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RESEARCH

Probiotic *Lactobacillus* and *Bifidobacterium* strains possess safety characteristics, antiviral activities and host adherence factors revealed by genome mining

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Abstract

Background Probiotics belonging to *Lactobacillus* and *Bifidobacterium* spp. have been exploited for their health benefits in treatment and prevention of many pathological conditions and promoting human health. Recent advances in understanding probiotics-human interaction through microbiome research in the context of various medical conditions suggest their provisional role in preventive, personalized, and predictive medicine. To streamline their application in disease prevention, development of personalized-based treatments, or their use as biomarkers for predictive diagnosis, in vitro screening for strains with potential probiotic properties should be performed. In this work, we aimed to emphasize the probiotic features of four *Lactobacillus* and two *Bifidobacterium* probiotic strains which showed antagonistic properties against microbial pathogens.

Methods Firstly, cytotoxicity assessment of cell-free preparations from these strains was performed using a baby hamster kidney (BHK) cells and cell viability was measured by means of sulfo-rhodamine B stain. Secondly, Newcastle disease (ND) and infectious bursal disease (IBD) viruses which pose a great threat in infected poultry were used for assessing antiviral activity of probiotics. Thirdly, the genomes of six probiotic strains were used to identify genes encoding host adherence factors that mediate interaction with human tissues.

Results Probiotic preparations exhibited insignificant toxicity as indicated by the high survival rate of BHK cells (surviving fraction varied from 0.82 to 0.99) as compared to the untreated control. Cell-free preparations of probiotics mixed with equal volume of ND and IBD viruses (10^6 and 10^4 Tissue Culture Infectious Dose 50, respectively) reduced the titer of ND and IBD viruses on chicken embryo fibroblast cells. Genome mining analysis revealed that the draft genomes of these strains were predicted to encode LPXTG-containing proteins, surface layer proteins, tight adherence pili, sortase-dependent pili, fibronectin, or collagen binding proteins and other factors that adhere to human tissues such as mucus. Such adherence factors enable probiotic bacteria to interact and colonize the host.

Conclusion Taken together, safety privileges, antiviral activities, and genomically encoded host interaction factors confirmed probiotic features of the six probiotic strains and their potential in promoting human health.

Keywords Probiotics · Preventive · Personalized and predictive medicine · Cytotoxicity · Antiviral activity · Genome mining · Host adherence proteins

Introduction

Probiotics are known as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [1]. Presence of adequate scientific evidence of safety, viability, and efficacy should exist to consider strains as probiotics, and this distinguish them from strains with probiotic potential or potentially beneficial microbes [1]. The potential health benefits of probiotics include improving immune responses [2], eliminating gastrointestinal microbial pathogens [3], strengthening intestinal mucosal barrier [4],

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reducing cholesterol level in serum [5], and treating many diarrheal and gastrointestinal diseases [6, 7].

A growing evidence about probiotics to be useful in medicine such as reducing incidence of traveler's diarrhea [8], reducing severity of rotavirus gastroenteritis [9], and remission from *Clostridium difficile* colitis [10] has been documented. Randomized controlled trials from human studies accumulate evidences for health effects of probiotics. However, it is plausible to distinguish such effects which are widely distributed among probiotics such as normalizing disturbed microbiota, exclusiveness of pathogens, colonization resistance, and prevention of gastrointestinal diseases [1] and those effects that are rare among probiotics. In this context, evidences for specific health benefits pertaining to specific probiotic strains are on the rise. For example, probiotics may reduce eczema-relative risk by 22% during late pregnancy [11] or reduce crying time in breastfed infants by average of 25 min per day [12]. Additionally, probiotics have been realized to impart beneficial effects at distant sites such as the airways, skin, brain, and heart [13].

Evidences for probiotics' benefits were based on using diverse microbial species in experimental and clinical settings. However, *Lactobacillus* and *Bifidobacterium* strains received enormous interest as therapeutic agents. Clinical studies showed efficacy of *L. rhamnosus* GG and *L. acidophilus* as well as *B. longum* in treatment of colon cancer and colorectal cancer, respectively [14, 15]. Strains belonging to *L. plantarum*, *L. reuteri*, and *L. brevis* were found efficient in treatment of vaginal disorders [16, 17]. In addition to this, there are evidences for efficacy of *Lactobacillus* and *Bifidobacterium* strains in treatment of hypercholesterolemia [18], inhibition of *Helicobacter pylori* infection [19], treatment of oxaluria [20], treatment of gastrointestinal pathologies [21], survival in gastrointestinal tract [22], and treatment of acute diarrhea [23]. The growing scientific evidence of using these two genera prompted us to explore some beneficial characteristics of six strains belonging to the same genera in the current study.

The aforementioned findings highlight the significant role of probiotics in prevention and treatment of human diseases and to be the game changers in preventive medicine. Despite the numerous studies focused on health-promoting benefits of probiotics [24], boosting the immune system and preventing infections [25], their interaction with host [26], their role in balancing the human gut microbiome [27], and the improving remedy of metabolic diseases [28], the development of personalized treatments which is based on truly patient profile are still lacking and screening for the strains to be used for such therapy under in vitro and in vivo conditions is mandatory. With regard to predictive medicine approaches, probiotics could serve as a basis for a panel of biomarkers for recognition of immunity-related, and respiratory diseases or cancer based on their role in the gut-brain axis or the intestinal microbiota-human interactions [29]. Being of natural origin, well-documented characterization of the potent probiotic strains will contribute to the move from reactive to preventive, personalized, and predictive medicine. In this regard, strains of probiotic *L. delbrueckii*, *L. casei*, and *B. animalis* were proven to reduce cholesterol levels in blood serum and recover liver morphology of obese BALB/c mice as described by Bubnov et al. [30]. In the same study, probiotics were able to decrease body weight and beneficially module gut microbiota of the fat-diet-fed mice. These findings suggest likelihood of using such strains in prevention and treatment of metabolic syndromes. However, consideration regarding strain-dependent properties (e.g., resistance to biological fluids and antibiotics) of *Lactobacillus* and *Bifidobacterium* strains should be made prior to their applications in individualized treatment [31]. Since we had evidence about the antagonistic and antibiofilm potential of the six probiotics strains, used in this study, we initiated current investigation to reveal additional beneficial traits that can expand the strains' applicability against microbial infections. This also will lay the ground for designing future animal studies to confirm their beneficial use.

The application of probiotics as therapy necessitates their safety and maintenance of host cell viability. According to the International Association of Probiotics and Prebiotics (ISAPP) consensus document, certain effects could be ascribed to probiotics as a general class based on well-designed trials, meta-analyses, and systematic reviews. In the same document, several bacterial species (e.g., *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, *B. longum*, and *B. bifidum*), from which some strains have been investigated here, were mentioned to be accepted by Health Canada for their contribution in gut microbiota health [1]. Collectively, this implicates their safety for human use. Safety of probiotic bifidobacteria and lactobacilli has been extensively overviewed. Di Cerbo et al. [32] summarized clinical evidences that support the therapeutic efficacy of lactobacilli in the treatment of numerous pathological conditions based on selected papers from 1950 to 2015. Different approaches have been proposed to measure safety of probiotics. Of these, monitoring toxicity in vivo via evaluation of some parameters such as blood parameters, assessment of bacterial translocation, signs of intestinal inflammation, or alterations in body or splenic weight [33]. Additional methods include measuring infectivity in vitro or in animal models [33]. However, little is known about the safety of the strains' supernatants after their growth in conventional media. This inspired our current work to assess the safety of cell-free preparations of six probiotics, belonging to *Lactobacillus* and *Bifidobacterium* spp., which demonstrated antibacterial and antibiofilm potential in our previous study against multidrug resistance *Escherichia coli* [34].

