

Interaction of probiotics and pathogens—benefits to human health?

Seppo Salminen¹, Sonja Nybom², Jussi Meriluoto²,
Maria Carmen Collado^{1,3}, Satu Vesterlund¹ and Hani El-Nezami⁴

The probiotic terminology has matured over the years and currently a unified definition has been formed. Lactic acid bacteria (LAB) and bifidobacteria have been reported to remove heavy metals, cyanotoxins and mycotoxins from aqueous solutions. The binding processes appear to be species and strain specific. The most efficient microbial species and strains in the removal of these compounds vary between components tested. However, it is of interest to note that most strains characterized until now do not bind positive components or nutrients in the diet. This has significant implications to future detoxification biotechnology development. In a similar manner, lactic acid bacteria and bifidobacteria interact directly with viruses and pathogens in food and water as well as toxin producing microbes and some toxins. This review updates information and aims to characterize these interactions in association. The target is to understand probiotic health effects and to relate the mechanisms and actions to future potential of specific probiotic bacteria on decontamination of foods and water, and diets. The same aim is targeted in characterizing the role of probiotics in inactivating pathogens and viruses of health importance to facilitate the establishment of novel means of disease risk reduction related health benefits.

Addresses

¹ Functional Foods Forum, University of Turku, 20014 Turku, Finland

² Department of Biochemistry and Pharmacy, Åbo Akademi University, Tykistökatu 6A, 20520 Turku, Finland

³ Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Burjassot, Valencia, Spain

⁴ School of Biological Sciences, University of Hong Kong, S5-13 Kadoorie Biological Sciences Building, Pokfulam, Hong Kong, SAR, China

Corresponding author: Salminen, Seppo (sepsal@utu.fi)

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Introduction

The probiotic terminology has matured over the years and currently a unified definition has been formed.

Derived from this development, the current definition used also in the European Union is mainly based on work of ILSI Europe and the WHO [1[•],2,3]. The WHO working group has defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [2]. This differs from the Japanese definition, where the term probiotic covers live microorganisms and cells of viable microorganisms, when a health benefit has been demonstrated [4[•]]. A large number of benefits have been ascribed also to heat-killed probiotic microbes [5].

There is a need to further define probiotics as we now understand that most of the microorganisms in the human gut are non-culturable using the current methodology. However, these bacteria are active in the gut demonstrating that viability *per se* is not a prerequisite for such health benefits, also non-viable lactic acid bacteria and bifidobacteria have been demonstrated to have health benefits. Probiotics usually interact with the intestinal microbiota and the intestinal wall receptors producing varying effects on the host. Viability *per se* is not a prerequisite for such health benefits, also non-viable lactic acid bacteria and bifidobacteria have been demonstrated to have health benefits.

Why does one observe health effects with non-viable microorganisms? One reason is our poor understanding of viability—many organisms can be unculturable but still demonstrating viability in different tests. Some organisms may require a sequence of different stimuli to be able to propagate or they may act in a surrounding composing of specific other microbes only. Such sequela, also indicated in the transfer of microbiota from the mothers gut into breast milk and consequently to the breastfed infant, also attest the different stages of viability and activity of a microbe, such as specific *Bifidobacterium* species [6].

The complex interaction of microbes with other members of the gut microbiota and also with the host also form the basis for assessing the bacteria probiotic interactions and the toxin probiotic interactions. The aim of this article is to characterize probiotics and their specific interactions with other potentially pathogenic bacteria, bacterial toxins, fungal toxins and viruses with the aim of defining future applications in human health promotion in this area.

Lactic acid bacteria [LAB] and bifidobacteria have been reported to remove heavy metals [7], cyanotoxins [8] and

mycotoxins [9] from aqueous solution *in vitro*. The binding process appears to be species and strain dependent. The most efficient microbial species and strains in the removal of these compounds vary between components tested, but surprisingly, most of them do not bind positive components or nutrients in the diet [8–10]. In a similar manner, lactic acid bacteria and bifidobacteria interact directly with pathogens and toxin producers where they may influence the health of the host. Our aim here is to characterize these interactions in association with different health effects and to relate the mechanisms and actions to future potential of specific probiotic bacteria on decontamination of foods and water as well as diets.

