Assessment of the Risk of Probiotics in Terms of the Food Safety and Human Health

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Abstract Probiotics are often referred to as microorganisms (bacteria or yeasts) that generally provide health benefits. There is great interest in probiotics for various medical reasons and millions of people around the world consume probiotic microorganisms daily with the perception that it is beneficial for health. Members of the *genus Lactococcus* and *Lactobacillus, Streptococcus, Enterococcus* strains, and some other LAB strains are generally accepted as safe (GRAS) status, although they contain some opportunistic pathogens. In addition, some of the spore forming bacteria have been researched and used as probiotics. However, nowadays theoretical concerns and side effects are discussed with regard to the safety of probiotics. Systemic infections, the risk of harmful metabolic activities, risk of adjuvant side effects, immunomodulation and gene transfer risk are among the theoretical concerns discussed. The most common side effects of probiotic microorganisms include gastrointestinal disorders such as nausea, diarrhea, bloating, abdominal pain and dyspepsia. Other side effects include respiratory tract infections, abscess, allergic reactions and severe medical conditions such as sepsis, endocarditis and fungemia. The safety of probiotics is related to the potential vulnerability of the consumer or the patient, the dose of use, duration of consumption and the frequency of consumption. The significance of negative probiotic effects will be better understood by understanding of the probiotic interaction mechanisms with host and colonizing microbes. In this chapter, the evaluation of the risk associated with the consumption of probiotic products has been discussed, based on epidemiological data and infected cases.

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Introduction

The term "probiotic" originates from the Greek words "pro" and "bio" and it means "for life". It was defined first by Lilly and Stillwell in 1965 as "the metabolites that are secreted by a microorganism and helps in the development of another living organism". In 1971, Sperti used this term for the tissue extracts contributing to the microbial reproduction (Yiğit 2009; Ebners et al. 2014). The most recent meaning of probiotics was used firstly in 1974 by Parker and it was defined as the microorganisms and metabolites protecting the intestinal microbial balance. Removing the term "metabolites" from the definition made by Parker, the current definition of the probiotic is obtained (Lee and Salminen 2009). According to Guarner and Schaafsma, the probiotics are the living microorganisms positively contributing to the health when taken at sufficient amount together with the probiotic foods, in addition to the nourishment (Guarner and Schaafsma 1998). The probiotics can be used as national support both in a healthy development and in the treatment of diseases. These bacteria compete against the harmful microorganisms for the nutrients and colonize on the intestinal surface. Besides that, they also positively contribute to the activities in the gastrointestinal system and the health. Moreover, in research of Metchnikoff carried out between 1845 and 1916, it is known that the probiotics have positive effects on the health by ensuring the microbial ecosystem balance in intestines (Metchnikoff 2004). The probiotics are defined as the living microorganisms positively contributing to the host's health when orally taken at sufficient amount (Özden 2005).

Some strains of *Lactobacillus* spp. (*Lactobacillus acidophilus*, *L. casei*, *L. brevis*, *L. bulgaricus*, *L. cellebiosus*, *L. delbrueckii*, *L. johnsonii*, *L. lactis*, *L. reuteri*, *L. rhamnosus*, *L. plantarum*, *L. fermentum*, *L. helveticus*, *L. curvatus*, *L. salivarius*, *L. gasseri*), *Bifidobacterium* spp. (*Bifidobacterium adolescentis*, *B. bifidum*, *B. breve*, *B. longum*, *B. infantis*, *B. thermophilum*), *Pediococcus* spp. (*P. cerevisiae*, *P. acidilactici*, *P. pentosaceus*), *Streptococcus* spp. (*S. cremoris*, *S. thermophilus*, *S. intermedius*, *S. lactis*, *S. diacetilactis*), *Bacillus* spp. (*B. subtilis*, *B. pumilus*, *B. lentus*, *B. licheniformis*, *B. coagulans*, *B. cereus*), *Bacteroides* spp. (*B. capillus*, *B. suis*, *B. ruminicola*, *B. amylophilus*), *Propionibacterium* spp. (*P. shermanii*, *P. freudenreichii*), *Leuconostoc* spp. (*L. mesenteroides*), some yeasts (*Saccharomyces cerevisiae*, *S. boulardii*, *Candida torulopsis*) and molds (*Aspergillus niger*, *A. oryzae*) are used in probiotic foodstuffs (Bozkurt and Aslım 2004; Toprak Kavas 2007).

The prebiotics are the nutrients improving the host health by positively contributing to the reproduction of gastrointestinal system bacteria and passing directly to the large intestines without being digested (Ceyhan and Alıç 2012). Some of the compounds having prebiotic character are inulin, fructo-oligosaccharides, lactulose, galacto-oligosaccharides, soya oligosaccharides, gluco-oligosaccharides and isomalto-oligosaccharides. Many plants synthesize the inulin. Onion, garlic, wheat, leek, and banana contain inulin (Gülmez and Güven 2002). The breast milk contains more than 130 sorts of oligosaccharides (Çoşkun 2006). The symbiotic is the com-

bined form of probiotics and prebiotics. The postbiotics are the biologically active by-products of the probiotic cultures and they are the materials such as short-chain fatty acid, which have positive effects on the health when added to the nutrients (Ceyhan and Alıç 2012).

The probiotic bacteria are of Gram-positive, asporogenic, and basil form, and they develop at 35–38 °C temperature and pH range of 5.5–6.0. *L. acidophilus* is an anaerobic or facultative anaerobic bacterium. The optimum temperature for the bifidobacteria is 37–43 °C, whereas the pH range is 6.5–7.0. Their development slows when ambient pH decreases below 4.5–5 or increases above 8–8.5. The bifidobacteria transform glucose into acetic acid and lactic acid. Thus, they are heterofermentative. An enzyme of this special mechanism, which is fructose-6-phosphate phosphoketolase (F6PKK), is routinely used in distinguishing the bifidobacteria from the other microorganisms (Ceyhan and Alıç 2012).

The probiotic bacteria can resist to the gastric acidity more than the other bacteria can. It is more resistant to the bile salt and lysozyme enzyme. The probiotic bacteria control the reproduction rate of the undesired bacteria in the intestines by producing antimicrobial materials such as lactic acid, acetic acid, and bacteriocin (Ceyhan and Alıç 2012).

Moreover, the expected characteristics of microorganism used as probiotic are as follows:

- It must be from the intestinal system microflora of a normal human.
- It must be easily metabolized in the intestinal system without being affected by the negative environmental factors such as low pH and bile salts.
- It must be able to live on intestinal epithelial cell surfaces and it must be able to colonize.
- The number of living cells on the intestinal surface must be at a high level.
- It must be capable of maintaining its vitality and activity during the production and storage.
- It must be capable of stimulating the immune system of the host.
- It must be safe and it must have no adverse effect,
- It must be capable of negatively influencing the carcinogenic and pathogenic bacteria.
- It must produce antimicrobial material.
- It has to have no pathogenic feature (Timmerman et al. 2004; Friedman 2005; Gönülateş 2008).

The retention of the probiotic bacteria on the epithelial and mucosal surfaces of the gastrointestinal system was reported to be the most important and essential characteristic in order for them to have a biological effect (Sağdıç et al. 2004). In order for the probiotics taken from the nutrients to show the expected benefit, they must resist to the bile salts and gastric acidity and bile salts, reach at the gastrointestinal system in living form, and they must be capable of living on and colonizing the epithelial cell surfaces of the gastrointestinal mucosa (Otles and Cagindi 2003). The food biotechnology aims to investigate and improve the tolerance of probiotics against the acidity and bile salts, as well as their capacity to retain on the intestinal

surface, their proteolytic characteristics, and their capacity of secreting lactic acid. These characteristics are also the most important characteristics of the probiotics (Maragkoudakis et al. 2006; Ranadheera et al. 2014).

Nowadays, the probiotics became very popular because of their protective effects against the diseases. As the probiotics become more popular, the studies on developing new probiotic products also gained speed. In many countries, the innovative probiotic products developed as a result of biotechnological studies compete with each other in order to dominate the biotechnology market. In the studies on probiotic market and consumption, it was reported that the 28 million USD has been spent in the USA in 2011 (Tall 2016). Yogurt, yogurt derived products, dairy based milks, dietary supplements are the nutrients having probiotic properties and being popularly consumed nowadays. *Lactobacillus* spp., (*L. johnsonii*, *L. paracasei*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. rhamnosus*) GG, *Bifidobacterium* spp. (*B. bifidum*, *B. animalis*, and *B. longum*), *S. thermophilus* are most common microorganisms found in the yogurt and yogurt-derived products (milk or soy based). Predominantly single strains, such as *L. casei* Shirota and *L. lactis* are commonly found in dairy-based drinks. Same as yogurt, generally single strains found in cheese, buttermilk and other dairy products. Same as yogurts, frequently single strains are found in non-dairy products such as fermented cereals, fruit drinks and raw sausages. Single strains of various species are found in dietary supplements (tablets, powders, drops an capsules) usually in combinations with vitamins. Single strains, such as *Escherichia coli* Nissle, *Enterococcus faecalis*, *S. boulardii* and *L. rhamnosus* GG are most common microorganisms found in medicine products used for human (Wassenaar and Klein 2008). Which bacteria can be used as probiotic was defined by FDA (Food and Drug Admiration) as GRAS (generally recognized as safe). However, the sensitivity to or resistance against the antibiotics is an important selection criterion for selecting the probiotic bacteria (Gismondo et al. 1999; Saarela et al. 2000; Yuksekdag and Aslim 2010). Wide and unconscious use of antibiotics caused the emergence of antibiotic-resistant microorganisms. Moreover, the potential of transferring the genes of antibiotic resistance from different microorganisms to especially the pathogenic bacteria is a very important health problem of today and the antibiotic resistance genes became an important risk factor. Besides that, the increasing use of antibiotics caused the development of resistance to the antibiotics used for the human and the erythromycin and tetracycline resistances were observed among the lactobacillus bacteria (Özteber 2013). It was reported that the antibiotic resistance of probiotic bacteria and the propagation of antibiotic resistance genes is an significant point that must be investigated for the reliability of these bacteria. As well as it can be a natural characteristic of the bacteria, the antibiotic resistance can also develop as a result of mutations or gene transfer. The possibility of the transfer of natural or mutant genes of antibiotic resistance is very low. Besides that, despite the high possibility of the resistance acquired due to a genetic mutation or a DNA transfer from the other bacteria and despite that these transferred genes are not solely a cause of disease, there also are risks such as the increase in the disease rates, the prolongation of the processes, and the increase in mortality rates together with the increasing antibiotic resistance of pathogen bacteria (Ammor

et al. 2007). In many studies, it was reported that the significant increase in the consumption of probiotic products and traditional fermented foods brought the risk of the antibiotic resistance gene transfer from the probiotic bacteria to the pathogenic ones and it may cause health problems among the people (Saarela et al. 2000).