The use of probiotics as antiviral agents was reported [35, 36]. For example, *B. adolescentis* SPM0212 exerted antiviral

activity against Hepatitis B virus [37] and *B. adolescentis* SPM1005 suppressed human papillomavirus–associated cervical cancer [38]. *Bifidobacterium* and *Lactobacillus* spp. proved to be antagonistic against many viruses such as herpes simplex virus, human influenza virus, and human immunodeficiency virus [39–41]. However, the efficacy of probiotics as antagonistic agents against some viruses was rarely investigated. Of these viruses are Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV). The former is the causative agent of Newcastle disease (ND) which is a highly infectious disease that has been reported globally and associated with multiple outbreaks in poultry [42]. On the other hand, IBDV is the causative agent of infectious bursal disease (IBD) which is highly contagious and acute in young chicken and turkeys [43, 44]. Currently, there is no effective treatment for NDV and IBDV, and protective vaccination is the only therapy adopted. This prompted us to assess the in vitro antiviral effect of probiotics against NDV and IBDV to gain insights about their possible use in further animal studies.

Genome mining is a powerful tool in unraveling the genetic determinants involved in host-microbe interactions. The increasing capabilities of DNA sequencing technologies enabled identifying majority of the genes in probiotic genomes [45, 46]. The approach has been utilized to advance the understanding of the probiotic traits such as host adhesion factors in VSL#3 multispecies marketed probiotic [45]. In addition, functional genomics elucidated genes of probiotic traits in *Bacillus coagulans* HS243 [46]. Furthermore, comparative genomic analysis of *Enterococcus* sp. genomes emphasized the genetic repertoire that confers probiotic features [47]. Genome mining analysis is utilized in current work to uncover the presence of genomically encoded adherence factors in the six probiotic strains that we investigated previously [34].

Our aim is to provide additional evidence for the probiotic traits, throughout evaluating the cytotoxicity and antiviral activity, of different probiotics belonging to *Lactobacillus* and *Bifidobacterium* spp. against Newcastle disease and infectious bursal disease viruses. Additionally, we will utilize genome mining tools to unravel the host adherence factors in these probiotics to emphasize their potential use in health promotion and prophylaxis.

Methods

Bacterial strains and growth conditions

Six strains of probiotics, obtained from Egyptian Microbial Culture Collection, Microbiological Resources Centre, Ain-Shams University, Egypt, were used in this work as shown in Table 1. Probiotic strains were grown on Man-Rogosa-Sharpe (MRS; Oxoid, Hampshire, England) agar for 24 h at 37 °C with 5% CO_2. Single colony from each strain was transferred into MRS broth under the same incubation conditions for 24 h for the preparation of the cell-free spent medium (CFSM).

Table 1 Probiotic strains used in this study

Strain number	Isolation source	Strain designation*	Accession number for whole genome sequence
Lactobacillus acidophilus	Human	ATCC4356 = EMCC 1324 = DSM 20079	JRUT01000000
Lactobacillus helveticus	Swiss cheese	ATCC15009 = EMCC 1654 = DSM 20075	NZ_ACL-M01000000
Lactobacillus plantarum ss. *plantarum*	Pickled cabbage	ATCC14917 = EMCC 1027= DSM 20174 = JCM 1149	AZEJ00000000.1
Lactobacillus rhamnosus	Unknown	ATCC7469 = EMCC 1105 = DSM 20021 = JCM 1136 = NBRC 3425	NZ_AZC-Q00000000.1
Bifidobacterium longum Reuter 1963[AL]	Intestine of an adult	ATCC 15707 = EMCC 1547 = DSM 20219 = ATCC 15707	FNRW00000000.1
Bifidobacterium bifidum (Tissier 1900)	Intestine of an adult	EMCC 1334 = DSM 20082 = JCM 1254	BBBT00000000.1

*Strain designations identified on NCBI databases for each strain will be used across the text. These are ATCC4356, DSM 20075, ATCC14917, DSM 20021, DSM 20219, and JCM 1254 for *L. acidophilus*, *L. helveticus*, *L. plantarum*, *L. rhamnosus*, *B. longum*, and *B. bifidum*, respectively

Preparation of the cell-free spent medium

The preparation of CFSM from each probiotic strain was performed as described previously [34, 48]. Briefly, overnight cultures of the six probiotic strains grown in MRS broth at 37 °C were diluted 1:100 with fresh medium and allowed to grow under same conditions to an optical density of 1.6 (~ 1×10^8 cells/mL), and the cells were then removed using centrifugation at 6000 *xg* at 4 °C for 10 min. The supernatant was filter-sterilized through 0.2-μm pore-size filter (Sigma Aldrich, Munch, Germany) and referred to as CFSM. The CFSM of all probiotic strains was stored at − 20 °C until use for cytotoxicity and antiviral assays.

Cytotoxicity assay for the CFSM of probiotics

The cytotoxicity assay was conducted using a Baby hamster kidney (BHK-A) cells (Sigma Aldrich) as described previously [49]. The BHK-A cells were grown in Dulbecco's modified Eagle's medium (DEME; Sigma scientific services, Cairo,

Egypt) supplemented with 200 μM glutamine and 10% fetal calf serum and incubated at 37 °C under 5% CO_2 for 24 h. The cells were inoculated at a density of 1×10^4 and allowed to grow until the cell has become confluent. Filtered CFSM at different dilutions (10%, 50%, 75%, and 100%) were added to the cell culture and incubated at the same conditions for 48 h. After incubation, the cells were fixed, washed, and stained with sulfo-rhodamine B stain. Excess stain was removed using acetic acid while the bound stain was recovered by Tris-EDTA buffer. The color intensity of the stain was measured in an ELISA reader (Sun Rise–TECAN, Clinilab Ltd., Egypt). The untreated cells were considered as a control. The surviving fraction of BHK cells in each treatment was calculated relative to the control.

Antiviral assay

Virus stock

Pure NDV and IBDV were implemented in this study and obtained kindly from The Virology Lab., Department of Microbiology, Faculty of Agriculture, Ain-Shams University, Egypt.

Cell line

Primary Chicken embryo fibroblast (CEF) cell culture (supplied by Virology Department, Animal Health Research Institute, Cairo, Egypt) was prepared using 9- to 10-day-old chicken embryos by warm trypsinization method [50]. Viability of the cells was determined by using "dye exclusion method," as described before [50, 51]. Micro-titration of NDV and IBDV was performed using the same cell line as described previously [52].

Seeding of CEF cell culture

For antiviral assay, 100 μl of CEF (1×10^5 cells/mL), grown in Eagle's minimal essential medium (MEM; GIBCO, Grand land, NY, USA) containing 10% inactivated calf serum and 100 μg/mL penicillin and streptomycin, were seeded in each well of the 96-well plates. The plates were then incubated at 37 °C for 72 h with 5% CO_2. Cells in each plate were regularly monitored under an inverted microscope (HUND, Germany) until they reached to a confluence state of 80–90% [50, 51].

Antiviral activity

The antiviral activity was investigated as reported before [50, 51]. Briefly, to determine any cytotoxic potential of CFSM on CEF cells, 100 μl of 10% probiotics' CFSM were added to confluent CEF cells grown in freshly prepared media, while CEFs grown only in Eagle's minimal essential medium (EMEM) served as positive control. Additionally, DMSO (20%) and EMEM media was used as negative control. For antiviral assay, 50 μl from each probiotic CFSM, at 10% final concentration, was mixed with 50 μl ND and IBD virus (10^6 and 10^4 tissue culture infectious dose (TCID) 50, respectively). The test concentrations (100 μl total) were added to each well containing CEF cells. Afterwards, plates were kept at 37° C in 5% CO_2. The development of cytopathic effects was observed every 24 h for 3 days. The reading of CPE was recorded, and the virus titer was calculated as described before [52].