Probiotic pathogen interactions

The protective role of probiotic bacteria against gastrointestinal pathogens and the underlying mechanisms have received special attention as such interaction has served as one criterion for selecting new probiotics for human use. The mechanisms by which probiotics exert their beneficial effects on the host are largely unknown. Among the identified mechanisms are the reduction of luminal pH, competition with pathogens for adhesion sites and nutritional sources, secretion of antimicrobial substances, toxin inactivation, and immune stimulation [11,12]. Host–bacteria interaction mechanisms include physical bacteria–epithelium interaction (adhesion to mucosal and epithelial cells, stimulation of mucus secretion, production of defensive molecules, reinforce of gut barrier function), bacteria–immune system interaction (modulation and regulation of immune responses) and also, bacteria–bacteria interaction (exclusion and inhibition of pathogens by prevention of adhesion, secretion of antimicrobial substances, competition for nutrients and anti-toxin effects).

Probiotics have defined targets and mechanisms both *in vitro* and *in vivo*. Different bacterial strains of the same genus and species may exert completely different effects on the host. Also the rapidly increased genomic information clearly defines the species and strain specific behaviour of both probiotic bacteria and other commensal bacteria associated with human intestinal surfaces. Such data also suggest that combinations of specific probiotics strains may prove more effective than a single probiotic strain depending to the specific targets [13,14].

Owing to the complexity of the intestinal microbiota probiotic combinations may be required for counteracting complex microbiota deviations at different sites of the intestinal tract. It is hypothesized that a combination of specific probiotics strains may enhance health effects on the host. However, the impact of combinations can be counteractive, if strains are not selected in a scientifically sound manner. There are few *in vivo* studies with probiotic combinations and reported health benefits [15–18]. The design and use of multiple genus, species and strain

combinations of probiotics should be assessed in preclinical settings to avoid challenges such as those reported in the study on pancreatitis patients [19].

Probiotic combinations can be tested *in vitro* and reasonably reliable results can be achieved. In some cases probiotic combinations show clear benefits as has been described in combinations counteracting the aggregation and adhesion of pathogens [20,21]. The adherence of bacteria to intestinal epithelium or invasion of pathogenic bacteria into the mucosa are preceding steps for both colonization and infection in the gastrointestinal tract. Adhesion is a complex process involving non-specific and specific mechanisms. Adhesion to intestinal mucosa is often a prerequisite for specific probiotic microorganisms to be effective and this property often differentiates probiotics from other lactic acid bacteria. Adhesion allows the colonization, although transient, of the human intestinal tract [22•]. In *in vitro* trials, the probiotic properties have mainly been tested alone or in combination with yoghurt bacteria such as *L. delbrueckii* and *L. acidophilus* [23]. Few studies are available on the interactions of probiotics regarding adhesion properties in the intestinal mucus system [23,24••]. The overall influence of combinations of probiotic strains in the adhesion and this influence may be positive (increase the adhesion) or negative (decrease) the adhesion when compared with the effects of single probiotic.

Combinations of known probiotic strains, such as *L. rhamnosus* GG and *B. lactis* Bb12, may exert an improved impact on the pathogen adhesion inhibition [23,24••]. Probiotic strains and their combinations were able to inhibit significantly the adhesion of *B. vulgatus*, *C. difficile*, *St. aureus* and *C. sakazakii* [21,24••]. Specific probiotic combinations were demonstrated more effective on the inhibition of pathogen adhesion and they were able to enhance the inhibition percentages than when the probiotic strains were tested alone. Thus, these studies demonstrate the potential application of probiotic combinations in the inhibition of pathogen adhesion to intestinal mucus but although further studies are needed to identify and clarify the mechanisms involved.

The next step along with the *in vitro* results is to characterize the properties in *in vivo* studies. Such studies should also take into consideration the impact of food matrix, processing of the probiotic strains and the impact of the normal intestinal microbiota on the preparation.

Recently, it has been reported that specific combinations of probiotic strains may complement or improve health benefits given by individual strains [15,16,26–29]. The best known probiotic combination VSL#3, a mixture of eight bacteria including *Lactobacillus* and *Bifidobacterium* strains, has been reported to be useful in several human diseases [26–29]. Some *in vitro* and *in vivo* studies

have assessed other combinations such as *Lactobacillus rhamnosus*, *Propionibacterium freudenreichii* and *Bifidobacterium lactis*. This combination has then been demonstrated effective in clinical studies on irritable bowel syndrome patients [25]. The same probiotic combination did not have an impact on allergy prevention in a study with infants while *L. rhamnosus* GG was previously demonstrated to be effective [30]. However, when the combination was mixed with oligosaccharides the effects were positive on the reduction in atopic eczema prevention [31].

For the future, probiotic combinations that inhibit and displace pathogens may be good candidates in case of specific microbiota aberrancies related to disease risk. The change of one strain in a combination may modify all the properties against pathogen adhesion regarding inhibition, displacement and competition mechanisms. Current data support the hypothesis that the use of probiotic combinations selected for specific targets may have a major impact on human health and this topic should be further assessed [32,33].