In other words, according to the criteria set by FAO/WHO (2002), the resistance gained against the antibiotics and the propagation of these antibiotic resistance genes pose an important problem in terms of the reliability of these bacteria (Herreros et al. 2005; Yuksekdag and Aslim 2010; Muñoz-Atienza et al. 2013). For this reason, the transferrable genes of antibiotic resistance must be controlled from the aspect of the resistance of antibiotics due to the potential of transfer from probiotic bacteria to pathogenic ones (Ammor et al. 2007; Dewan and Tamang 2007; Ouoba et al. 2008). Metabolic activity (Biogenic amine production, bile salt 7-hydroxylased D- vs. L-Lactate production, mucin degradation, cholyl glycine hydrolase, bile salt 7-hydroxylased azoreductase, nitro reductase, β-glucuronidase, β-glucosidase, N-nitrosation), infectivity (Platelet aggregation, adhesion, haemolysis, aggregation of erythrocytes, epidemiology, lethally irradiated mice), gene transfer (Plasmid transfer) and immune functions (Phagocytosis, congenital immune deficient mice) of probiotic bacteria are the possible safety assessment criteria (O'Brien et al. 1999).

According to a Report of 2002 published by Food and Agriculture Organization (FAO) and World Health Organization (WHO) of United Nations, "the probiotics can be held responsible theoretically for four types of adverse effects:

1. Systemic infections.
2. Harmful activities of metabolism.
3. Over stimulation of immunity among the sensitive individuals.
4. Gene transportation"

Moreover, the small gastrointestinal syndromes were also documented.

WHO/FAO study group mentioned that the toxin production, antibiotic resistance, and metabolic activities such as hemolytic potential, synthesis of D-lactate and deconjugation of bile salts should be tested, that the human studies should be maintained in order to evaluate the adverse effects and the surveillance of commercial consumers, that the contagiousness should be examined by using the probiotic organism on the animals with ideally suppressed immune system, and that the novel probiotic microorganisms should be analyzed from the aspect of reliability.

Besides the antibiotic resistance, there also are theoretical concerns about the safety and adverse effects of probiotics. The systemic infections caused by the probiotic microorganisms, the harmful metabolic activity risks, adjuvant adverse effect risk, immunomodulation risk, and gene transfer risk are some of the subjects that are theoretically discussed. The importance of these negative probiotic effects would be better understood when the mechanisms of the interaction between host and colonizing microorganisms are better understood. In this chapter, by making use of the epidemiological data and contagious cases, it was aimed to evaluation of the safety of the probiotic product consumption.

Theoretical Adverse Effects of the Use of Probiotics

There are some negative theoretical concerns about the use of probiotics by the humans (Salminen et al. 1998; Ishibashi and Yamazaki 2001; Reid 2002; Clancy 2003; Henriksson et al. 2005; Senok et al. 2005; Boyle et al. 2006). They have negative effects on the translocation potential, colonization of probiotics, metabolic/physiologic effects, and gastrointestinal physiology and function (Saarela et al. 2000; Henriksson et al. 2005; Senok et al. 2005). Moreover, the local and propagating undesired immunologic effects, the possibility of the transfer of antibiotic-resistance gene from the commensal/probiotic bacteria to the other ones and potential pathogens within the gastrointestinal system, and the potential of this antibiotic-resistance gene to the bacteria and potential pathogens are also among these theoretical adverse effects (Saarela et al. 2000; Ishibashi and Yamazaki 2001; Salyers et al. 2004; Senok et al. 2005).

Translocation Potential

The indigenous bacteria normally do not exist in the spleen, mesenteric lymph nodes or the blood of healthful animals. The immune system of the host annihilates these indigenous bacteria, which translocate through the mucosal epithelial tissue. For this reason, most of the studies, in which the probiotics were applied to the healthy subjects at high doses, showed that the probiotic translocation did not occur. In fact, even while translocating from the gastrointestinal system, the probiotics infrequently promote severe diseases among the healthful subjects. The observational reports presented in Lactic Acid Bacteria Industries Platform (LABIP) organized by the European Union showed that, except for the enterococcus, the risk of general infection arising from the lactic acid bacteria (LAB) is quite low (Adams and Marteau 1995). This observation was supported with various safety assessments performed for the probiotics consumed as food supplements. Making use of a rat model, Huang et al. (2003) studied the reliability of the application of *Propionibacterium jensenii* 702, which is a new probiotic, for a healthy human. The researchers determined that, at the doses as high as 10^{10} CFU/rat/day, live cells of *P. jensenii* 702 was not acquired from the blood, mesenteric lymph nodes, spleen or liver of the rats, hence no disease- or treatment-related mortality was observed. These results were supported by another study reporting that, when the dose of 10^{11} CFU/rat/day corresponding to 50 g/kg/day for rats, the translocation of the LAB did not occur. These lactic acid amounts are much higher than the amounts normally consumed by the people, and this suggests that the strains examined here are safe for human health (Zhou et al. 2000a, b). Likewise, Shu et al. (1999) applied 5×10^{10} CFU/rat/day dose and reported that there was no *Lactobacillus* or bifidobacteria in the spleen and kidneys of the healthy rats. This

dose is much higher than the regular daily intake and it corresponds to the dose 10^9–10^{11} CFU/day recommended for a human weighing 75 kg. Under the light of data they obtained, the researchers claimed that the use of probiotics among the humans might be safe.

Zhou et al. carried out a comprehensive study on the probiotic translocation (Zhou et al. 2000a, b). In that study, the researchers applied *Lactobacillus* and bifidobacteria at a dose as high as 10^{12} CFU/kg body weight/day to the healthy mice. *B. lactis*, *L. acidophilus* and *L. rhamnosus* strains are given to the mice together with their meal during 4 consecutive weeks. According to the study results, it was determined that these probiotics had no negative effect on the hematology and blood chemistry of those mice and on the intestinal mucosal histology. In a different study made by Asahara et al. (2003), probiotics are evaluated like commensal microorganisms. They transferred seven different probiotic microorganisms to Japanese rabbits (male) having no specific pathogen infection. The researchers determined that one strain of *L. casei* and four strains of the *L. rhamnosus* colonized and developed in liver and spleen at 10^2–10^4 CFU/g and 10^8–10^9 CFU/g concentrations, respectively. Besides that, no rabbit observed to have colonization even after 14 days did die. Moreover, the authors did not report the *L. gasseri* DSM 20243, *L. casei* Shirota and *L. acidophilus* ATCC 4356 infectivity.

Despite the reports stating that there was no probiotic translocation in healthful subjects, the enterococci or lactobacilli or were defined as the most common species settling in the mesenteric lymph nodes of the healthful mice having no pathogenic infection (Berg and Garlington 1979). The reason for frequent translocations of lactobacilli and enterococci is that they normally show colonization at high population levels in the gastrointestinal system. Rodriguez et al. (2001) reported that, in case of the healthful mice were given 2×10^9 living *L. rhamnosus* suspension orally on daily basis. Seven days after, the living bacteria were detected in spleen and liver of the mice. In that study, isolated bacterium colonies from liver, biochemically characterized. Then they were exposed to casually reproduced polymorphic DNA, the amplification patterns of five strains showed similar characteristics with *L. rhamnosus* ATCC 7469. Similarly, Perdigon et al. (1997) determined the *L. rhamnosus* translocation after giving to the healthful mice. The researchers reported that the translocation was induced 2 days after the application of the dose of 10^{11} CFU/day/mouse.

Despite these findings, in many of the studies carried out on the healthy subjects, no strict infection induced by the probiotic microorganism was reported even if they translocated from the intestinal system. The reason is still unknown but there are several theories on this subject. One of these theories is that the probiotic microorganisms are much more sensitive to intracellular killing by the macrophages after the translocation because the phagocytes are known as effect protectively after the initiation of infective endocarditis induced by the Gram-positive bacterium (Duffy 2000; Veltrop et al. 2000).