Genome mining analysis for host adherence proteins

The whole genome sequences of the six probiotic strains were retrieved from the NCBI using the accession numbers listed in Table 1. The annotated genomes were screened for host adherence factors using the NCBI sequence set browser. The adherence factors were selected based on previous reports that defined the genetic determinants necessary for probiotic *Lactobacillus* and *Bifidobacterium* spp. to adhere to host tissues [45, 53]. The retrieved protein sequences of adherence factors in the probiotic strains were screened for similarity using online NCBI's BLASTp tool. In addition, protein sequences of adherence factors were analyzed for similarity with translated nucleotide sequences from genomes of commercial probiotic strains using tBLASTn tool. For *B. bifidum* JCM 1254, the annotated protein sequences were not available on NCBI database and the genetic determinants encoding adherence proteins in the strain's genome were identified based on sequence similarity with the closest reference genome. Genes encoding adherence factors in *B. bifidum* JCM 1254 genome were screened for sequence similarity with the reference genome in addition to a marketed probiotic *B. animalis* BB-12 strain using BLASTn tool. Genes encoding bile salt tolerance were identified using tBLASTn tool. Antibiotic resistance genes (ARGs) in the probiotic strains were analyzed against the comprehensive antibiotic resistance database (CARD).

Results

Cell-free spent media and cytotoxicity

The cytotoxic activities of four different dilutions of the cell-free preparations of the six probiotic strains were investigated using BHK cell line in vitro. The different preparations of CFSM exhibited very low toxicity as indicated by the high survival rate of BHK cells (surviving fraction ranged from 0.82 to 0.99) as compared to the control as shown in Table 2. Only, in case of the CFSM of *L. helveticus*, the cell

Table 2 Cytotoxicity results for the cell free preparations of six probiotic strains on BHK cells

Concentration (v:v) of the diluted CFSM	Surviving fraction of the BHK cells					
	L. acidophilus ATCC4356	*L. helveticus* DSM 20075	*L. plantarum* ATCC14917	*L. rhamnosus* DSM 20021	*B. longum* DSM 20219	*B. bifidum* JCM 1254
0	1.0	1.0	1.0	1.0	1.0	1.0
10	0.97	0.93	0.93	0.93	0.90	0.93
50	0.82	0.81	0.92	0.97	0.88	0.91
75	0.86	0.70	0.90	0.99	0.92	0.89
100	0.87	0.58	0.90	0.93	0.93	0.87

viability was lower than the other strains with a survival fraction of 0.58 and 0.70 at 75% and 100% of this bacterium's CFSM (Table 2). These data show that very low cytotoxic effects could be exhibited by CFSM preparations of the probiotic strains except for *L. helveticus* strain which demonstrated toxicity at high CFSM concentrations (75% and 100%).

Additional safety criteria of the probiotic strains

Our preliminary data indicates the ability of two different hosts (mice and poultry) to survive well after oral administration of the whole cultures of the six probiotic strains (data are not shown). Additionally, the analysis of the probiotic genomes against CARD was executed using strict Resistance Gene Identifier (RGI) criteria to decipher the presence of ARGs. Interestingly, genomes of the four *Lactobacillus* strains were void of ARGS while two ARG families conferring resistance to mupirocin and rifamycin were identified in *B. bifidum* JCM 1254 and only rifamycin-resistant gene family was found in *B. longum* DSM 20219 (Supplementary file 1). However, such susceptibility of *Bifidobacterium* strains to mupirocin and rifamycin requires further experimental validation.

Titration of NDV and IBDV on cell culture

Infectivity titers of NDV and IBDV were determined by infecting CEF cell line with increasing dilutions of virus material, and the highest dilution producing cytopathic effect in 50% of the inoculated cells was determined. The 50% end point dilution expressed as TCID 50/mL was calculated using Reed-Muench formulae. Data showed that CEF infected with NDV and IBDV showed CPE after 4 and 5 days post-infection, respectively. The virus infectivity titer was 10^6 and 10^4 TCID 50/mL for NDV and IBDV, respectively.

Effect of probiotics on NDV and IBDV titer

Non-toxic concentration (10%) of CFSM of probiotics was used to treat the CEF cells infected with NDV or IBDV. Reduction in titers of NDV and IBDV in the presence CFSM of probiotics varied with species as shown in Table 3. The percent decrease in virus titer in case of exposure to CFSM of *B. bifidum* JCM 1254, *L. plantarum* ATCC 14917, and *L. rhamnosus* DSM 20021 was 98.3, 98.5, and 97.6%, respectively, followed by *B. longum* 20219 that caused reduction of 96.3% in NDV titer. The inhibitory effect observed by

Table 3 Reduction of NDV and IBDV titer by cell free preparations of probiotics

Treatment	% decrease in virus titer After 72 h	
	NDV	IBDV
Virus only (control)	Zero	Zero
B. longum DSM 20219	96.31	79.36
L. acidophilus ATCC4356	90.86	63.75
B. bifidum JCM 1254	98.33	61.90
L. plantarum ATCC14917	98.50	71.31
L. helveticus DSM 20075	89.65	70.10
L. rhamnosus DSM 20021	97.64	69.10
Mixture of six strains	99.43	83.61
Untreated cell line (negative control)	Zero	Zero
Probiotic CFSM only on cell line	Zero	Zero

probiotic strains was much higher in case of NDV as compared to IBDV. Intriguingly, treatment with a mixture of all strains' CFSM resulted in the highest reduction (99.4 and 83.6% decrease) in titer of NDV and IBDV, respectively. These results suggest the ability of the six probiotic strains to repress in vitro NDV and IBDV infection.

Mining of host adherence proteins in probiotic genomes

Probiotic *Lactobacillus* and *Bifidobacterium* spp. produce host-specific adherence proteins to signal and interact with the host. The whole genomes of the six probiotic strains encoded multiple surface components that adhere to the host. Of these, cell wall anchor domain proteins harboring LPXTG motifs were found in the four *Lactobacillus* spp., with 5, 2, 1, and 1 motifs for *L. helveticus* DSM 20075, *L. rhamnosus* DSM 20021, *L. plantarum* ATCC 14917, and *L. acidophilus* ATCC 4356, respectively (Tables 4, 5, 6, and 7). All LPXTG motifs were highly similar to corresponding motifs in other strains of *Lactobacillus* species. Interestingly, LPXTG motifs showed less identity to similar motifs in marketed probiotic strains. Among these, *L. helveticus* DSM 20075's LPXTG motif, EEW68618.1 (product length of 851 amino acids) and *L. rhamnosus* DSM 20021's LPXTG motif, and KRK28219.1 (product length of 143 amino acids) are less similar to those in the commercial strains. On the other hand, *L. rhamnosus* DSM 20021's LPXTG motif, KRK32331.1, with a high molecular weight had 100% coverage to marketed *L. rhamnosus* GG but low similarity (67% identity) as shown in Table 5.

Fibronectin binding proteins were found in all probiotic *Lactobacillus* strains with high similarity to the closest *Lactobacillus* species. A similar trend was observed with the marketed probiotic *Lactobacillus* spp. (Tables 4, 5, 6, and 7). A collagen adhesion protein was found only in *L. rhamnosus* DSM 20021.

Sortases are enzymes which anchor surface proteins in cell wall of *Lactobacillus*, and *Bifidobacterium* spp. were encoded in *L. helveticus* DSM 20075, *L. rhamnosus* DSM 20021, *L. plantarum* ATCC 14917, and *L. acidophilus* ATCC 4356 with 1, 2, 1, and 1 gene products per genome, respectively (Tables 4, 5, 6, and 7).

Bacterial S-layer proteins were encoded in *L. helveticus* DSM 20075, *L. plantarum* ATCC 14917, and *L. acidophilus* ATCC 4356 (1, 1, and 4 gene products per genome, respectively). The S-layer protein, EEW68127.1, in *L. helveticus* DSM 20075 showed low similarity (54% identity) compared to the closest motif in the marketed probiotic *L. helveticus* R0052 (Table 4).

The whole genome of *L. rhamnosus* DSM 20021 encoded 4 WxL domain surface proteins with high similarity to other NCBI's *L. rhamnosus* strains and the commercial *L. rhamnosus* GG. Additionally, ATCC469 strain encoded 1 surface protein, involved in host adherence, called "isopeptide-forming domain-containing fimbrial protein" (Table 5). The protein did not exist in the other three studied *Lactobacilli*. Glyceraldehyde 3-phosphate dehydrogenase, the enzyme involved in plasma adherence, is encoded in *L. plantarum* ATCC 14917 genome and exhibited similarity to *L. paraplantarum* species (Table 6). Twelve mucus-binding proteins were encoded *L. acidophilus* ATCC4356 with high similarity (> 89% identity) to those encoded by other *L. acidophilus* strains (Table 7).