Probiotics and viruses

Viruses are an increasing threat to human health causing enormous disease burden in both high-income and low-income countries. The estimation is that from all human infections, 60% is caused by viruses, and especially by enteric and respiratory viruses [34]. Viruses are problematic as they evolve constantly, which makes it difficult to develop means for protection of humans from them. Wide serological diversity among virus population as well as limited knowledge of their pathogenesis makes it difficult to develop vaccines and drugs for prophylactics or treatment. Clinical intervention studies have shown that specific probiotic strains may be able to shorten the duration or reduce the risk of certain viral infections. One of the best demonstrated human health benefits of specific probiotics is shortening the duration of viral diarrhoea, and reducing the risk of viral diarrhoea especially in infants and children [35–38,39[•]]. Moreover, in recent studies selected probiotics have been effective also against respiratory tract infections of viral origin [40–42].

In the host, epithelia represent the first barrier against pathogens, including viruses, followed by the response of the mucosal immune system. Intestinal epithelia interface with a dense and diverse community of microorganisms. Although the standard treatment of acute diarrhoea remains to be an oral rehydration solution (ORS), probiotics have gained an important role as adjuvant therapy. A large number of randomized and controlled trials have shown that probiotics have antidiarrhoeal effects particularly in children. It is well documented that certain probiotics, as *L. rhamnosus* GG (LGG), *L. casei* Shirota, *L. reuteri*, *B. lactis* Bb-12 and a number of other probiotic

strains, are effective against rotavirus by shortening the recovery time from diarrhoea approximately by 1–1.5 days, reducing the shedding of rotaviruses or increasing the production of rotavirus-specific antibodies [37,38,39[•]].

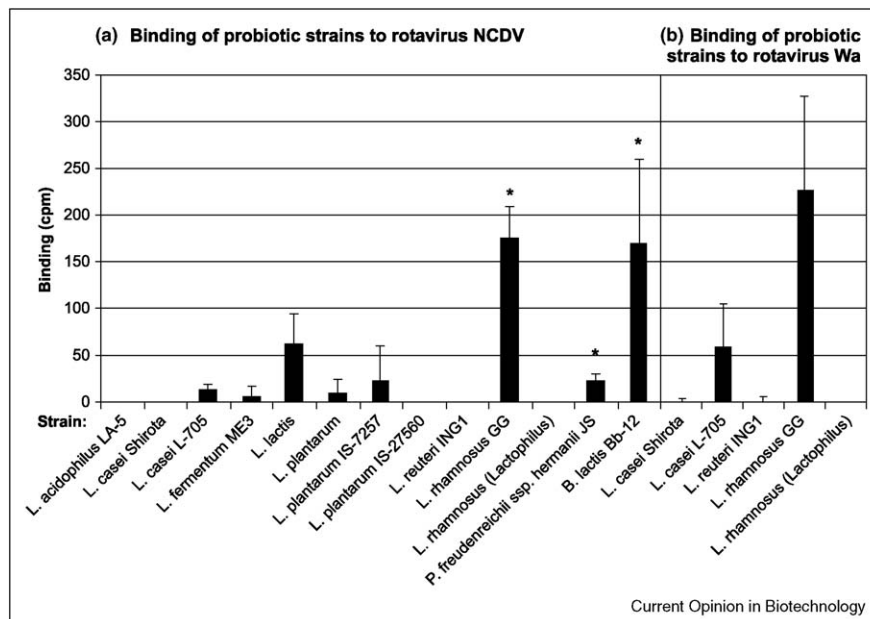
Respiratory diseases cause morbidity and mortality worldwide especially in aged population. Recent multicentric, double-blind and controlled study involved 1072 elderly volunteers (median age of 76 years) who consumed fermented dairy product containing *Lactobacillus casei* DN-114 001 for three months [45]. As a result was shown that consumption was associated with a decreased duration of common infectious diseases in comparison with the control group, especially for upper respiratory tract infections. Probiotic administration in preschool children has been shown to reduce incidence and/or duration of respiratory tract infections [41,46,47]. In another study by Leyer *et al.* (2009) probiotic consumption effects on cold and influenza-like symptom incidence and duration were evaluated in 326 healthy children during the winter season. In this double-blind, placebo-controlled study, children received *Lactobacillus acidophilus* NCFM, *L. acidophilus* NCFM in combination with *Bifidobacterium animalis* subsp *lactis* Bi-07 or placebo twice daily for 6 months. Single and combination probiotics reduced fever, rhinorrhoea, and cough incidence and duration and antibiotic prescription incidence, as well as the number of missed school days attributable to illness. A trend for a broader protective effect was obtained when combination was used. Winkler *et al.* [48] reports that probiotic consumption may reduce the incidence and the severity of symptoms in common cold infections in otherwise healthy adults due to the stimulated cellular immunity. Similarly, de Vrese *et al.* [43,44[•]] reported that consumption of probiotics significantly shortened common cold episodes and reduced the severity of symptoms in intervention study with 479 healthy adults.