Bacteremia and Endocarditis Potential

It is known that the LAB containing the *Bifidobacterium* spp. are isolated as the promoters of endocarditis and bacteremia (Kalima et al. 1996; Oggioni et al. 1998; Kunz et al. 2004; Cannon et al. 2005; De Groote et al. 2005; Henriksson et al. 2005). Some of the endocarditis- or bacteremia-related organisms are *L. plantarum*, *L. rhamnosus*, *L. casei*, *L. paracasei*, *L. acidophilus*, *L. salivarius* and many other *Lactobacillus* species (Cannon et al. 2005). Moreover, in addition to the *Leuconostoc* and *L. lactis*, also the *Pediococcus* spp. were found to cause endocarditis and bacteremia. Some of *Bifidobacterium* spp. were also isolated from the blood and from the endocarditis patients (Spinosa et al. 2000). *Enterococcus* species were well-known to cause the bacteremia and endocarditis (Vergis et al. 2001).

Given the sepsis cases related with probiotics, it was determined that there were three children with short bowel syndrome having bacteremia due to *Lactobacillus* GG, one central endocarditis case, two endocarditis cases, and one liver abscess case with bacteremia related with *Lactobacillus* GG (Kalima et al. 1996; Oggioni et al. 1998; Rautio et al. 1999; De Groote et al. 2005; Salminen 2006). Moreover, there also was an endocarditis case caused by the *L. rhamnosus* strain, subcharacteristics of which have not been exactly determined. Five bacteremia cases were reported to be related with *Bacillus subtilis* (Richard et al. 1988). Moreover, in a patient with Hodgkin disease and HIV infection, the *L. acidophilus* bacteremia and the *Lactobacillus* infection after the bone marrow transplantation were detected (Kalima et al. 1996).

Lactobacillus GG bacteremia cases were detected also in patients with short gout disease (Kunz et al. 2004; De Groote et al. 2005; Land et al. 2005). All the cases are defined with the central venous catheters and the bowel feeding tube. Two out of four isolated strains are proven to be *Lactobacillus* GG by using PFGE and one of four strains was proven to be *Lactobacillus* GG by using both of PFGE and PCR. Only one isolate was not proven as *Lactobacillus* GG specifically. Central venous catheter infections were detected in two of four cases and the positive catheter culture results were obtained. These studies emphasize the *Lactobacillus* GG bacteremia risk related with the short bowel syndrome. The origin of these organisms is thought to be the central venous catheters contamination throughout the handling, particularly along feeding.

The surveillance information of Finland did not show expand in *Lactobacillus* bacteremia between 1990 and 2000 (Salminen et al. 2002). In this period, the *Lactobacillus* species constituted 0.02% of all the positive blood cultures. In another study carried out in National Public Health Laboratory, it was determined that the *Lactobacillus* was found in 0.24% of all the positive blood cultures referred to the laboratory (Salminen et al. 2004). Although these cultures were reported to be *Lactobacillus*, only 27% of them were proven. In these analyses, it was determined that *Lactobacillus* GG constituted 11 of 26 *L. rhamnosus* strains isolated from blood. *L. rhamnosus* constituted 54% of all the isolated *Lactobacillus*. Given the fact that *Lactobacillus* GG intake in Finland during the working period increased

from 1 to 6 L/person/year, it is attention-grabbing that there was no change in the prevalence of *Lactobacillus* bacteremia, especially in the prevalence of *Lactobacillus* GG, in the last decade (Salminen et al. 2002).

The *Lactobacillus* bacteremia in Sweden was examined for 6 years and it was determined that three probiotic microorganisms were entered into clinical use in this period (Sullivan and Nord 2006). The probiotics that were examined were *Lactobacillus* GG, *L. paracasei* and *L. acidophilus* NCFB 1478. The researchers defined that most of the lactic acid bacteremia cases were polymicrobial in reality.

Besides that, there also are probiotic-related sepsis cases. The most significant ones were related to *S. boulardii* (Bassetti et al. 1998; Hennequin et al. 2000; Perapoch et al. 2000; Lherm et al. 2002; Cassone et al. 2003). In their study carried out on 23 patients, 16 candidemia cases were detected. Some of these patients experienced septic shock. The molecular diagnosis and the confirmation of probiotic strain could be performed to a certain level in many of the cases (Fredenucci et al. 1998; Cesaro et al. 2000).

Gastrointestinal Toxicity Studies

When the possible effect of the probiotic microorganism use on the intestinal physiology is examined, it can be seen that the reproduction of undesired metabolites is possible especially for the patients with the small intestine syndrome (Marteau et al. 1990). It is claimed that the probiotic bacteria are theoretically risky because the deconjugation of bile salts cause the malabsorption and, thus, they might extend the colon cancer risk. Besides that, there is no clinic or epidemiological proof supporting that hypothesis. There also are theoretical information showing that the probiotics have an inhibiting effect on the colon cancer in animals (Snydman 2008).

Among the additional toxicity potency, it is also theoretically possible that the development of lactic acidosis might cause D-lactate secretion. The studies were carried out on the healthy people with ileostomy and it was determined in these studies that the *L. acidophilus* and *Bifidobacterium* species transformed the conjugated bile acid to non-toxic secondary salts (Connolly et al. 2005). Among the patients with the small intestine syndrome, the bile acid metabolites may accumulate in the bowels and cause malabsorption (Bongaerts et al. 2000). This may cause the lactate accumulation and it poses risk for the colon cancer. Moreover, it is theoretically possible that the deteriorations might occur in the intestinal mucosa (Ruseler-van Embden et al. 1995). Moreover, in both *in vitro* studies and in those carried out on gnotobiotic rats, no evidence suggesting that the probiotics would deteriorate the intestinal mucus could be found (Ishibashi and Yamazaki 2001; Snydman 2008).

The studies showed that the probiotics could alter the immune responses of the individuals, increase their responses to vaccination or change the natural history of the allergic response. The probiotic bacterium can alter the non-specific immune, cellular and humoral responses. In addition, they can also affect the local immune

response, in addition to the local secretion of cytokines. Some of these responses are thought to be specific to the strain and host (Senok et al. 2005). The gastrointestinal microbiota role in the growth of intestines recommended that the manipulations caused by the probiotics might theoretically have a negative immune-modulator effect. Another population, in which an adverse immunologic response may theoretically occur, is the pregnant women. Besides that, the probiotic microorganism use in pregnancy and in children and newborn has not been related to any immunological effect (Snydman 2008).

Antibiotic Resistance Transfer

The most important concern about the theoretical risks of the probiotics is the potential transfer of antibiotic resistance between probiotics and pathogen bacterium in the intestinal canal (Salyers et al. 2004; Mathur and Singh 2005). Given the antibiotic resistance transfer in LAB, the plasmid existence having antibiotic-resistant genes containing the genes coding the macrolide-lincosamide streptogramin, tetracycline and chloramphenicol resistance was reported (Lin et al. 1996). These plasmids of resistance were found in *L. reuteri*, *L. acidophilus*, *L. plantarum* and *L. fermentum* isolated from animal feces, raw meat and silage (Gevers et al. 2003). Streptomycin, tetracycline, and chloramphenicol resistances and 214 plasmids were detected in *L. lactis* isolated from soft cheese and raw milk. The resistance of tetracycline was detected in *L. plantarum* 5057 (Snydman 2008).

The natural *Lactobacillus* plasmids transfer is rarely seen. Fermentation of lactose plasmids were transferred to *L. casei* (Ahn et al. 1992) and the bacteriocin reproduction was transferred to *L. johnsonii*. There are several evidences suggesting that the *Leuconostoc* and *Pediococcus* species can be accepted as the wide-interval antibiotic-resistant plasmids when compared to the *Lactococcus* species (Snydman 2008). The conjugation from enterococci to *Lactococcus* and lactobacilli may take place in animal bowels, and also it occurs *in vitro*. Besides that, the lactobacilli transfer is very rare (Dessart and Steenson 1991; Mathur and Singh 2005).

Moreover, the attempts were made in order to molecularly identify the genes with resistance to vancomycin in lactobacilli. For this purpose, five *L. reuteri* strains and one *L. rhamnosus* strain were examined in terms of vanA, vanB and vanC genes and none of these genes was found in any of the strains (Klein et al. 2000). *Lactobacillus* GG was specifically investigated and no evidence related with vanA, vanB, vanH, vanS, vanX, vanY and vanZ genes was found by using PCR (Tynkkynen et al. 1998).

The Infection Risks of Probiotics Among the Healthy Individuals and the Individuals with Suppressed Immune System

Lactobacillus *and* Bifidobacterium *Safety*

Some of the probiotic foodstuffs (such as cheese, yogurt, cabbage pickle, and other fermented herbs and olives) have had the history of safe use for a long time (Shortt 1999). Among the healthy persons, the normal concentrations of lactobacilli are 10^3–10^4 CFU/g in the oral cavity, 10^3–10^7 CFU/g in ileum, and 10^4–10^8 CFU/g in the colon. In addition these microorganisms are also the dominant microorganisms of the vagina (Borriello et al. 2003).

Most of the rarely seen *Lactobacillus* infection cases develop in patients having rigorous underlying conditions (Gasser 1994; Saxelin et al. 1996; Husni et al. 1997); most of these patients die within 1 year after the development of infection (Husni et al. 1997). Lactobacillemia is a frequently seen indicator of an underlying lethal or severe disease (Gasser 1994; Saxelin et al. 1996; Husni et al. 1997). The patients who has the suppressed immune system are usually more sensitive to the pathogenic microorganism infection and the prevalence of opportunistic infection is very high. Besides that, there is no study proving that the intake of probiotics including *Lactobacillus* or bifidobacteria increased the risk of opportunistic infection among these persons. Furthermore, two clinical studies were carried out on small groups of patients with a suppressed immune system (i.e., the patients with HIV infection) to evaluation of the reliableness of probiotics and the data obtained from the study supports the reliability of the probiotic microorganisms consumed by patients (Wolf et al. 1998; Cunningham-Rundles et al. 2000).

