, Porayko MK, Osmon DR, Ilstrup DM, Keating MR. Lactobacillemia in liver transplant patients. Clin Infect Dis **1994**; 18:207–12.
12. Antony SJ, Stratton CW, Dummer JS. *Lactobacillus* bacteremia: description of the clinical course in adult patients without endocarditis. Clin Infect Dis **1996**; 23:773–8.
13. Pedone CA, Bernabeu AO, Postaire ER, Bouley CF, Reinert P. The effect of supplementation with milk fermented by *Lactobacillus casei* (strain DN-114 001) on acute diarrhoea in children attending day care centres. Int J Clin Pract **1999**; 53:179–84.
14. Vanderhoof JA, Young RJ. Use of probiotics in childhood gastrointestinal disorders. J Pediatr Gastroenterol Nutr **1998**; 27:323–32.
15. Guandalini S, Pensabene L, Zikri MA, et al. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhoea: a multicenter European trial. J Pediatr Gastroenterol Nutr **2000**; 30: 54–60.
16. Codex Alimentarius Commission. Codex Alimentarius, vol. IX, suppl. 1. Codex standards for dietetique foods or diet foods, comprising foods destined for feeding of young infants, and a code relating to their hygienic utilisation. Geneva: World Health Organization, **1987**.
17. European Parliament and Council directive of 20 February 1995 on food additives other than colours and sweeteners. In: Official Journal of the European Communities. Luxemburg City, Luxemburg: Commission of the European Communities, **1995**; L61:1–40. Publication 95/2/EC.
18. Commission directive on processed cereal-based foods and baby foods for infants and young children. In: Official Journal of the European Communities. Luxemburg City, Luxemburg: Commission of the European Communities, **1996**; L49:17–96. Publication 96/5/EC.

19. Commission directive on infant formulae and follow-on formulae. In: Official Journal of the European Communities. Luxemburg City, Luxemburg: Commission of the European Communities, **1991**; L175: 35–49. Publication 91/321/EEC.

20. Hennequin C, Kauffmann-Lacroix C, Jobert A, et al. Possible role of catheters in *Saccharomyces boulardii* fungemia. Eur J Clin Microbiol Infect Dis **2000**; 19:16–20.

21. Bongaerts GPA, Tolboom JJM, Naber AHJ, et al. Role of bacteria in the pathogenesis of short bowel syndrome–associated D-lactic acidemia. Microb Pathog **1997**; 22:285–93.

22. Coronado BE, Opal S, Yoburn DC. Antibiotic-induced D-lactic acidosis. Ann Intern Med **1995**; 122:839–42.

23. Vanderhoof JA, Young RJ, Murray N, Kaufman SS. Treatment strategies for small bowel bacterial overgrowth in short bowel syndrome. J Pediatr Gastroenterol Nutr **1998**; 27:155–60.

24. Holzapfel WH, Haberer P, Geisen R, Bjorkroth J, Schillinger U. Taxonomy and important features of probiotic microorganisms in food and nutrition. Am J Clin Nutr **2001**; 73:S365–73

25. Andrighetto C, De Dea P, Lombardi A, Neviani E, Rossetti L, Giraffa G. Molecular identification and cluster analysis of homofermentative thermophilic lactobacilli isolated from dairy products. Res Microbiol **1998**; 149:631–43.

26. International Life Sciences Institute Risk Science Institute Pathogen Risk Assessment Working Group. A conceptual framework to assess the risks of human disease following exposure to pathogens. Risk Anal **1996**; 16:841–8.

27. Rautio M, Jousimies-Somer H, Kauma H, et al. Liver abscess due to a *Lactobacillus rhamnosus* strain indistinguishable from *L. rhamnosus* strain GG. Clin Infect Dis **1999**; 28:1159–60.

28. Mackay AD, Taylor MB, Kibbler CC, Hamilton-Miller JM. *Lactobacillus* endocarditis caused by a probiotic organism. Clin Microbiol Infect **1999**; 5:290–2.

29. Organization for Economic Cooperation and Development. Guidelines for the testing of chemicals. Paris: Organization for Economic Cooperation and Development, **1995**.

30. Regulation (EC) of the European Parliament and of the Council of 27 January concerning novel foods and novel food ingredients. In: Official Journal of the European Communities. Luxemburg City, Luxemburg: Commission of the European Communities, **1997**; L043:1–11. Publication 258/97/EC.

31. Salminen S, von Wright A, Morelli L, et al. Demonstration of safety of probiotics—a review. Int J Food Microbiol **1998**; 44:93–106.

32. O'Brien J, Crittenden R, Ouwehand AC, et al. Safety evaluation of probiotics. Trends in Food Science and Technology **1999**; 10:418–24.

33. Wagner RD, Warner T, Roberts L, Farmer J, Balish E. Colonization of congenitally immunodeficient mice with probiotic bacteria. Infect Immun **1997**; 65:3345–51.

34. Wagner RD, Pierson C, Warner T, et al. Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice. Infect Immun **1997**; 65:4165–72.

35. Hill MJ, ed. Role of gut bacteria in human toxicology and pharmacology. London: Taylor & Francis, **1995**.

36. Working Group of the Bundesinstitut fuer gesundheitlichen Verbraucherschultz und Veterinaermedizin. Probiotic cultures in foods. Berlin: **1999**.

37. Tynkkynen S, Singh KV, Varmanen P. Vancomycin resistance factor of *Lactobacillus rhamnosus* GG in relation to enterococcal vancomycin resistance *(van)* genes. Int J Food Microbiol **1998**; 41:195–204.