The results from several clinical studies suggest that regular consumption of probiotic product may aid the immune response to vaccination. A probiotic fermented dairy drink was shown to improve antibody response to influenza vaccination in the elderly in two randomised controlled trials [49,50].

Virus probiotic interactions

Clinical studies have demonstrated that certain probiotics are effective in viral infections, but the underlying mechanisms are not understood. Specific probiotics fight viral infections presumably owing to their ability to: exclude viruses, strengthen the tight junctions between enterocytes, produce antimicrobial and potentially antiviral substances and stimulate host-cell immune defences [51,52]. Although exclusion of bacterial pathogens by probiotic bacteria is shown by numerous *in vitro* and clinical studies, the exclusion effect has not been reported for viruses.

Figure 1



Specific probiotic bacteria with clinically documented efficacy in rotavirus diarrhoea treatment, that is *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb-12 (a or b), can bind rotaviruses. Nebraska Calf Diarrhoea Virus (NCDV) (a) and Human rotavirus Wa (b) were selected as model viruses. Binding is measured by counting the radioactivity by liquid scintillation (as counts per minute, cpm) of virus-bound radiolabelled bacteria. Results are means + SEM of four independent experiments with four replicates and non-specific binding (binding to blocking reagent, bovine serum albumin) is subtracted from the results. Statistically significant specific binding is indicated with * (P ; 0.05).

It has been of interest to our consortium to characterize probiotic and virus interactions in a manner similar to those reported for pathogenic bacteria. We discovered that specific probiotic bacteria can bind and inactivate rotaviruses (Figure 1). Interestingly, best binding or adhesion of virus particles was obtained with those specific probiotic strains that have clinically documented efficacy in rotavirus diarrhoea treatment and prevention, that is *L. rhamnosus* GG and *B. lactis* Bb-12. Probiotic strains demonstrating this specific efficacy may offer new potential in the fight against viral gastroenteritis. The demonstration provides the basis for novel approaches to inactivate viruses and to reduce the risk of mucosa-associated viral infections.

As reported earlier, specific probiotics clearly reduce the risk of viral infections or reduce the duration of viral diarrhoea. A priority for the future is to screen probiotic combinations that interact with viruses and potentially inactivate them or prevent their contact with epithelial cells. Such combinations should be the target of future efforts and could have significant potential for human health. The challenges and practical benefits lie especially in decontamination of potable water in areas where other procedures may not be accessible.

Toxins from food pathogens

Mycotoxins are of great concern because of their acute and long-term toxicity. Once activated metabolically,

many mycotoxins can become carcinogenic, mutagenic, teratogenic and immunosuppressive in nature [53]. An attractive opportunity for intervention to reduce exposure to dietary mycotoxins is presented by the bacterial strains identified in mycotoxin binding earlier. Two such strains, *L. rhamnosus* strain GG (*L. rhamnosus* GG) and *L. rhamnosus* strain LC-705 (*L. rhamnosus* LC705) have been extensively studied and are shown to have the greatest aflatoxin removal capacity described to date. These strains of lactic acid bacteria are currently used in food products and present a potential cost effective and commercially viable approach to detoxifying aflatoxin contaminated products. Information on their ability to remove aflatoxins and other mycotoxins both *in vitro* and *in vivo* is an area of rapid development. The application of this technology to industry and the development of functional foods may form important areas of detoxification and decontamination biotechnology in the future.

Binding of mycotoxins by lactic acid bacteria

Specific strains of lactic acid bacteria have proved highly effective in removing aflatoxin B1 (AFB1) in model systems. It was found, however, that in a given genus and even within a given species, not all strains were equivalent in terms of toxin binding. Unlike the removal of other dietary mutagens by lactic acid bacteria [54], the capacity for AFB1 removal was a characteristic of only

specific strains, with efficacy varying markedly [55,56]. The results also indicated that aflatoxins are not removed from solution by bacterial metabolism, but rather are bound to the bacteria. These results have been confirmed by Oatley *et al.* [57], reporting that bifidobacteria bound from 25% to nearly 60% of the AFB1 added.