Many attempts have been made in order to assess the factors that might predispose the persons, who have severe diseases, to the *Lactobacillus* or bifidobacteria infections (Patel et al. 1994; Husni et al. 1997). In some cases, together with the chronic immune-suppressive and antibiotic treatment, the invasive procedures involving the gastrointestinal system (the *Lactobacillus* and bifidobacteria have large communal populations) and the other organs contributed the increasing risk (Antony et al. 1996). Besides that, the statistical analyses were generally not used and, when used, the studies were carried out on very few cases in order to enable the general suggestions. As far as we know, there is no medical guideline submitted on the inpatient patients' consumption of probiotic or the other products including applicable lactobacilli or bifidobacteria. Although there are guidelines on the probiotic yeast preparations, the current evidences do not guarantee the probiotic lactobacilli and bifidobacteria in the nutrients.

Safety of Probiotic Enterococci

The enterococci safety is an important point but the enterococci were reported to have healthy and important benefits. Some probiotic enterococcus species have long-term reliable usage history. On the other hand, it is also known that the enterococci are opportunist pathogens and they play an important role in hospital infections. Moreover, the antibiotic resistance (they may have multiple antibiotic resistances) characteristic is generally coded by the transferrable elements. Because of this harmful characteristic, there are concerns regarding their use in probiotic foods (Franz et al. 2001).

The *Enterococcus* spp. are significant nosocomial pathogenic bacteria causing bacteremia, urinary tract infection, endocarditis and other infections (Murray 1990; Morrison et al. 1997). The *Enterococcus* spp. generally known as the opportunist pathogens causing infections among the individuals having a rigorous underlying diseases or immune deficiency (Morrison et al. 1997). One of the factors contributing to pathogenicity and causing worldwide concerns is the resistance to a large scale of antibiotics, that hinders the number of treatment options (Murray 1990; Landman and Quale 1997; Leclercq 1997). The enterococci, which are especially resistant to vancomycin, induced global hospital crises (Willems et al. 2001; Ruiz-Garbajosa et al. 2006; Werner et al. 2008).

Besides that, the enterococci virulence cannot be defined solely with the resistance of antibiotic. The enterococci virulence factors are playing role in the strain pathogenicity include the circumstances related with the colonization and the host tissue invasions, such as the resistance mechanisms to non-specific and specific host protection mechanisms. Moreover, these virulent species should cause pathologic alterations directly via production of toxin or implicitly via inflammation (Johnson 1994). The enterococcus virulence factors have been intensively investigated in recent years and some of the virulence factors have been well defined. The other "more detailed" virulence determinants are still being investigated. The virulence factors that have been defined to date are related with the colonization, the invasion, and the pathologic changes.

The various studies on the food-origin enterococci's virulence factors showed that the personal virulence factors' existence and prevalence are specific to the strain (Eaton and Gasson 2001; Franz et al. 2001; Yousif et al. 2005; Lepage et al. 2006; Pérez-Pulido et al. 2006; Aakra et al. 2007; Serio et al. 2007; Abriouel et al. 2008; McGowan-Spicer et al. 2008; Valenzuela et al. 2008; Martin-Platero et al. 2009). It is reported that the virulence is not occur from the specific virulence determinant presence though it is a more complex procedure. Interestingly, the virulence strains, inversion sequence elements, phages, transposons, and mobile genetic elements like a pathogenic island are seen to acquire specific virulence-related genes or the antibiotic resistance genes (Lepage et al. 2006; Aakra et al. 2007; McBride et al. 2007; Solheim et al. 2011). Thus, the virulence strains developed as a result of acquiring the genetic materials allowing the increase of their vitality when they adapt themselves to the host (Lepage et al. 2006). As a result, it was determined that

the new sorts of antibiotic resistance entered into the species and the different genetic strains of *E. faecalis*, in which the virulence properties are different, emerged (McBride et al. 2007). The clonal clusters (CCs), which are some strains called CC9, CC8 and CC2 have antibiotic resistance and pathogenic island genes more than the other strains and they spread over the entire world (McBride et al. 2007). Solheim et al. (2011) defined a series of genes enhanced in CC2 strains and related with mobile elements such as *faj03*, Tn*916*, and efaB5.

Several probiotic strains have been well-investigated from safety and functional aspects. Two of the best defined in terms of safety are *E. faecalis* Symbioflor 1 (produced by SymbioPharm, Herborn, Germany) and *E. faecium* SF68 (NCIMB 10415 produced by Cerbios Pharma SA, Barbengo, Switzerland). These probiotics have safe and long usage histories (longer than 20 years each) without any harmful effect. Kayser (2003) reported the safety of *E. faecium* SF68 produced by Cerbios Pharma and investigated the absence of virulence determinants. According to the research results, *E. faecium* SF68 does not contain a plasmid that is sensitive to the sex pheromone. *E. faecalis* strain is Symbioflor 1 (produced by SymbioPharm) CC25 strain (Solheim et al. 2011). The whole genome of this strain was arranged in a raw and then compared to *E. faecalis* V583, a pathogenic strain. Although there is a general synteny between the sequences of both strains, the detailed analyses showed the absence of a large genomic zone, which indicates the loss of a gene, in the chromosome of the probiotic strain. The genes that do not exist in *E. faecalis* Symbioflor 1 are cytolysin, Esp, gelatinase, hyaluronidase, and peptide antibiotic AS-48. Besides the reproduction of AS and the collagen-adhesive proteins, the different determinants such as reactive oxygen anions and resistance to capsule formation were detected. All of these characteristics are thought to be the colonization factors that provide a competitive advantage to the probiotic strain and, thus, supporting the probiotic character and activity (Domann et al. 2007). Although these probiotics have been commonly used at the highest doses to date, no infection related with these two probiotic enterococci was reported.

Safety of Probiotic Bacillus *Species*

Bacillus clausii, *B. subtilis*, *B. pumilus*, *B. coagulans* (generally mislabeled as '*Lactobacillus sporogenes*') and *B. cereus*, which are probiotics from spore-forming *Bacillus* species, are the less-known ones among the lactobacilli and bifidobacteria (Sanders et al. 2003; Hong et al. 2005). The addition of *B. subtilis* as a food additive was approved in minimum one European country (Italy). However, it is not the case for the other species, except for *B. clausii* which is licensed in that country as "Enterogermina" product, which is a prophylactic medication (produced by Sanofi-Aventis, Milan, Italy). The use of *Bacillus* spp. rises many safety questions since it is known that many species including *B. cereus* and *B. anthracis*, *B. pseudomycoides*, *B. thuringiensis*, *B. pseudomycoides* and *B. weihenstephanesis* are known as pathogenic. *B. cereus* is a factor playing role in food poisoning, which is

well-documented to arise from the one or more enterotoxins production (Granum and Lund 1997; Granum 2002; Guinebretiere et al. 2002). Besides that, the pathogenicity is specific to the strain since there are some *B. cereus* species producing no enterotoxin and, as specified before, they are used as probiotic for humans and animals. Although the diseases caused by *B. weihenstephanensis* and *B. thuringiensis* strains are not understood well, they possibly arise from the production of enterotoxins similar to those produced by *B. cereus*.

Few things are known about the other *Bacillus* species but there are few reports related with *Bacillus* spp. under clinic conditions (De Boer and Diderichsen 1991; Osipova et al. 1998; Salminen et al. 1998; Sanders et al. 2003; Logan 2004). The prevalence of the diseases related with *Bacillus* is rare but, in most cases, they may be misdiagnosed by recycling the "spores" from the clinical samples. The opportunist infections were reported (for instance) in the patients with a suppressed immune system. However, these infections may frequently exist together with the members of other "non-pathogenic" species. Besides that, in some reports, it is stated that the isolates of *Bacillus* species have toxigenic characteristics (Rowan et al. 2001; Phelps and McKillip 2002; From et al. 2005). In conclusion, the dose of consumed bacteria is a significant factor playing role in the growth of the disease (Kramer et al. 1982; Duc et al. 2005).

De Boer and Diderichsen (1991) investigated the safety of *B. subtilis* and *B. amyloliquefaciens*. In this review, the published cases of *Bacillus* infections were specifically investigated. These infections do not arise directly from the digestion of *Bacillus* but the other sources. The results showed that the infections were mainly observed in the persons having immune-suppressed endocarditis history or recently undergone surgical operation. Although it is stated in the report that the prevalence of the food poisoning cases arising from the *B. subtilis* is very low, it is also emphasized that it is very difficult to obtain exact and reliable numbers. This is because the hospitals do not make an absolute distinction between *B. cereus* and other *Bacillus* species as food poisoning agents. The *Bacillus* species were related to the nosocomial bacteremia (Richard et al. 1988).