Table 4 Host adhesion and interaction proteins in probiotic *Lactobacillus helveticus* DSM 20075 as determined by genome mining

General function	Protein accession	Locus_tag	Product length	Product name	Closest hit by BLASTp coverage/identity*	Marketed probiotic** coverage/identity
Adherence and binding to human extracellular proteins	EEW68912.1	HMPREF0518_0183	56	LPXTG-motif cell wall anchor domain protein	100/100	100/71
	EEW68618.1	HMPREF0518_0430	851	LPXTG-motif cell wall anchor domain protein	100/100	66/58
	EEW68498.1	HMPREF0518_0555	466	LPXTG-motif cell wall anchor domain protein	100/100	100/89
	EEW68322.1	HMPREF0518_0725	135	LPXTG-motif cell wall anchor domain protein	100/100	100/85
	EEW67461.1	HMPREF0518_1574	78	LPXTG-motif cell wall anchor domain protein	100/100	100/94
	EEW68980.1	HMPREF0518_0083	229	Sortase family protein	100/100	100/89
	EEW68127.1	HMPREF0518_0914	472	Bacterial surface layer protein	100/100	100/54
Adherence to fibronectin of epithelial cells	EEW69041.1	HMPREF0518_0004	564	Fibronectin binding protein A domain protein	100/100	100/90

*The closest species identified for all gene products was *L. helveticus*

**The marketed probiotic used for comparison was *L. helveticus* R0052 (accession number; CP003799)

Table 5 Host adhesion and interaction proteins in probiotic *Lactobacillus rhamnosus* DSM 20021 as determined by genome mining

General function	Protein accession	Locus_tag	Product length	Product name	Closest hit by BLASTp coverage/identity*	Marketed probiotic coverage/identity**
Adherence and binding to human extracellular proteins	KRK30998.1	Q777_GL002528	233	Sortase (surface protein transpeptidase)	100/100	100/89
	KRK30857.1	Q777_GL002684	222	WxL domain surface protein	100/100	100/53
	KRK32234.1	Q777_GL000216	262	WxL domain surface protein	100/100	100/89
	KRK30861.1	Q777_GL002689	232	WxL domain surface protein	100/100	100/93
	KRK30572.1	Q777_GL000358	268	WxL domain surface protein	100/100	91/88
	KRK30112.1	Q777_GL000983	274	Sortase (surface protein transpeptidase)	100/100	90/92
	KRK30113.1	Q777_GL000984	517	Surface protein (isopeptide-forming domain-containing fimbrial protein)	100/100	100/94
	KRK32331.1	Q777_GL000323	2076	LPXTG-motif cell wall anchor domain protein	100/100	100/67
	KRK28219.1	Q777_GL001762	143	LPXTG-motif protein cell wall anchor domain protein	100/99	79/73
Adherence to fibronectin of epithelial cells	KRK30506.1	Q777_GL000539	577	Fibronectin binding protein A	100/100	100/95
Adherence to host collagen	KRK30425.1	Q777_GL000700	1649	Collagen adhesion protein	100/99	65/34

*The closest species identified for all gene products was *L. rhamnosus*

**The marketed probiotic used for comparison was *L. rhamnosus* GG (Accession number, NC_013198.1)

Table 6 Host adhesion and interaction proteins in probiotic *Lactobacillus plantarum* ATCC14917 as determined by genome mining

General function	Protein accession	Locus_tag	Product length	Product name	Closest hit by BLASTp coverage/identity*	Marketed probiotic** coverage/identity
Adherence and binding to human extracellular proteins	KRL35623.1	FC76_GL002094	955	Cell-surface protein precursor, LPXTG-motif cell wall anchor	100/99	100/95
	KRL33595.1	FC76_GL000951	234	Sortase	100/99	100/93
	KRL34462.1	FC76_GL002889	340	Glyceraldehyde 3-phosphate dehydrogenase	100/99	100/96
	KRK32234.1	FC76_GL000366	85	S-layer family protein and secreted protein with 1 GW repeat fusion protein	88/92	98/79
Adherence to fibronectin of epithelial cells	KRL36306.1	FC76_GL000267	215	Fibronectin binding protein	100/99	92/98

*The closest species identified for all gene products was *L. plantarum* except for glyceraldehyde 3-phosphate dehydrogenase which showed closest similarity to *L. paraplantarum*

**The marketed probiotic used for comparison was *L. plantarum* JDM1 (accession number, NC_012984)

Table 7 Host adhesion and interaction proteins in probiotic *Lactobacillus acidophilus* ATCC4356 as determined by genome mining

General function	Protein accession	Locus_tag	Product length	Product name	Closest hit by BLASTp coverage/identity*	Marketed probiotic coverage/identity**
Adherence and interaction with human proteins	KHE29327.1	NH13_08900	438	LPXTG cell wall anchor protein	100/100	94/88
	KHE29965.1	NH13_06240	229	Sortase	100/100	100/85
	KHE30278.1	NH13_04685	508	Mucus-binding protein	100/100	100/100
	KHE30367.1	NH13_05180	185	Mucus-binding protein	100/100	100/100
	KHE30368.1	NH13_05185	294	Mucus-binding protein	100/100	100/100
	KHE30369.1	NH13_05190	346	Mucus-binding protein	100/100	100/99
	KHE30370.1	NH13_05195	2650	Mucus-binding protein	100/100	100/91
	KHE29946.1	NH13_06125	697	Mucus-binding protein	100/100	100/94
	KHE29700.1	NH13_06860	1017	Mucus-binding protein	100/99	92/99
	KHE29713.1	NH13_06935	4326	Mucus-binding protein	100/100	100/94
	KHE29774.1	NH13_07255	339	Mucus-binding protein	100/100	100/100
	KHE29525.1	NH13_07960	643	Mucus-binding protein	100/100	100/94
	KHE29426.1	NH13_08195	1174	Mucus-binding protein	100/100	95/89
	KHE29473.1	NH13_08490	1208	Mucus-binding protein	100/100	100/90
	KHE30826.1	NH13_00935	357	S-layer protein	100/100	86/88
	KHE30641.1	NH13_01170	172	S-layer protein	100/100	100/75
	KHE30642.1	NH13_01175	177	S-layer protein	100/100	100/91
	KHE29957.1	NH13_06190	167	Lactocepin S-layer protein	100/100	100/100
Adherence to fibronectin of epithelial cells	KHE29547.1	NH13_07425	991	Fibronectin binding protein	100/100	100/90

*The closest species identified for all gene products was *L. acidophilus*

**The marketed probiotic used for comparison was *L. acidophilus* NCFM (accession number, NC_006814)

Bifidobacterium spp. possesses extracellular structures; pili that play a role in host adherence. Two types of pili, namely tight adherence (Tad) pili and sortase-dependent pili, were found in *B. longum* DSM 20219 and *B. bifidum* JCM 1254. Four genes identified in *B. longum* DSM 20219 genome were found to encode two TadE-like protein, TadA and TadB proteins (Table 8). Interestingly, three sortase A genes were identified in the DSM 20219 genome, in addition to fimbrial isopeptide formation D2 domain-containing protein. Tad proteins and sortase-dependent system were similar in other *B. longum* strains found in NCBI database (Table 8) but with low similarity to the marketed *B. longum* JDM301. In a similar fashion, three Tad proteins (TadA, TadE, and TadZ) involved in pili formation were identified in *B. bifidum* JCM 1254. Additionally, fimbriae formation was elucidated by presence of *fimA* gene in *B. bifidum* JCM1254 strain; however, no sortase coding gene was found. Moreover, two collagen binding cell-surface proteins were identified in the JCM 1254 genome (Table 9). All these results document the well-defined capabilities of the probiotic *Lactobacillus* and *Bifidobacterium* strains to adhere, colonize, and interact with the human host.