Bacterial concentrations must exceed 10⁹ bacteria/mL for effective removal of AFB1 [55]. The total number of AFB1 molecules that can be bound to a single viable bacterium has been estimated to exceed 10⁷.

Besides AFB1, the binding of other aflatoxins, AFB2, AFB2a, AFM1, AFM2, AFG1, AFG2, have also been studied [58,59,60,62]. In general, these aflatoxins are not bound as effectively as the more toxic and more commonly found AFB1. Another mycotoxin, Ochratoxin A, was also removed (36–76%) by *L. rhamnosus* GG and *L. rhamnosus* LC705, however, not as effectively as AFB1 (77–92%) [54].

The ability of selected strains of *Lactobacillus* and *Propionibacterium* has been assessed in the removal of common *Fusarium* toxins, ‘trichothecenes’, from liquid media [60]. The trichothecenes studied were deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), nivalenol (NIV), fusarenon (FX), diacetoxyscirpenol (DAS), T-2 and HT-2 toxins. The bacteria, *L. rhamnosus* GG, *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *Shermanii* JS were incubated in PBS-buffer containing 20 µg of toxin/mL for one hour at 37 °C, and after centrifugation the concentration of the toxins in the supernatant fraction was measured. Both viable and heat-killed forms of *L. rhamnosus* GG and *P. freudenreichii* ssp. *Shermanii* JS were more efficient than *L. rhamnosus* LC705 in removing the toxins from liquid media. *L. rhamnosus* GG and *P. freudenreichii* ssp. *Shermanii* JS removed four of the seven tested toxins (the percentage of removal varying from 18 to 93%) and *L. rhamnosus* LC705 removed two toxins (in 10–64%). While DAS was removed by all three bacteria, 3-AcDON was not removed by any of the bacteria, and HT-2 was not removed by non-viable *L. rhamnosus* GG and was only slightly removed by non-viable *L. rhamnosus* LC705. Binding is postulated as the mechanism of the removal, since no difference was observed between the ability of viable and heat-killed bacteria to remove trichothecenes, and, no degradation products of the toxins were detected in the gas chromatography–mass spectrophotometric analysis [60]. Thus, significant differences exist in the ability of the bacteria to bind trichothecenes *in vitro*.

The interaction between another two *Fusarium* mycotoxins, zearalenone (ZEN) and its derivative α -zearalenol (α -ZOL), and two food grade strains of *Lactobacillus* has also been reported [60]. When incubated with either *L. rhamnosus* GG or *L. rhamnosus* LC705 a considerable proportion (38–46%) of both toxins was recovered from

the bacterial pellets. No degradation products were detected in either the supernatant of the culturing media or the methanol extract of the pellet. Both heat-killed and acid-killed bacteria were capable of removing the toxins indicating that binding, not metabolism, is the verified mean of toxin removal. Binding of ZEN or α -ZOL by lyophilized *L. rhamnosus* GG and *L. rhamnosus* LC705 was a rapid reaction, with approximately 55% of the toxins being bound directly after mixing with the bacteria. As expected, binding was dependent on the bacterial concentration. Co-incubation of ZEN and α -ZOL with the bacteria significantly affected the percentage of toxin bound, indicating that these toxins may share the same binding site [60,61].

Mycotoxin removal mechanisms

Characterization of mechanism involved is of crucial importance for the safe use of specific probiotics for detoxification. AFB1 appears to bind to the bacterial surface of both *L. rhamnosus* GG and *L. rhamnosus* LC705 [62–64]. This was indicated by the accessibility of bound AFB1 to a polyclonal anti-AFB1 antibody in an indirect competitive inhibition enzyme-linked immunosorbent assay (ELISA). The recovery of up to 99% of bound AFB1 from the bacteria by solvent extraction [62] is further evidence for bacterial surface binding. Transmission electron microscopy reports involvement of surface exopolysaccharides for both strains when optimal binding occurs. Judging by the effects of pronase E, lipase and m-periodate on binding [63], it is suggested that the binding occurs predominantly with carbohydrate and protein components. The effect of the anti-hydrophobic agent urea on binding also suggests that hydrophobic interactions are important [63]. Studies over a range of ionic strength (up to 3), using monovalent (NaCl) and divalent (CaCl₂) metal ions, and a range of pH (2.5–8.5), showed no substantial effects on AFB1 binding [63], implying that electrostatic interactions and hydrogen bonding do not play a major role.