Some infection cases arising from the consumption of the *Bacillus* probiotic were reported. Oggioni et al. (1998) reported a septicemia case caused by the *B. subtilis* strains from a probiotic preparation used by a patient with a suppressed immune system. Spinosa et al. (2000) examined two samples of *Bacillus* infections that might be related with a commercial probiotic preparation. A contiguous agent, which has been found in a cholangitis case in France in 1996 and in a recurrent septicemia case in Italy in 1998, could not be distinguished from a *Bacillus* spp. found in an Italian probiotic foodstuff (*B. clausii*). Besides that, the authors could not confirm the causative role of Italian probiotic in the infections. Both of the infections developed in the patients with a suppressed immune system (French patient has undergone kidney transplantation and Italian patient has received chemotherapy). Attempts were made in order to define the antibiotic resistance of *Bacillus* spp. used as probiotic foodstuff. Both Ciffo (1984) and Mazza et al. (1992) investigated four *B. clausii* strains including Enterogermina, which is a commercial product, from the aspect of the resistance to therapeutic antibiotics. Mazza

et al. (1992) continued testing the transferability and stability of these resistance characteristics. The resistance to quinolones, cephalosporins and macrolides was observed to be stable, but it was also determined that these resistance phenotypes were not transferred to the other bacteria *in vitro* or *in vivo*.

A significant aspect of ensuring the safety of probiotics is the taxonomical characterization of the bacteria included in the foodstuff. Green et al. (1999) analyzed two commercial products (Enterogermina and Biosubtyl) and they showed that none of them consisted of *B. subtilis* as the manufacturers claim. This result was based on various important phenotypic characteristics (alkaline formation and amylase activity) and totally 16S rDNA sequences. Enterogermina strain, which is at closest alignment with *Sperolactobacillus* group (a subgroup of *B. alcalophilus*), and Biosubtyl strain were found to be in relation with *B. pumilus*.

It is necessary to accurately represent the probiotic product, which is capable of forming spore, to the customers. One way of this is to improve and use objective and scientific guidelines for the commercial products by the industry. As an alternative, the governments may realize the necessity of making stronger regulations on specific microorganisms. The list of suggestions presented below is based on the current guidelines (Donohue and Salminen 1996; Przyrembel 2001) and global organizations (SCAN 2002a, b; FAO/WHO 2001). It is recommended for the companies to consider the minimal safety information, which is provided below, for introducing the spore-forming bacteria to the market in form of probiotic products.

1. Each bacteria strain in the product must be isolated, named, and taxonomically identified. The most current and valid method must be used in order to ensure the exact speciation. In general, the combination of the gene-based and phenotypic methods is necessary. In comparing all the strains, the best available method for most of the microorganisms is 16 rRNA genes identified from the well-known culture collections such as ATCC, European culture collections (DSMZ, LMG, CIP, NCIMB) or Japanese collection (IAM). If the identity of a strain in a product is suspicious, then no decision can be made about the safety of that product.
2. The identification of the bacteria must be in harmony with the scientifically known names. The use of old or misleading names for a long time in the product tags is unacceptable. A "Validation List Declaration" confirming the names of bacteria must be prepared. The Validation List must be published in *Intl J of Syst* and *Evolutionary Microbiol*.
3. The in-vitro characterization of each strain including the antibiotic resistance profile, emetic or enterotoxin production, gastric acid or bile salt resistance must be enough. The bacterial strains exhibiting transferrable resistance of antibiotic must not be used. A elaborated schema for testing the production of toxin is already provided by the Scientific Committee of Animal Nourishment (SCAN 2002b). The strains that are capable of producing toxin must not be used as probiotic. The bacteria must be defined by the existence of plasmids or transferrable DNA vectors. It was determined that the *Bacillus* plasmids mediate the transfer of antibiotic resistance between the zones (Koehler and Thorne 1987) and one must avoid this risk.

4. The safety assessment must be performed by a professional, who has experience in this field. Depending on the species and genus being used, the dose being aimed, and the target audience, the safety characterization might be made as follows; the capability of remaining attached to appropriate human cell lines and invading and modulating capabilities must be tested. The strongly adhesive or invader bacteria species, which are in vegetative or spore forms, must not be used as probiotic. The acute and embryonic toxicity studies would contribute to the safety approach. Each strain must be tested in concentrated form (in both vegetative and spore forms) and in final product on minimum one mammalian species. The dosage chronic toxicity studies, which must be continued for a minimum 9 months, must be performed for each strain in concentrated form (in both vegetative and spore forms) and in final product on minimum two mammalian species (preferably a rodent and a larger species; i.e., a rodent and pig, rabbit or cat).
5. On the tag and marketing literature, the contraindications for the *Bacillus* species or spore-forming bacteria must be listed, including the specific references for the patients or customers with suppressed immune system because of HIV infection, chemotherapy, or allograft treatment.
6. The marketing literature or product tag must provide the information below:
   (a) Usage indications supported by the clinical evidence.
   (b) Net definitions of species, genus, strain, and concentration of each element of the bacterium.
   (c) A phone number for negative feedbacks must be provided on the product tag.

Mortality Related with Probiotic Infection (Mortality Rate)

The deaths related with the probiotic infections, especially those involving healthy individuals, are very rare. Although they cause low-grade infection and endocarditis among the individuals with the suppressed immune system, the rate of mortality related with *Lactobacillus* spp. is very low (Olano et al. 2001).

In some studies, it is emphasized that the correlativity between probiotic infections and death is very weak. Cannon et al. (2005) reviewed 92 research papers involving 241 *Lactobacillus* spp. infection cases [localized infection (39 cases), bacteremia (129 cases) and endocarditis (73 cases)] and reported that none of these infections was related with the mortality. Antony et al. (1996) analyzed 53 probiotic infection cases and the researchers reported that only three of the deaths could possibly be explained with the *Lactobacillus* spp. infection. In another study involving 45 patients, Husni et al. (1997) reported that the *Lactobacillus* spp. might possibly have contributed to the bacteremia, which caused only one death. The researchers emphasized that *Lactobacillus* spp. bacteremia rarely threatens the life and it is an indicator of a severer underlying disease.

One of the enhanced studies on probiotic bacterium was carried out by Salminen et al. (2004) and it involves 89 cases. The researchers reported that the mortality-related *Lactobacillus* bacteremia cases are generally accompanied by additional underlying severe diseases, that there were severe underlying diseases in 82% of all the cases. Despite that, at the end of research, the mortality rate was found to be 26% at the end of 1 month and 48% within 1 year after the onset of disease. It was determined that the mean survival time of *L. rhamnosus* GG, which is widely used in probiotic preparations, in *L. rhamnosus* bacteremia patients is approx. 2.5 months. This duration is statistically significantly shorter than the patients with bacteremia induced by other specific *Lactobacillus* species (8.9 months) or mean survival time of the patients with bacteremia caused by uncharacterized lactobacilli (34 months) ($p < 0.0425$). Although *L. rhamnosus* is realized as the most lethal *Lactobacillus*, there are evidences asserting the contrary. It was reported that the mortalities related with *L. casei* (32.6%), *L. plantarum* (30.8%) and *L. acidophilus* (28.6%) were much higher than the mortality related with *L. rhamnosus* (13.6%) (Cannon et al. 2005).

McNaught et al. (2002) carried out a randomized research on 129 selected surgical patients in order to examine the effects of *L. plantarum* 299v on the barrier function of bowels (probiotic group n = 64). The researchers determined that *L. plantarum* 299v application did not influence the bacterial translocation, stomach colonization or postoperative septic morbidity incidence. Besides that, the mortality rate was found to be slightly higher in the probiotic group when compared to the control group, and the difference was not statistically significant.

Since the relationship between death and probiotic infection is difficult to reveal in human, although the use of animal models would be a better option to obtain more data. For instance; Wheeler et al. (2003) used a mutant probiotic yeast (*S. cerevisiae*) with a demolishing SSD1 gene altering the joint of cellular surfaces and the architecture of cellular wall, and they revealed that the yeast became more virulent and less sensitive to being destroyed by the macrophages. It was determined that the deceased mice have a high amount of living yeast ($>10^6$ yeasts) in kidneys. These results suggest that the yeasts cause death because of their development and the interaction with the host.

Difficulties in Identifying the Probiotic Infections

It has always been hard to verify the ineffectiveness of probiotic bacterium because of their anaerobic structure. Because of their low contagiousness, the probiotics are seldom suspected of infection among healthy people. For this reason, it is desired to detect the pathogenic potential of a microorganism before the application. One of the most reliable methods is the use of chronic and acute toxicity tests that may yield information also about the toxicity. Zhou et al. (2000a, b) asserted that the feed consumption, activity status and rate of growth in an animal model are the most susceptible parameters that can be used in examining the acute toxicity of the strains being tested. Moreover, the lethal doses of strains being tested can also be used as

an toxicity indicator. The reports showed that *B. longum* BB536 is safe and, even given at a dose higher than the maximum oral dose of 5×10^{13}/kg, exhibited no toxicity (Ishibashi and Yamazaki 2001; Liong and Shah 2005). *B. longum* BB536's safety was proven after applying the dose of 2.5×10^{11} kg/day for a year and detecting no toxicity.

The preliminary detection of the toxicity is very complicated because the procedure is very long. When the probiotic strains cause infection, it generally cannot be determined in the laboratory because their long-term safety history eliminated the suspicions on them. Moreover, it is difficult to selectively define the probiotic microorganisms in laboratories since most of them are anaerobic. In order to maintain the anaerobic structure in elective counting, the redox decreasing compounds such as L-cysteine · HCl (Liong and Shah 2005) were used and, because of their adaptation to the laboratory environment, the sub-enculturation was minimized in order to prevent any change. The exact identification of the rod-shaped anaerobic probiotic microorganisms such as *Lactobacillus* is very hard since most of the commercially available identification systems are inadequate for identifying *Lactobacillus* (Murray et al. 2003). While examining the field of effect of *Lactobacillus* bacteremia in Central Hospital of Helsinki University, Salminen et al. (2002) isolated 66 cultures, which were characterized at the beginning, as *Lactobacillus*. Besides that, when further analyses were performed for identifying the species, 18 of 66 isolates were found to be other microorganisms (four *Actinomyces*, four *Clostridium*, three *Bifidobacterium*, one *Weissella confusa*, and one *Carnobacterium*). It was observed that microscopic identification is very difficult. The morphology of *Lactobacillus* is similar to the other members of species including *Corynebacterium*, *Clostridium*, *Nocardia* and *Streptococcus* (McNaught et al. 2002; Cannon et al. 2005). Based on the Gram-staining identification, the probiotic microorganisms such as *Lactobacillus* were perplexed with diphtheroid (Gallemore et al. 1995). Similarly, since both of two are aminopeptidase-positive and they may have resemble morphologies based on the Gram staining from blood agar discs, *Enterococcus* spp. was confused with *W. confusa* (Olano et al. 2001). Thus, a detailed identification such as those performed by using a specific polymerase chain reaction and pulsed-field gel electrophoresis (Ouwehand et al. 2004) is needed.