Discussion

Emerging resistance to the current antimicrobial drugs poses a great concern and is considered as a growing public health concern globally. Such resistance has been reported for bacterial, fungal, and viral drugs. Therefore, the search for a novel,

Table 8 Host adhesion and interaction proteins in probiotic *Bifidobacterium longum* DSM 20219 as determined by genome mining

General function	Protein accession	Locus_tag	Product length	Product name	Closest hit by BLASTp coverage/identity*	Marketed probiotic coverage/identity**
Adherence and interaction with human proteins	SEB40549.1	SAMN04489748_0988	309	Sortase A	100/100	86/42
	SEB51646.1	SAMN04489748_1310	327	Sortase A	100/100	71/44
	SEB55405.1	SAMN04489748_1494	377	Sortase A	100/100	100/79
	SEB51666.1	SAMN04489748_1311	529	LPXTG-motif cell wall anchor domain-containing protein	100/100	87/29
	SEB55278.1	SAMN04489748_1488	125	TadE-like protein	100/100	100/80
	SEB55302.1	SAMN04489748_1489	116	Helicase/secretion neighborhood TadE-like protein	100/100	100/95
	SEB51395.1	SAMN04489748_1297	149	tRNA-adenosine deaminase (TadA protein)	100/100	100/82
	SEB55214.1	SAMN04489748_1485	218	Tight adherence (Tad) protein B	100/100	100/98
	SEB28668.1	SAMN04489748_0023	352	von Willebrand factor type A domain-containing protein	100/99	100/90

*The closest species identified for all gene products was *B. longum*

**The marketed probiotic used for comparison was *B. longum* JDM301 (accession number, NC_014169.1)

safe, and unconventional drugs is of a rising interest. Data obtained from the current work suggest six probiotic strains with potential health benefits that could be exploited in provisional applications in preventive treatments, combating microbial diseases, and formulation of probiotics-enriched diets according to patient phenotypic characteristics. The strains are lactic acid producers and possess genomic determinants involved in bile salt tolerance (Supplementary file 2). These are pivotal criteria for probiotics to survive the gastrointestinal tract. To emphasize the therapeutic use of the six probiotic

Table 9 Host adhesion and interaction proteins in probiotic *Bifidobacterium bifidum* JCM 1254 as determined by genome mining

General function	Reference strain	Gene name (Gene ID) in the reference stain	Locus_tag in the reference strain	Product name	*B. bifidum* JCM 1254 vs. reference genome (coverage/identity)**	*B. bifidum* JCM 1254 vs. marketed probiotic# (coverage/identity)+
Adherence and binding to human proteins	*B. bifidum* PRL2010*	*tadA* (9889934)	BBPR_1811	Cytosine/adenosine deaminase	100/99	32/80
		tadE (9889878)	BBPR_1756	TadE-like protein	100/100	10/83
		tadZ (9889884)	BBPR_1762	TadZ-like protein	100/99	10/83
		BBPR_0810 (9888954)	BBPR_0810	Sortase	100/98	48/84
		fimA (9888452)	BBPR_0283	Fimbrial subunit FimA	100/98	9/82
Adherence to host collagen		BBPR_1822 (9889945)	BBPR_1822	Cell-surface protein (collagen binding)	100/99	22/84
		BBPR_0282 (9888451)	BBPR_0282	Cell-surface protein (collagen binding)	100/99	9/82

**B. bifidum* PRL2010 was used as a reference strain because the coverage/identity of this strain vs. *B. bifidum* JCM 1254 is 99/98% as shown by microbial genome Blast

**BLASTn tool was used to determine the sequence similarity of each gene in the reference strain vs. *B. bifidum* JCM 1254

The marketed probiotic used for comparison was *B. animalis BB*-12 (accession number, CP001853)

+ BLASTn tool was used to determine the sequence similarity of each gene in *B. bifidum* JCM 1254 vs. the commercial *B. animalis BB*-12

strains used in this work, the cytotoxicity assessment was conducted. The CFSM of all probiotics demonstrated relatively very low cytotoxicity on BHK-2 cells which encourage their applications for further in vivo studies. Likewise, previous work concluded that the cell viability of Vero cells was around 80% when exposed to probiotic *B. longum* SPM1205 and SPM1206 and *L. ruminis* SPM0211 [54]. In addition, cell extract of *B. adolecentis* SPM0212 had no cytotoxicity on HepG2.2.15 [37]. These findings are in agreement with our results and augment the safety potential of the six strains studied in this work. In addition, genome mining analysis revealed the absence of ARGs in the strains except for *Bifidobacterium* strains which harbored up to two ARGs. However, this resistance pattern requires validation. For example, putative antibiotic resistance genes were identified in strains of the multispecies VSL#3 marketed probiotic product [45]. However, the strains showed sensitivities to the corresponding antibiotics within the recommended minimum inhibitory concentration values after conducting micro-dilution antibiotic susceptibility testing.

Resistance to drugs pertaining to treat enteric, avian, respiratory, and sexually transmitted viruses is on rise [55]. In the last decade, many studies highlighted the potential of probiotics such as lactic acid bacteria and their bioactive metabolites as antiviral drugs [56, 57]. The protective effect and immune-modulatory activity of probiotics against respiratory viruses in mice was clearly described [56]. The antiviral compounds produced by probiotics were identified as non-proteinaceous such as hydrogen peroxide and lactic acid as well as proteinaceous compounds such as bacteriocins [55]. The antiviral activities of probiotics vary by species, where *L. fermentum* ACA-DC179, *E. faecium* PCK38, *L. plantarum* PCA236, *L. pentoses* PCA227, *B. animalis* subsp. *Lactis* BB-12, *L. casei* Shirota, and *B. longum* SP07/3 were examples with the highest antiviral activities [55]. In this work, CFSM of all probiotic strains were investigated for their antiviral activities against NDV and IBDV. The six probiotic strains were able to reduce the viral titer of NDV and IBDV, which might be attributed to the ability of probiotics to produce antiviral metabolites which reduce the pH (lactic acid) or interfere with the adsorption of these viruses to their host cells (bacteriocins) [55] or increasing the CEF resistance against viral infection by reducing viral replication throughout produced exopolysaccharides [58]. However, this assumption needs more investigations. Wang et al. [59] revealed that probiotics could inhibit the replication rate of viruses. The current results suggest beneficial use of probiotics' cell-free preparations or purified compounds in protecting hosts such as poultry against viral infection. In addition, genome mining revealed presence of numerous cell wall-associated components such as S-layer proteins and affirmed a well-defined adhesive potential to host tissues. These criteria could contribute to the antiviral potential of the strains themselves regardless of their metabolites [60, 61].

Besides the antiviral and safety characteristics observed for the 6 *Lactobacillus* and *Bifidobacterium* strains investigated in this work, genome mining analyses revealed the wealth of genomic information that document additional probiotic features of these strains. The whole genome sequences of the strains encoded surface structures that are known to mediate host-microbe interaction and adherence. Long extracellular structures such as pili (Tad and sortase-dependent pili) and fimbriae were encoded in *Lactobacillus* and *Bifidobacterium*. Tad pili were found to be essential for gut colonization in *B. breve* [62]. Sortase-dependent pili that encode for FimB were capable of binding to xylan and other matrices that are important for interaction with the host. Furthermore, sortase-dependent pili proteins expressed in *Lactococcus lactis* enhanced the adhesion to human enterocytes [63].

LPXTG motifs harbored in cell-surface-associated proteins were encoded in all *Lactobacillus* strains as well as the marketed probiotic species used for comparison. Cell wall containing LPXTG motifs were found in different *Lactobacillus* and *Bifidobacterium* spp. that constitute the VSL#3 marketed probiotics and considered as important players in adherence and interaction with the host [45]. S-layer proteins were found in *L. helveticus* DSM 20075, *L. plantarum* ATCC 14917, and *L. acidophilus* ATCC 4356. These proteins played a role in T cell functioning and signaling dendritic cells [64, 65]. Genes encoding fibronectin binding proteins and collagen adhesins were found in some of the probiotic strains. These proteins were identified to play potential role in host adherence of *L. plantarum* BP06, *L. acidophilus* BA05, and *B. animalis* BL03 [66].