The stability of the AFB1 complexes formed with specific probiotics, in both viable and non-viable (heat-killed or acid-killed) forms reports that after five extractions a significant amount of AFB1 remained bound, with non-viable bacteria retaining the highest amount of AFB1 [62]. Autoclaving and sonication did not release detectable AFB1. Variation in temperature (4–37 °C) and pH (2–10) did not have any significant effect on the amount of AFB1 released. In all cases binding is of a reversible nature and the stability of the complexes formed depends on the bacterial strain, bacterial treatment and environmental conditions.

Animal studies on mycotoxin binding

L. rhamnosus GG, *L. rhamnosus* LC705 and *P. freudenreichii* ssp. *Shermanii* JS proved capable of reducing the absorption of AFB1 from ligated duodenal loops of chicks as

indicated by a reduced content of AFB1 in the soluble fraction of the luminal fluid, reduced mucosal uptake of AFB1 and increased content of AFB1 in the bacterial pellet of the luminal fluid [64]. The finding was significant as it indicated a potential reduction in the bioavailability of AFB1 through a reduction in its absorption via the intestinal mucosa. The complex formed between the bacteria and AFB1 was stable under luminal conditions suggesting that dietary decontamination can be accomplished by the addition of specific non-viable probiotic lactic acid bacteria to animal feeds, enabling the binding of aflatoxin in the gastrointestinal tract and its removal via the faeces, without harmful effects to the host animal (Figure 1). Such an approach could easily be adapted for use in poultry and other feeds with potential benefits in terms of the enhanced productivity of non-contaminated animals and the production of non-contaminated food products [64] (Figure 2).

Human studies

Only a few studies have been reported on probiotic administration to subjects naturally exposed to aflatoxin via diet. An intervention trial in Guangzhou, China demonstrated that dietary supplementation of specific probiotic bacteria to Chinese subjects exposed to aflatoxin B1 via diet resulted in reduced urinary excretion of one aflatoxin metabolite, known to be a biomarker for liver cancer risk [65,66]. Intervention studies are ongoing with specific probiotics supplemented to pregnant Egyptian women starting during the past month of pregnancy and continuing throughout the first year of lactation. It is

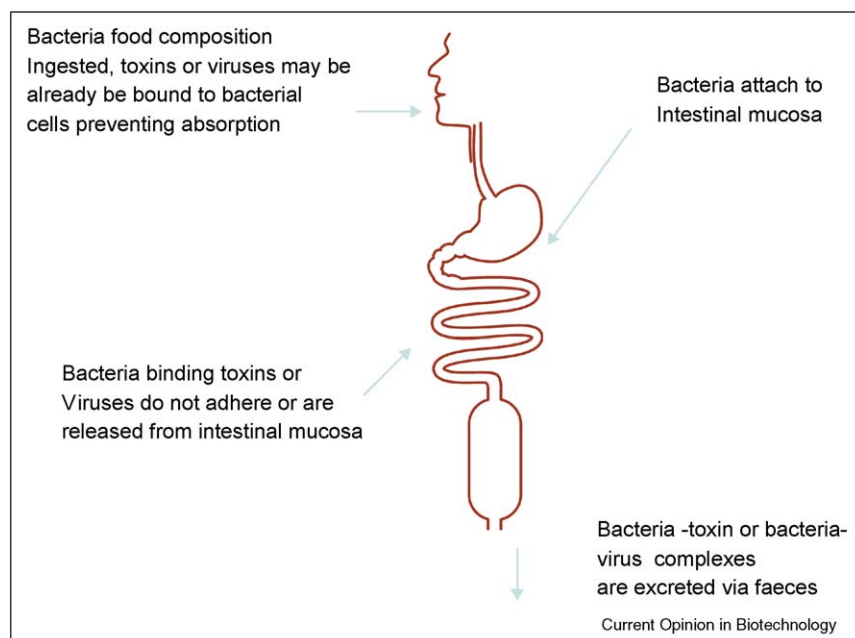
hypothesized that probiotic supplementation will lead to a reduction in exposure of both foetus and infants to aflatoxins.

Water pathogens

Cyanobacteria produce toxins that may present a hazard for drinking water safety. Mass occurrences of cyanobacteria are common in aquatic environments worldwide. These blooms are often toxic, owing to the presence of hepatotoxins or neurotoxins. Toxic and non-toxic strains of cyanobacteria co-occur and cannot be easily differentiated. Toxic cyanobacteria have been reported in natural and artificial freshwater reservoirs in several countries. Microcystins are cyclic heptapeptide hepatotoxins synthesized by several species of the freshwater cyanobacterial genera, for example *Microcystis*, *Anabaena*, *Planktothrix* and *Nostoc*, which occur in eutrophic waterbodies worldwide [67]. Microcystins consist of a seven-membered peptide ring, which is made up of five non-protein amino acids and two protein amino acids. The most common and highly toxic is microcystin-LR contains the amino acids leucine (L) and arginine (R) in the variable positions. Microcystins are a significant public health problem due to both their acute and chronic toxicities. Their potent hepatotoxicity and tumour-promoting activity is considered to be caused by inhibition of protein phosphatases 1 and 2A [68,69].