Conclusion

Although they have a safe usage history and several health benefits, the probiotic microorganisms may have harmful effects especially on the individuals with a suppressed immune system. It may be misleading to identify a probiotic or product as GRAS by making use of the safety history. Thus, the GRAS status must be determined in accordance with the purpose of use. For regulatory purposes, the probiotic strains' translocation, antibiotic resistance, and capability of causing infection must be taken into consideration.

References

Aakra Å, Nyquist L, Snipen L et al (2007) Survey of genomic diversity among *Enterococcus faecalis* strains by microarray-based comparative genomic hybridisation. Appl Environ Microbiol 73(7):2207–2217

Abriouel H, Benomar N, Molinos AC et al (2008) Comparative analysis of genetic diversity and incidence of virulence factors and antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water, soil, and clinical samples. Int J Food Microbiol 123(1-2):38–49

Adams MR, Marteau P (1995) On the safety of lactic acid bacteria from food. Int J Food Microbiol 27(2-3):263–264

Ahn C, Collins-Thompson D, Duncan C et al (1992) Mobilization and location of the genetic determinant of chloramphenicol resistance from *Lactobacillus plantarum* caTC2R. Plasmid 27(3):169–176

Ammor MS, Florez AB, Mayo B (2007) Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. Food Microbiol 24(6):559–570

Antony S, Stratton CW, Dummer JS (1996) Lactobacillus bacteremia: description of the clinical course in adult patients without endocarditis. Clin Infect Dis 23(4):773–778

Asahara T, Takahashi M, Nomoto K et al (2003) Assessment of safety of lactobacillus strains based on resistance to host innate defense mechanisms. Clin Diagn Lab Immunol 10(1):169–173

Bassetti S, Frei R, Zimmerli W (1998) Fungemia with Saccharomyces cerevisiae after treatment with *Saccharomyces boulardii*. Am J Med 105(1):71–72

Berg RD, Garlington AW (1979) Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in the gnotobiotic Mouse model. Infect Immun 23(2):403–411

Bongaerts G, Bakkeren J, Severijnen R et al (2000) Lactobacilli and acidosis in children with short small bowel. J Pediatr Gastroenterol Nutr 30(3):288–293

Borriello SP, Hammes WP, Holzapfel W et al (2003) Safety of probiotics that contain lactobacilli or bifidobacterial. Clin Infect Dis 36(6):775–780

Boyle RJ, Robins-Browne RM, Tang ML (2006) Probiotic use in clinical practice: what are the risks? Am J Clin Nutr 83(6):1256–1264

Bozkurt H, Aslım B (2004) İmmobilizasyonun Probiyotik Kültürlerde Kullanımı. Orlab On-Line Mikrobiyoloji Dergisi 2(7):01–14

Cannon JP, Lee TA, Bolanos JT et al (2005) Pathogenic relevance of *Lactobacillus*: a retrospective review of over 200 cases. Eur J Clin Microbiol Infect Dis 24(1):31–40

Cassone M, Serra P, Mondello F et al (2003) Outbreak of *Saccharomyces cerevisiae* subtype *boulardii* fungemia in patients neighboring those treated with a probiotic preparation of the organism. J Clin Microbiol 41(11):5340–5343

Cesaro S, Chinello P, Rossi L et al (2000) *Saccharomyces cerevisiae* fungemia in a neutropenic patient treated with *Saccharomyces boulardii*. Support Care Cancer 8(6):504–505

Ceyhan N, Alıç H (2012) Bağırsak Mikroflorası ve Probiyotikler. Türk Bilimsel Derlemeler Dergisi 5(1):107–113

Ciffo F (1984) Determination of the spectrum of antibiotic resistance of the *Bacillus subtilis* strains of Enterogermina. Chemioterapia 3(1):45–52

Clancy R (2003) Immunobiotics and the probiotic evolution. FEMS Immunol Med Microbiol 38(1):9–12

Connolly E, Abrahamsson T, Bjorksten B (2005) Safety of D(-)-lactic acid producing bacteria in the human infant. J Pediatr Gastroenterol Nutr 41(4):489–492

Çoşkun T (2006) Pro-, Pre- ve Sinbiyotikler. Çocuk Sağlığı ve Hastalıkları Dergisi 49(2):128–148

Cunningham-Rundles S, Ahrné S, Bengmark S et al (2000) Probiotics and immune response. Am J Gastroenterol 95(1):S22–S25

De Boer AS, Diderichsen B (1991) On the safety of *Bacillus subtilis* and *B. amyloliquefaciens*: a review. Appl Microbiol Biotechnol 36:1–4

De Groote MA, Frank DN, Dowell E et al (2005) *Lactobacillus rhamnosus* GG bacteremia associated with probiotic use in a child with short gut syndrome. Pediatr Infect Dis J 24(3):278–280

Dessart SR, Steenson LR (1991) High frequency intergeneric and intrageneric conjugal transfer of drug resistance plasmids in *Leuconostoc mesenteroides* ssp. *cremoris*. J Dairy Sci 74(9):2912–2919

Dewan S, Tamang JP (2007) Dominant lactic acid bacteria and their technological properties isolated from the himalayan ethnic fermented milk products. Antonie Van Leeuwenhoek 92(3):343–352

Domann E, Hain T, Ghai R et al (2007) Comparative genomic analysis for the presence of potential enterococcal virulence factors in the probiotic *Enterococcus faecalis* strain Symbioflor 1. Int J Med Microbiol 297(7-8):533–539

Donohue DC, Salminen S (1996) Safety of probiotic bacteria. Asia Pac J Clin Nutr 5:25–28

Duc LH, Dong TC, Logan NA et al (2005) Cases of emesis associated with bacterial contamination of an infant breakfast cereal product. Int J Food Microbiol 102(2):245–251

Duffy LC (2000) Interactions mediating bacterial translocation in the immature intestine. J Nutr 130(2):432–436

Eaton TJ, Gasson MJ (2001) Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. Appl Environ Microbiol 67(4):1628–1635

Ebners S, Smug LN, Kneifel W et al (2014) Probiotics in dietary guidelines and clinical recommendations outside the European Union. World J Gastroenterol 20(43):16095–16100

FAO/WHO (2001) Food and Agriculture Organization/World Health Organization. Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Available from http://www.fao.org/es/ESN/food/foodandfood_probio_en.stm. Accessed 1 July 2003

FAO/WHO. (2002) Food and Agriculture Organization/World Health Organization. Guidelines for the evaluation of probiotics in foods, report of a joint FAO/WHO working group, London, Ontario, Canada, April 30 and May 1 2002. Available from ftp://ftp.fao.org/es/esn/food/wgreport2.pdf

Franz CMAP, Muscholl-Silberhorn AB, Yousif NMK (2001) Incidence of virulence factors and antibiotic resistance among enterococci isolated from food. Appl Environ Microbiol 67(9):4385–4389

Fredenucci I, Chomarat M, Boucaud C et al (1998) *Saccharomyces boulardii* fungemia in a patient receiving Ultra-levure therapy. Clin Infect Dis 27(1):222–223

Friedman G (2005) Probiotics, prebiotics, and commensal bacteria: perspectives and clinical applications in gastroenterology. Gastroenterol Clin North Am 34(3):13–16

From C, Pukall R, Schumann P et al (2005) Toxin-producing ability among *Bacillus* spp. outside the *Bacillus cereus* group. Appl Environ Microbiol 71(3):1178–1183

Gallemore GH, Mohon RT, Ferguson DA (1995) *Lactobacillus fermentum* endocarditis involving a native mitral valve. J Tenn Med Assoc 88(8):306–308

Gasser F (1994) Safety of lactic-acid bacteria and their occurrence in human clinical infections. Bull Inst Pasteur 92:45–67

Gevers D, Danielsen M, Huys G et al (2003) Molecular characterization of tet(M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. Appl Environ Microbiol 69(2):1270–1275

Gismondo MR, Drago L, Lombardi A (1999) Review of probiotics available to modify gastrointestinal flora. Int J Antimicrob Agents 12(4):287–292

Gönülateş N (2008) Kefirin insanlar üzerindeki immünomodülatör etkilerinin araştırılması. (Doctoral dissertation, SDÜ Tıp Fakültesi)

Granum PE (2002) *Bacillus cereus* and food poisoning. In: Berkeley R, Heyndrickx M, Logan NA, de Vos P (eds) Applications and systematics of bacillus and relatives. Blackwell Science, Oxford, pp 37–46

Granum PE, Lund T (1997) *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Lett 157(2):223–228

Green DH, Wakeley PR, Page A et al (1999) Characterization of two *Bacillus* probiotics. Appl Environ Microbiol 65(9):4288–4291