Four WxL domain surface proteins were encoded in *L. rhamnosus* ATCC7469 and showed similarity to those encoded in the well-studied *L. rhamnosus* GG. The WxL domains are cell wall-associated proteins and recognized to play a role in cell wall binding in *E. faecalis* [67]. Glyceraldehyde-3-phosphate dehydrogenase identified in *L. plantarum* DSM 20021 was also found in *L. plantarum* LA 318 and proved to mediate high adhesion to human colonic mucin [68]. The whole genome of *L. acidophilus* ATCC 4356 was rich in mucus-binding proteins. Similarly, putative factors adhering to mucus were identified in *L. acidophilus* NCFM [69]. Besides the key role of adherence factors in host colonization and interaction, they might modulate the host immune system. Our data showed presence of numerous cell-surface components such as sortase-dependent proteins. A study conducted by Call et al. [70] demonstrated that *L. acidophilus* sortase gene (*srtA*) mutant induced lower quantities of IL-12 and TNF-α from the murine DC cells compared to the parent strain. Furthermore, bacterial cell wall elasticity in *Lactobacillus* and *Bifidobacterium* strains proved to induce

immuno-modulatory cytokines (e.g., IL-12 and INF-Y) as well as nitric oxide production in macrophages but the induction increased with mounting cell wall elasticity [71]. Overall, scientific-driven evidences supported the provisional role of probiotics in influencing innate immune responses, reducing immunity-related metabolic conditions such as obesity [29].

In conclusion, six probiotics belonging to the genera, *Lactobacillus* and *Bifidobacterium*, exhibit probiotic traits as summarized in Fig. 1. The strains were found to have very low cytotoxicity and a strong in vitro antiviral activity against two avian viruses; NDV and IBDV which encourage further in vivo studies to expand their applicability. Furthermore, the whole genomes of the probiotic strains were found to be rich in adherence and interaction factors which indicate their well-colonization and host interaction capabilities. These findings indicate the tested probiotic strains to be good candidates in therapeutic purposes and their beneficial use in further in vivo investigations.

Probiotics and personalized, preventive, and predictive medicine

The interest in personalized medicine is on the rise because there is a need for the patient phenotypic characteristics to determine the right medication at the right dose in an attempt to maximize the treatment efficacy [72]. Probiotics could be exploited in personalized medicine through the development of probiotic supplements according to the patients' metabolic patterns which vary by lifestyle, diet, environment, and individual genes in each patient. Being a part of the healthy gut microbiome, probiotics could be used for the development of personalized treatment against metabolic syndrome disease(s) which is associated with altered microbiome-host interaction. Additionally, microbiome analysis of the gut microbiota in individuals could be used for predictive diagnosis of metabolic, infectious, or inflammatory diseases provided that high-throughput imaging techniques are implemented. Probiotics could act as the biomarkers for human diseases associated with dysbiosis of the gut microbiota composition and dynamics. However, this will depend on the cost of microbiome sequencing and the availability of the technology throughout the clinical labs. Moreover, the exploitation of probiotics in treatment as alternative to antimicrobial drugs against multi-drug resistance bacterial pathogens also showed promising findings [73]. Furthermore, the role of probiotics in preventive medicine has a growing interest and there are accumulative studies that emphasize their role in improving human health [24]. For example, evidences for their ability in prevention and treatment of urinary tract infections [74], and candida vaginitis [75], are promising. Probiotics could be useful in prevention of dental caries, respiratory tract infections, inflammatory bowel disease, and necrotizing enterocolitis [25]. Additional health benefits were reported to improve growth in healthy and malnourished persons. However, the health-promoting effects are dependent on strain type, dosing regimen, and patient's individual responses.

Findings from the current study support the feasibility of using such probiotic strains in the treatment of microbial infections. Data provided in this work, in addition to our previous study [34], indicate that oral administration of these strains in mice or poultry might protect the corresponding host against bacterial or viral pathogens throughout the antagonistic properties and the adherence capabilities to host tissues. The latter provides colonization resistance and competitive

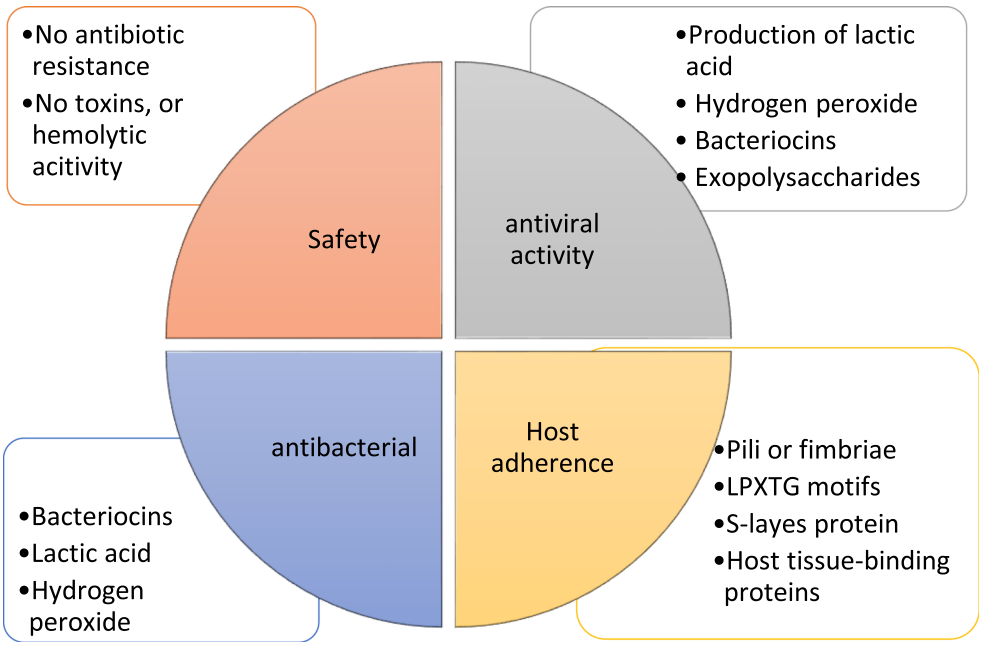

Fig. 1 Summary of the potential probiotic mechanisms attributed to the studied *Lactobacillus* and *Bifidobacterium* strains

exclusiveness against invading pathogens. Based on currently available literature, taxonomically similar strains to those used in this study were effective in treatment of numerous pathological conditions in clinical trials [32]. Similar benefits could be ascribed to our strains and these encourage further investigations to validate such aspects.

Limitations

Although evidence for the health-promoting functions of probiotics used in this study accumulates, further investigations to emphasize their safety, viability, and efficacy in animal and clinical settings are needed. In addition, optimizing preparation methods (e.g., traditional vs. encapsulation) of probiotics is worth the consideration. Strain-specific properties should be considered when selecting strains for individualized treatments. Despite the growing evidence about effective use of probiotics in treatment of human diseases, legislative rules complicate their way toward approval and commercialization. However, the advancement of current high-throughput technologies could facilitate probiotics' full characterization and shorten the time needed to gather information about their implications in human health.