The accumulation of toxins in drinking water, water used for food processing or in plants and animals used for human consumption creates a risk of poisoning. Evidence

Figure 2



Probiotic interactions with toxins and viruses in the human gut.

of human poisonings by cyanobacterial toxins ranges from health effects after recreational exposure to poisonings following intake of contaminated drinking water. Water bodies containing potentially toxic cyanobacteria are unfortunately often used for drinking water production. Eighty percent of microcystin exposure has been estimated to occur through drinking water [70].

The occurrence of cyanobacterial toxins in water bodies used in production of drinking water also causes problems for water purification. No effective method has been proposed for degrading microcystins in the natural environment. Microcystins are structurally stable against physical and biological factors such as temperature, sunlight and enzymes under natural conditions [71]. Prevention of bloom formation is the most efficient method for avoiding cyanobacterial toxin in drinking water.

Human intoxications by cyanotoxins have occurred frequently, in some cases even lethal [72]. In 1996, one hundred patients developed acute liver failure after exposure to microcystin-containing dialysis water at a haemodialysis clinic in Caruaru, Brazil, and 76 of them died within 20 months [73,74]. Owing to the growing concern about the health effects of cyanotoxins, the World Health Organization has established a provisional guideline value of 1 µg/L for microcystin-LR in drinking water in 1998 (WHO, 1998). This guideline value is based on the acute toxicity of microcystins, and does not take into account possible long-term exposures to microcystins. The consequences of low-level chronic exposure to microcystins, especially in combination with other dietary toxins, are not well understood. Removal of cyanotoxins in drinking water treatment processes is, therefore, of increasing concern worldwide.

Removal of toxins produced by water pathogens—current knowledge and future perspectives

Humans are potentially exposed to microcystins through drinking water produced using contaminated resources and therefore toxins present in the water supply are a serious cause of health concern. It has recently been demonstrated that specific strains of probiotic bacteria are able to eliminate cyanobacterial toxins from aqueous solutions [8,75]. The research has been focused on removal of toxins by probiotic bacteria and evaluating their potential for water decontamination. The specific aims have been to characterize the capacity of specific strains of probiotic lactobacilli and bifidobacteria in toxin removal, to identify the potential of the strains to decontaminate toxin-containing drinking water, to investigate different conditions optimal for the removal and to study the potential mechanisms of toxin removal.

We have uncovered the cyanotoxin-removal properties of 11 probiotic bacterial strains of which *L. rhamnosus* strains GG and LC-705, *Bifidobacterium longum* 46, *B. lactis* 420

and *B. lactis* Bb12 were shown to be the most effective in removal of the cyanobacterial peptide toxin microcystin-LR among 11 tested strains [8]. The removal efficiency was dependent on incubation temperature, pH, cell viability and bacterial cell density. Optimal toxin removal was achieved at 37 °C with all five strains. The removal percentages increased with increasing temperature; at 4 °C practically no removal of microcystins could be observed. This could be explained by the fact that at 4 °C, the bacterial cells are metabolically inactive, but at higher temperatures they become metabolically active, which is required for enzymatic activity and metabolism. The bacterial cell density should exceed 10⁹ CFU/mL for significant cyanotoxin removal by viable lactobacilli and bifidobacteria.

For the above-described probiotics, the capacity of the strains in the removal of several other cyanotoxins, and when several microcystins were present simultaneously, was also efficient [76]. The removal was quite rapid, and up to 80% of the cyanotoxins were removed within 24 h of incubation. By addition of glucose as a source of nutrient to the bacterial cells the removal could further be improved [75]. From these findings it can be concluded that glucose as an energy source plays an important role in the metabolic activity of the investigated probiotic strains and thereby influence their microcystin elimination ability. Furthermore, the requirement of cell viability for efficient toxin removal was assessed. The bacteria were inactivated with different treatments and the removal capacity of the non-viable bacteria was then assessed and compared with viable bacteria. For all strains studied viable bacteria were shown to be more effective in toxin removal than non-viable bacteria [8]. These findings suggest an active biological degradation of the toxins by viable probiotics as one pathway to decontamination.

In a study on a combination of specific probiotic strains *L. rhamnosus* GG, *L. rhamnosus* LC-705 and *B. longum* 46 were tested individually. Most efficient toxin elimination was achieved with a combination of the probiotic bacteria [76]. As a result of variations in toxin-removal efficiency of different probiotic strains, combinations of bacteria may be beneficial for efficient removal of microcystins from solution.