Guarner F, Schaafsma GJ (1998) Probiotics. Int J Food Microbiol 39(3):237–238

Guinebretiere MH, Broussolle V, Nguyen-The C (2002) Enterotoxigenic profiles of food-poisoning and food borne *Bacillus cereus* strains. J Clin Microbiol 40:3053–3056

Gülmez M, Güven A (2002) Probiyotik prebiyotik ve sinbiyotikler. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 8:83–89

Hennequin C, Kauffmann-Lacroix C, Jobert A et al (2000) Possible role of catheters in *Saccharomyces boulardii* fungemia. Eur J Clin Microbiol Infect Dis 19(1):16–20

Henriksson A, Borody T, Clancy R (2005) Probiotics under the regulatory microscope. Expert Opin Drug Saf 4(6):1135–1143

Herreros MA, Sandoval H, González L et al (2005) Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). Food Microbiol 22(5):455–459

Hong HA, Duc LH, Cutting SM (2005) The use of bacterial spore formers as probiotics. FEMS Microbiol Rev 29(4):813–835

Huang Y, Kotula L, Adams MC (2003) The in-vivo assessment of safety and gastrointestinal survival of an orally administered novel probiotic, *Propionibacterium jensenii* 702, in a male Wistar rat model. Food Chem Toxicol 41(12):1781–1787

Husni RN, Gordon SM, Washington JA et al (1997) Lactobacillus bacteremia and endocarditis: review of 45 cases. Clin Infect Dis 25(5):1048–1055

Ishibashi N, Yamazaki S (2001) Probiotics and safety. Am J Clin Nutr 73(2):465–470

Johnson AP (1994) The pathogenicity of enterococci. Int J Antimicrob Agents 33(6):1083–1089

Kalima P, Masterton RG, Roddie PH (1996) *Lactobacillus rhamnosus* infection in a child following bone marrow transplant. J Infect 32(2):165–167

Kayser FH (2003) Safety aspects of enterococci from the medical point of view. Int J Food Microbiol 88(2-3):255–262

Klein G, Hallmann C, Casas IA (2000) Exclusion of vanA, vanB and vanC type glycopeptide resistance in strains of *Lactobacillus reuteri* and *Lactobacillus rhamnosus* used as probiotics by polymerase chain reaction and hybridization methods. J Appl Microbiol 89:815–824

Koehler TM, Thorne CB (1987) Bacillus subtilis (natto) plasmid pLS20 mediates interspecies plasmid transfer. J Bacteriol 169(11):5271–5278

Kramer JM, Turnbull PCB, Munshi G et al (1982) Identification and characterization of *Bacillus cereus* and other *Bacillus* species associated with foods and food poisoning. In: Corry JEL, Roberts D, And Skinner FA (eds) Isolation and identification methods for food poisoning organisms. Academic Press, London, pp 261–286

Kunz AN, Noel JM, Fairchok MP (2004) Two cases of Lactobacillus bacteremia during probiotic treatment of short gut syndrome. J Pediatr Gastroenterol Nutr 38(4):457–458

Land MH, Rouster-Stevens K, Woods CR et al (2005) Lactobacillus sepsis associated with probiotic therapy. Pediatrics 115(1):178–181

Landman D, Quale JM (1997) Management of infections due to resistant enterococci: a review of therapeutic options. J Antimicrob Chemother 40:161–170

Leclercq R (1997) Enterococci acquire new kinds of resistance. Clin Infect Dis 24(Suppl.1):80–84

Lee YK, Salminen S (2009) Handbook of probiotics and prebiotics. John Wiley & Sons, New York

Lepage E, Brinster S, Caroin C et al (2006) Comparative genomic hybridization analysis of *Enterococcus faecalis*: identification of genes absent from food strains. J Bacteriol 188(9):6858–6868

Lherm T, Monet C, Nougiere B et al (2002) Seven cases of fungemia with *Saccharomyces boulardii* in critically ill patients. Intensive Care Med 28(6):797–801

Lin CF, Fung ZF, Wu CL et al (1996) Molecular characterization of a plasmid-borne (pTC82) chloramphenicol resistance determinant (cat-TC) from *Lactobacillus reuteri* G4. Plasmid 36(2):116–124

Liong MT, Shah NP (2005) Bile salt deconjugation and BSH activity of five bifidobacterial strains and their cholesterol co-precipitating properties. Food Res Int 38(2):135–142

Logan NA (2004) Safety of aerobic endospore-forming bacteria. In: Ricca E, Henriques AO, Cutting SM (eds) Bacterial spore formers: probiotics and emerging applications. Horizon Bioscience, Norfolk, pp 93–106

Maragkoudakis PA, Zoumpopoulou G, Miaris C et al (2006) Probiotic potential of Lactobacillus strains isolated from dairy products. Int Dairy J 16(3):189–199

Marteau P, Pochart P, Flourie B et al (1990) Effect of chronic ingestion of a fermented dairy product containing Lactobacillus acidophilus and Bifidobacterium bifidum on metabolic activities of the colonic flora in humans. Am J Clin Nutr 52(4):685–688

Martin-Platero AM, Maqueda M, Valdivia E et al (2009) Polyphasic study of microbial communities of two Spanish farmhouse goats' milk cheeses from Sierra de Aracena. Food Microbiol 26(3):294–304

Mathur S, Singh R (2005) Antibiotic resistance in food lactic acid bacteria-a review. Int J Food Microbiol 105(3):281–295

Mazza P, Zani F, Martelli P (1992) Studies on the antibiotic resistance of Bacillus subtilis strains used in oral bacteriotherapy. Boll Chim Farm 131(11):401–408

McBride SM, Fischetti VA, LeBlanc DJ et al (2007) Genetic diversity among Enterococcus faecalis. PLoS One 2(7):e582

McGowan-Spicer LL, Fedorka-Cray PJ, Frye JG et al (2008) Antimicrobial resistance and virulence of Enterococcus faecalis isolated from retail food. J Food Prot 71(4):760–769

McNaught CE, Woodcock NP, MacFie J et al (2002) A prospective randomised study of the probiotic Lactobacillus plantarum 299V on indices of gut barrier function in elective surgical patients. Gut 51:827–831

Metchnikoff II (2004) The prolongation of life: optimistic studies. Springer Publishing Company, New York

Morrison D, Woodford N, Cookson B (1997) Enterococci as emerging pathogens of humans. J Appl Microbiol Symp Suppl 83:89–99

Muñoz-Atienza E, Gómez-Sala B, Araújo C et al (2013) Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. BMC Microbiol 13(1):15

Murray BE (1990) The life and times of the Enterococcus. Clin Microbiol Rev 3(1):46–65

Murray PR, Baron E, Jorgenson JH et al (2003) Manual of clinical microbiology. ASM Press, Washington, pp 857–869

O'Brien J, Crittenden R, Ouwehand AC et al (1999) Safety evaluation of probiotics. Trends Food Sci Technol 10:418–424

Oggioni MR, Pozzi G, Valensin PE et al (1998) Recurrent septicemia in an immunocompromised patient due to probiotic strains of Bacillus subtilis. J Clin Microbiol 36(1):325–326

Olano A, Chua J, Schroeder S et al (2001) Weissella confusa (Basonym: Lactobacillus confusus) bacteremia: a case report. J Clin Microbiol 39(4):1604–1607

Osipova IG, Sorokulova IB, Tereshkina NV et al (1998) Safety of bacteria of the genus Bacillus, forming the base of some probiotics. Zh Mikrobiol Epidemiol Immunobiol 6:68–70

Otles S, Cagindi O (2003) Kefir: a probiotic dairy-composition, nutritional and therapeutic aspects. Pak J Nutr 2(2):54–59

Ouoba LII, Lei V, Jensen LB (2008) Resistance of potential probiotic lactic acid bacteria and Bifidobacteria of African and European origin to nantimicrobials: determination and transferability of the resistance genes to other bacteria. Int J Food Microbiol 121(2):217–224

Ouwehand AC, Saxelin M, Salminen S (2004) Phenotypic differences between commercial Lactobacillus rhamnosus GG and L. rhamnosus strains recovered from blood. Clin Infect Dis 39(12):1858–1860

Özden A (2005) Gastrointestinal sistem ve probiyotik-prebiyotik-sinbiyotik. Güncel Gastroenteroloji 9(3):124–133

Özteber M (2013) Fermente süt ürünlerinden izole edilen laktik asit bakterilerinin antibiyotik dirençliliklerinin fenotipik ve genotipik yöntemlerle belirlenmesi. Master's thesis, Adnan Menderes Üniversitesi, Fen Bilimleri Enstitüsü

Patel R, Cockerill FR, Porayko MK et al (1994) Lactobacillemia in liver transplant patients. Clin Infect Dis 18:207–212

Perapoch J, Planes AM, Querol A et al (2000) Fungemia with Saccharomyces cerevisiae in two newborns, only one of whom had been treated with ultra-levura. Eur J Clin Microbiol Infect Dis 19(6):468–470

Perdigon G, Alvarez S, Aquero G et al (1997) Interactions between lactic acid bacteria, intestinal microflora and the immune system. In: Martins MT, Zanoli Sato MI, Tiedje JM, Norton Haggler JB, Dobereiner J, Sanchez P (eds) Proceedings of the 7th International Symposium of Microbial Ecology, Santos, Brazil, pp 311–316

Pérez-Pulido R, Abriouel H, Ben Omar N et al (2006) Safety and potential risks of enterococci isolated from traditional fermented capers. Food Chem Toxicol 44(12):2070–2077