Recommendations

- Evidence-based knowledge of the role of probiotics in preventive and personalized medicine should be considered with caution because the beneficial effect is strain-dependent.
- Whole genome sequencing of probiotic strains provide privileges to identify, throughout genome mining tools, the safety-related genes, probiotics features of the strains, and their ability to interact with the host. This enables reasonable expectations about the potential probiotic strains considered for animal studies or clinical trials. It also allows the comparison with commercially available strains whose genomes are fully characterized.
- Screening for probiotic properties under in vitro conditions narrow down the strains that could be exploited for further in vivo experiments.
- Probiotics that showed promising results under in vitro and in vivo experiments will be good candidates for clinical trials.
- Evidence-based knowledge about the health-promoting probiotic strains could be gained first through laboratory validation followed by in vivo experiments using animal models.
- Data regarding the significant role of probiotics in preventive medicine and treatment of several human diseases are accumulating; however, the contribution to personalized medicine needs addition investigations.
- Metabolic profiling of probiotics individually and within a community of intestinal microbiota is crucial for development and interpretation of biomarkers required for disease diagnostics.
- Translating the obtained data in the current work into provisional application of the probiotic strains in preventing diseases and design of personalized diets for promoting health is an interesting area of further research.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Consent for publication Not applicable.

Ethical approval This article does not have any studies with animals or patients performed by any of the authors

References

1. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–14.
2. Ljungh A, Wadström T. Lactic acid bacteria as probiotics. Curr Issues Intest Microbiol. 2006;7:73–89.
3. Amalaradjou MAR, Bhunia AK. Modern approaches in probiotics research to control foodborne pathogens. Adv Food Nutr Res. 2012;67:185–239.
4. Ohland CL, Macnaughton WK. Probiotic bacteria and intestinal epithelial barrier function. Am J Physiol Liver Physiol. 2010;298: G807.
5. Sharma M, Devi M. Probiotics: a comprehensive approach toward health foods. Crit Rev Food Sci Nutr. 2014;54:537–52.
6. Rolfe RD. The role of probiotic cultures in the control of gastrointestinal health. J Nutr. 2000;130:396S–402S.
7. Guandalini S. Probiotics for prevention and treatment of diarrhea. J Clin Gastroenterol. 2011;45:S149–53.
8. Hilton E, Kolakowski P, Singer C, Smith M. Efficacy of *Lactobacillus* GG as a diarrheal preventive in travelers. J Travel Med. 1997;4:41–3.
9. Lee DK, Park JE, Kim MJ, Seo JG, Lee JH, Ha NJ. Probiotic bacteria, *B.longum* and *L.acidophilus* inhibit infection by rotavirus in vitro and decrease the duration of diarrhea in pediatric patients. Clin Res Hepatol Gastroenterol. 2015;39:237–44.
10. Surawicz CM. Role of probiotics in antibiotic-associated diarrhea, *Clostridium difficile*-associated diarrhea, and recurrent *Clostridium difficile*-associated diarrhea. J Clin Gastroenterol. 2008;42(Suppl 2):S64–70.
11. Zhang G-Q, Hu H-J, Liu C-Y, Zhang Q, Shakya S, Li Z-Y. Probiotics for prevention of atopy and food hypersensitivity in early childhood. Medicine (Baltimore). 2016;95:e2562.
12. Sung V, D'Amico F, Cabana MD, Chau K, Koren G, Savino F, et al. Lactobacillus reuteri to treat infant colic: a meta-analysis. Pediatrics. 2018;141:e20171811.

13. Reid G, Abrahamsson T, Bailey M, Bindels LB, Bubnov R, Ganguli K, et al. How do probiotics and prebiotics function at distant sites? Benefic Microbes. 2017;8:521–33.
14. Zhang J-W, Du P, Yang B-R, Gao J, Fang W-J, Ying C-M. Preoperative probiotics decrease postoperative infectious complications of colorectal Cancer. Am J Med Sci. 2012;343:199–205.
15. Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, Karlsson PC, et al. Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. Am J Clin Nutr. 2007;85:488–96.
16. Petricevic L, Unger FM, Viernstein H, Kiss H. Randomized, double-blind, placebo-controlled study of oral lactobacilli to improve the vaginal flora of postmenopausal women. Eur J Obstet Gynecol Reprod Biol. 2008;141:54–7.
17. Mastromarino P, Macchia S, Meggiorini L, Trinchieri V, Mosca L, Perluigi M, et al. Effectiveness of Lactobacillus-containing vaginal tablets in the treatment of symptomatic bacterial vaginosis. Clin Microbiol Infect. 2009;15:67–74.
18. Ataie-Jafari A, Larijani B, Alavi Majd H, Tahbaz F. Cholesterol-lowering effect of probiotic yogurt in comparison with ordinary yogurt in mildly to moderately hypercholesterolemic subjects. Ann Nutr Metab. 2009;54:22–7.
19. Sheu BS, Wu JJ, Lo CY, Wu HW, Chen JH, Lin YS, et al. Impact of supplement with *Lactobacillus*- and *Bifidobacterium*-containing yogurt on triple therapy for *Helicobacter pylori* eradication. Aliment Pharmacol Ther. 2002;16:1669–75.
20. Campieri C, Campieri M, Bertuzzi V, Swennen E, Matteuzzi D, Stefoni S, et al. Reduction of oxaluria after an oral course of lactic acid bacteria at high concentration. Kidney Int. 2001;60:1097–105.
21. Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, et al. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther. 1999;13:1103–8.
22. Del Piano M, Carmagnola S, Andorno S, Pagliarulo M, Tari R, Mogna L, et al. Evaluation of the intestinal colonization by microencapsulated probiotic bacteria in comparison with the same uncoated strains. J Clin Gastroenterol. 2010;44:S42–6.
23. Dinleyici EC, Dalgic N, Guven S, Ozen M, Kara A, Arica V, et al. The effect of a multispecies synbiotic mixture on the duration of diarrhea and length of hospital stay in children with acute diarrhea in Turkey: single blinded randomized study. Eur J Pediatr. 2013;172:459–64.
24. Iannitti T, Palmieri B. Therapeutical use of probiotic formulations in clinical practice. Clin Nutr. 2010;29:701–25.
25. Minocha A. Probiotics for preventive health. Nutr Clin Pract. 2009;24:227–41.
26. Thomas LV, Ockhuizen T, Suzuki K. Exploring the influence of the gut microbiota and probiotics on health: a symposium report. Br J Nutr. 2014;112:S1–S18.
27. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Ther Adv Gastroenterol. 2013;6:39–51.
28. Shapiro H, Suez J, Elinav E. Personalized microbiome-based approaches to metabolic syndrome management and prevention. J Diabetes. 2017;9:226–36.
29. Bubnov RV, Spivak MY, Lazarenko LM, Bomba A, Boyko NV. Probiotics and immunity: provisional role for personalized diets and disease prevention. EPMA J. 2015;6:14.
30. Bubnov RV, Babenko LP, Lazarenko LM, Mokrozub VV, Demchenko OA, Nechypurenko OV, et al. Comparative study of probiotic effects of *Lactobacillus* and *Bifidobacteria* strains on cholesterol levels, liver morphology and the gut microbiota in obese mice. EPMA J. 2017;8:357–76.
31. Bubnov RV, Babenko LP, Lazarenko LM, Mokrozub VV, Spivak MY. Specific properties of probiotic strains: relevance and benefits for the host. EPMA J. 2018;9:205–23.
32. Di Cerbo A, Palmieri B, Aponte M, Morales-Medina JC, Iannitti T. Mechanisms and therapeutic effectiveness of lactobacilli. J Clin Pathol. 2016;69:187–203.
33. Sanders ME, Akkermans LMA, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, et al. Safety assessment of probiotics for human use. Gut Microbes. 2010;1:164–85.
34. Abdelhamid AG, Esaam A, Hazaa MM. Cell free preparations of probiotics exerted antibacterial and antibiofilm activities against multidrug resistant *E. coli*. Saudi Pharm J. 2018;26:603–7.
35. Kim MJ, Lee DK, Park JE, Park IH, Seo JG, Ha NJ. Antiviral activity of *Bifidobacterium adolescentis* SPM1605 against Coxsackievirus B3. Biotechnol Biotechnol Equip. 2014;28:681–8.
36. Lehtoranta L, Pitkäranta A, Korpela R. Probiotics in respiratory virus infections. Eur J Clin Microbiol Infect Dis. 2014;33:1289–302.
37. Lee DK, Kang JY, Shin HS, Park IH, Ha NJ. Antiviral activity of *Bifidobacterium adolescentis* SPM0212 against Hepatitis B virus. Arch Pharm Res. 2013;36:1525–32.
38. Cha M-K, Lee D-K, An H-M, Lee S-W, Shin S-H, Kwon J-H, et al. Antiviral activity of *Bifidobacterium adolescentis* SPM1005-A on human papillomavirus type 16. BMC Med. 2012;10:72.
39. Kwak M-K, Liu R, Kwon J-O, Kim M-K, Kim AH, Kang S-O. Cyclic dipeptides from lactic acid bacteria inhibit proliferation of the influenza a virus. J Microbiol. 2013;51:836–43.
40. Patel AR, Shah NP, Prajapati JB. 2014. Immunomodulatory effects of probiotics in the treatment of human immunodeficiency virus (HIV) infection. Biomed Prev Nutr. 2014;4:81–4.
41. An HM, Lee DK, Kim JR, Lee SW, Cha MK, Lee KO, et al. Antiviral activity of *bifidobacterium adolescentis* SPM 0214 against herpes simplex virus type 1. Arch Pharm Res. 2012;35:1665–71.
42. Garcia SC, Navarro Lopez R, Morales R, Olvera MA, Marquez MA, Merino R, et al. Molecular epidemiology of Newcastle disease in Mexico and the potential spillover of viruses from poultry into wild bird species. Appl Environ Microbiol. 2013;79:4985–92.
43. Berg TP, Gonze M, Meulemans G. Acute infectious bursal disease in poultry: isolation and characterisation of a highly virulent strain. Avian Pathol. 1991;20:133–43.
44. Razmyar J, Peighambari SM. Isolation and characterization of a very virulent infectious bursal disease virus from Turkey. Acta Virol. 2009;53:271–6.
45. Douillard FP, Mora D, Eijlander RT, Wels M, de Vos WM. Comparative genomic analysis of the multispecies probiotic-marketed product VSL#3. PLoS One. 2018;13:e0192452.
46. Kapse NG, Engineer AS, Gowdaman V, Wagh S, Dhakephalkar PK. Functional annotation of the genome unravels probiotic potential of *Bacillus coagulans* HS243. Genomics; 2018. https://doi.org/10.1016/j.ygeno.2018.05.022.
47. Guo L, Li T, Tang Y, Yang L, Huo G. Probiotic properties of *Enterococcus* strains isolated from traditional naturally fermented cream in China. Microb Biotechnol. 2016;9:737–45.
48. Bayoumi MA, Griffiths MW. In vitro inhibition of expression of virulence genes responsible for colonization and systemic spread of enteric pathogens using *Bifidobacterium bifidum* secreted molecules. Int J Food Microbiol. 2012;156:255–63.
49. Skehan P, Storeng R, Scudiero D, Monks A, Mcmahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990;82:1107–12.
50. Freshney IR. Culture of animal cell; a manual of basic technique and specialized applicaitons, vol. 1. Hoboken: Wiley; 2010. p. 676.
51. Raheel R, Ashraf M, Ejaz S, Javeed A, Altaf I. Assessment of the cytotoxic and anti-viral potential of aqueous extracts from different