Microcystins and nodularin are cyclic peptides and highly resistant to degradation. However, biodegradation of the toxins occurs and several bacterial strains capable of toxin degradation have previously been identified. Different bacterial strains, other than lactobacilli or bifidobacteria, have previously been shown to be able to degrade microcystins enzymatically. Bacterial degradation of microcystins has been reported for some *Sphingomonas* strains isolated from the environment [77–79]. The degradation of microcystin-LR has been shown to be mediated by intracellular hydrolytic enzymes and genes involved in

the bacterial degradation have been detected and characterized [80]. The breakdown of microcystin-LR results in linear chain of seven amino acids and thereby also the toxicity is significantly reduced. Two more enzymes cause further breakdown of the peptide to single amino acids. The mechanisms involved in toxin removal by probiotic bacteria are still but the data suggest that microcystins could be degraded enzymatically in the presence of metabolically active probiotic bacteria.

Probiotic potential for decontamination and human health promotion

Cyanobacteria frequently form blooms that present a problem for water used for drinking or recreation. The main threat for animal and human health is caused by the potential occurrence of cyanobacterial toxins, the most common of which are the hepatotoxins, microcystins and nodularins. Guideline values may promote more careful observation of drinking and recreational water, but water decontamination still remains an important aspect. Providing the human population with safe drinking water is an important issue in public health and methods for decontamination are needed. A promising novel tool in cyanotoxin decontamination is the use of metabolically active probiotic bacterial strains. Efficient cyanotoxin removal has been observed with several studied probiotic bacteria. The main human exposure route of microcystins is considered to be the oral route. The removal of microcystins in drinking water will be of great importance and specific probiotic bacteria are promising candidates for decontamination of water and diets. Probiotic dietary supplements could possibly be used as a personal defence against cyanotoxins in the gastrointestinal tract and to reduce the health risks caused by microcystins in drinking water. Furthermore, by the results obtained in recent studies, probiotic bacteria show potential in biological decontamination of cyanotoxin-containing water. Therefore it is hoped that the probiotic bacterial strains or possible enzymes identified in the removal process can be used in a bioremediation process to remove toxins from drinking water supplies and some future.

Conclusion

Probiotic combinations that inhibit and displace pathogens may be good candidates in case of specific microbiota aberrancies related to disease risk reduction. In a similar manner, it may be worthwhile to characterize the properties of specific probiotics and probiotic combinations on heavy metals, toxins of microbial origin in food and water and other harmful substances such as allergens. As stated previously it is important to take into account the high specificity of these processes being necessary to characterize the properties of the strains in order to select the best combination for a specific application. Knowledge on the surface properties and genomics of probiotic bacteria increases continuously providing new ways of understanding the mechanisms

involved as has been seen with *L. rhamnosus* GG [81^{**},82^{**}]. The change of one strain in a combination may modify all the properties against pathogen adhesion regarding inhibition, displacement and competition mechanisms. All together, these studies would allow the development of new probiotic combinations for the treatment or prevention of specific diseases by targeting the adhesion of specific pathogens. These results supported the idea that the use of probiotic combinations selected for specific targets may have a major impact on the disease.

The era of 'omics' and related technologies will contribute to the expansion of probiotic knowledge by enabling us to analyse and compare whole genomes, transcriptomes, proteomes and metabolites from specific probiotic and their combinations. Such information will lead to the identification and characterization of probiotic properties (alone and in different combinations) and understanding potential mechanisms of action for their impact on human health. This development underlines the focus on adhesion and adhesion interaction with pathogens, viruses and normal microbiota as means of modulating the composition and metabolic activity of microbiota in the intestinal epithelium. It will allow identification of new probiotics and probiotic combinations to target challenges in pathogen adhesion prevention and enhancement of the intestinal barrier functions.

Even at present, a rapid increase in the knowledge on the role of specific probiotic bacteria in binding and metabolizing some harmful components present in water and food has occurred. It is also clear from human intervention studies that specifically selected probiotics may have a positive effect in reducing the risk of diseases associated with toxin, virus, heavy metal or pathogen intake.

Using this knowledge as a platform to continue searching new treatment technologies could lead to characterization and development of low-cost probiotic bacteria and specific probiotic combinations that would be able to bind or inactivate both mycotoxins, cyanotoxins, heavy metals, viruses and pathogenic bacteria. Designing such combinations for food and water use could provide new options for high and low income countries to combat food and water contamination needs in the future.

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