Phelps RJ, McKillip JL (2002) Enterotoxin production in natural isolates of Bacillaceae outside the Bacillus cereus group. Appl Environ Microbiol 68(6):3147–3151

Przyrembel H (2001) Consideration of possible legislation within existing regulatory frameworks. Am J Clin Nutr 73(2):471–475

Ranadheera CS, Evans CA, Adams MC (2014) Effect of dairy probiotic combinations on in vitro gastrointestinal tolerance, intestinal epithelial cell adhesion and cytokine secretion. J Funct Foods 8:18–25

Rautio M, Jousimies-Somer H, Kauma H et al (1999) Liver abscess due to a Lactobacillus rhamnosus strain indistinguishable from L. rhamnosus strain GG. Clin Infect Dis 28(5):1159–1160

Reid G (2002) Safety of lactobacillus strains as probiotic agents. Clin Infect Dis 35(3):349–350

Richard V, Van der Auwera P, Snoeck R et al (1988) Nosocomial bacteremia caused by Bacillus species. Eur J Clin Microbiol Infect Dis 7(6):783–785

Rodriguez AV, Baigorí MD, Alvarez S et al (2001) Phosphatidylinositol-specific phospholipase C activity in *Lactobacillus rhamnosus* with capacity to translocate. FEMS Microbiol Lett 204(1):33–38

Rowan NJ, Deans K, Anderson JG et al (2001) Putative virulence factor expression by clinical and food isolates of *Bacillus* spp. after growth in reconstituted infant milk formulae. Appl Environ Microbiol 67(9):3873–3881

Ruiz-Garbajosa P, Bonten MJ, Robinson DA et al (2006) Multilocus sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic complexes in a background of high rates of evolution. J Clin Microbiol 44(6):2220–2228

Ruseler-van Embden JG, van Lieshout LM, Gosselink MJ et al (1995) Inability of *Lactobacillus casei* strain GG, *L. acidophilus*, and *Bifidobacterium bifidum* to degrade intestinal mucus glycoproteins. Scand J Gastroenterol 30(7):675–680

Saarela M, Mogensen G, Fonden R (2000) Probiotic bacteria: safety, functional and technological properties. J Biotechnol 84(3):197–215

Sağdıç O, Küçüköner E, Özçelik S (2004) Probiyotik ve Prebiyotiklerin Fonksiyonel Özellikleri, Atatürk Üniv. Ziraat Fak Dergisi 35(3-4):221–228

Salminen MK (2006) Lactobacillus bacteremia, with special focus on the safety of probiotic *Lactobacillus rhamnosus* GG (doctoral dissertation). University of Helsinki, Helsinki

Salminen S, von Wright A, Morelli L et al (1998) Demonstration of safety of probiotics – a review. Int J Food Microbiol 44(1-2):93–106

Salminen MK, Tynkkynen S, Rautelin H et al (2002) Lactobacillus bacteremia during a rapid increase in probiotic use of *Lactobacillus rhamnosus* GG in Finland. Clin Infect Dis 35(10):1155–1160

Salminen MK, Rautelin H, Tynkkynen S et al (2004) Lactobacillus bacteremia, clinical significance, and patient outcome, with special focus on probiotic *L. rhamnosus* GG. Clin Infect Dis 38(1):62–69

Salyers AA, Gupta A, Wang Y (2004) Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol 12(9):412–416

Sanders ME, Morelli L, Tompkins TA (2003) Sporeformers as human probiotics: bacillus, sporolactobacillus, and brevibacillus. Compr Rev Food Sci Food Saf 2(3):101–110

Saxelin M, Chuang NH, Chassy B (1996) Lactobacilli and bacteremia in southern Finland, 1989–1992. Clin Infect Dis 22(3):564–566

SCAN (2002a) Scientific Committee on Animal Nutrition. Report of the Scientific Committee on Animal Nutrition. Opinion of the Scientific Committee on Animal Nutrition on the safety of use of Bacillus species in animal nutrition. European Commission, Health & Consumer Protection Directorate-General, Brussels

SCAN (2002b) Scientific Committee on Animal Nutrition. Report of the Scientific Committee on Animal Nutrition, Opinion of the SCAN on the criteria for assessing the safety of microorganisms resistant to antibiotics of human, clinical, and veterinary importance. European Commission Health & Consumer Protection Directorate-General, Brussels. http://europa.eu.int/comm/food/fs/sc/scan/out64_en.pdf. Accesses 1 July 2003

Senok AC, Ismaeel AY, Botta GA (2005) Probiotics: facts and myths. Clin Microbiol Infect 11(12):958–966

Serio A, Paparella A, Chaves-López C (2007) Enterococcus populations in Pecorino Abruzzese cheese: biodiversity and safety aspects. J Food Prot 70(7):1561–1568

Shortt C (1999) The probiotic century: historical and current perspectives. Trends Food Sci Technol 10(12):411–417

Shu Q, Zhou JS, Rutherfurd KJ (1999) Probiotic lactic acid bacteria (Lactobacillus acidophilus HN017, Lactobacillus rhamnosus HN001 and Bifidobacterium lactis HN019) have no adverse effects on health of mice. Int Dairy J 9(11):831–836

Solheim M, Brekke MC, Snipen LG (2011) Comparative genomic analysis reveals significant enrichment of mobile genetic elements and genes encoding surface-structure proteins in hospital-associated clonal-complex 2 Enterococcus faecalis. BMC Microbiol 11(1):3

Spinosa MR, Wallet F, Courcol RJ (2000) The trouble in tracing opportunistic pathogens: cholangitis due to Bacillus in a French hospital caused by a strain related to an Italian probiotic? Microb Ecol Health Dis 12(2):99–101

Sullivan A, Nord CE (2006) Probiotic lactobacilli and bacteraemia in Stockholm. Scand J Infect Dis 38(5):327–331

Snydman, D. R. (2008). The safety of probiotics. Clinical infectious diseases, 46(Supplement_2), S104–S111.

Tall BD (2016) We are what we eat: should food microbiology take the lead on understanding how the homeostasis of the gut microbiome influences human health and disease? IAFP Annual Meeting, Des Moines

Timmerman HM, Koning CJM, Mulder L (2004) Monostrain, multistrain and multispecies probiotics - a comparison of functionality and efficacy. Int J Food Microbiol 96(3):219–233

Toprak Kavas S (2007) Probiyotik Mikroorganizmaların Gastrointestinal Sistem Uyumluluğu Ve Enterik Patojenlere Etkisi. Pamukkale Üniversitesi Tıp Fakültesi İnfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Anabilim Dalı, Uzmanlık Alan Tezi

Tynkkynen S, Singh KV, Varmanen P (1998) Vancomycin resistance factor of Lactobacillus rhamnosus GG in relation to enterococcal vancomycin resistance (van) genes. Int J Food Microbiol 41(3):195–204

Valenzuela AS, Omar NB, Abriouel H (2008) Risk factors in enterococci isolated from foods in Morocco: determination of antimicrobial resistance and incidence of virulence traits. Food Chem Toxicol 46(8):2648–2652

Veltrop MHAM, Bancsi MJLM, Bertina RM et al (2000) Role of monocytes in experimental Staphylococcus aureus endocarditis. Infect Immun 68(8):4818–4821

Vergis EN, Hayden MK, Chow JW et al (2001) Determinants of vancomycin resistance and mortality rates in enterococcal bacteremia. A prospective multicenter study. Ann Intern Med 135(7):484–492

Wassenaar TM, Klein G (2008) Safety aspects and implications of regulation of probiotic bacteria in food and food supplements. J Food Prot 71(8):1734–1741

Werner G, Coque TM, Hammerum AM et al (2008) Emergence and spread of vancomycin resistance among enterococci in Europe. Eurosurveillance 13(47):1–11

Wheeler RT, Kupiec M, Magnelli P et al (2003) A Saccharomyces cerevisiae mutant with increased virulence. Proc Natl Acad Sci U S A 100(5):2766–2770

Willems RJ, Homan W, Top J (2001) Variant esp gene as a marker of a distinct genetic lineage of vancomycin-resistant Enterococcus faecium spreading in hospitals. Lancet 357(9259):853–855

Wolf BW, Wheeler KB, Ataya DG (1998) Safety and tolerance of Lactobacillus reuteri supplementation to a population infected with the human immunodeficiency virus. Food Chem Toxicol 36(12):1085–1094

Yiğit T (2009) Süt ve Süt Ürünlerinden Probiyotik Bakterilerden İzolasyonu. Anadolu Üniversitesi,Yüksek Lisans Tezi, Eskişehir

Yousif NM, Dawyndt P, Abriouel H (2005) Molecular characterization, technological properties and safety aspects of enterococci from 'Hussuwa', an African fermented sorghum product. J Appl Microbiol 98(1):216–228

Yuksekdag ZN, Aslim B (2010) Assessment of potential probiotic-and starter properties of Pediococcus spp. isolated from Turkish-type fermented sausages (sucuk). J Microbiol Biotechnol 20(1):161–168

Zhou JS, Shu Q, Ritherford KJ et al (2000a) Acute oral toxicity and bacterial translocation studies on potentially probiotic strains of lactic acid bacteria. Food Chem Toxicol 38(2-3):153–161

Zhou JS, Shu Q, Rutherfurd KJ et al (2000b) Safety assessment of potential probiotic lactic acid bacteria strains Lactobacillus rhamnosus HN001, Lb. Acidophilus HN017 and Bifidobacterium lactis HN019 in BALB/c mice. Int J Food Microbiol 56(1):87–96