parts of *Acacia nilotica* (Linn) Delile against Peste des petits ruminants virus. Environ Toxicol Pharmacol. 2013;35:72–81.
52. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Epidemiol. 1938;27:493–7.
53. Sánchez B, Bressollier P, Urdaci MC. Exported proteins in probiotic bacteria: adhesion to intestinal surfaces, host immunomodulation and molecular cross-talking with the host. FEMS Immunol Med Microbiol. 2008;54:1–17.
54. Kang JY, Lee DK, Ha NJ, Shin HS. Antiviral effects of *Lactobacillus ruminis* SPM0211 and *Bifidobacterium longum* SPM1205 and SPM1206 on rotavirus-infected Caco-2 cells and a neonatal mouse model. J Microbiol. 2015;53:796–803.
55. Al Kassaa I, Hober D, Hamze M, Chihib NE, Drider D. Antiviral potential of lactic acid Bacteria and their Bacteriocins. Probiotics Antimicrob Proteins. 2014;6:177–85.
56. Maeda N, Nakamura R, Hirose Y, Murosaki S, Yamamoto Y, Kase T, et al. Oral administration of heat-killed *Lactobacillus plantarum* L-137 enhances protection against influenza virus infection by stimulation of type I interferon production in mice. Int Immunopharmacol. 2009;9:1122–5.
57. Guillemard E, Tondu F, Lacoin F, Schrezenmeir J. Consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-114001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial. Br J Nutr. 2010;103:58–68.
58. Kanmani P, Albarracin L, Kobayashi H, Hebert EM, Saavedra L, Komatsu R, et al. Genomic characterization of *Lactobacillus delbrueckii* TUA4408L and evaluation of the antiviral activities of its extracellular polysaccharides in porcine intestinal epithelial cells. Front Immunol. 2018;9:2178.
59. Wang Z, Zhang P, Fu W, Zhang Y, Li T, Pan B, et al. Effect of probiotics on Newcastle disease virus. Wei Sheng Wu Xue Bao. 2010;50:1664–9.
60. Conti C, Malacrino C, Mastromarino P. Inhibition of herpes simplex virus type 2 by vaginal lactobacilli. J Physiol Pharmacol. 2009;60(Suppl 6):19–26.
61. Mastromarino P, Cacciotti F, Masci A, Mosca L. Antiviral activity of *Lactobacillus brevis* towards herpes simplex virus type 2: role of cell wall associated components. Anaerobe. 2011;17:334–6.
62. O'Connell Motherway M, Zomer A, Leahy SC, Reunanen J, Bottacini F, Claesson MJ, et al. 2011. Functional genome analysis of *Bifidobacterium breve* UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. Proc Natl Acad Sci. 2011;108:11217–22.
63. Turroni F, Serafini F, Foroni E, Duranti S, O'Connell Motherway M, Taverniti V, et al. 2013. Role of sortase-dependent pili of *Bifidobacterium bifidum* PRL2010 in modulating bacterium-host interactions. Proc Natl Acad Sci. 2013;110:11151–6.
64. Konstantinov SR, Smidt H, de Vos WM, Bruijns SCM, Singh SK, Valence F, et al. 2008. S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. Proc Natl Acad Sci. 2008;105:19474–9.
65. Hynönen U, Palva A. 2013. *Lactobacillus* surface layer proteins: structure, function and applications. Appl Microbiol Biotechnol. 2013;97:5225–43.
66. Sengupta R, Altermann E, Anderson RC, McNabb WC, Moughan PJ, Roy NC. The role of cell surface architecture of *Lactobacilli* in host-microbe interactions in the gastrointestinal tract. Mediat Inflamm. 2013;2013:1–16.
67. Brinster S, Furlan S, Serror P. C-terminal WxL domain mediates cell wall binding in *Enterococcus faecalis* and other gram-positive bacteria. J Bacteriol. 2007;189:1244–53.
68. Kinoshita H, Uchida H, Kawai Y, Kawasaki T, Wakahara N, Matsuo H, et al. Cell surface *Lactobacillus plantarum* LA 318 glyceraldehyde-3-phosphate dehydrogenase (GAPDH) adheres to human colonic mucin. J Appl Microbiol. 2008;104:1667–74.
69. Buck BL, Altermann E, Svingerud T, Klaenhammer TR. Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. Appl Environ Microbiol. 2005;71:8344–51.
70. Call EK, Goh YJ, Selle K, Klaenhammer TR, O'Flaherty S. Sortase-deficient lactobacilli: effect on immunomodulation and gut retention. Microbiology. 2015;161:311–21.
71. Mokrozub VV, Lazarenko LM, Sichel LM, Babenko LP, Lytvyn PM, Demchenko OM, et al. The role of beneficial bacteria wall elasticity in regulating innate immune response. EPMA J. 2015;6:13.
72. Golubnitschaja O, Baban B, Boniolo G, Wang W, Bubnov R, Kapalla M, et al. Medicine in the early twenty-first century: paradigm and anticipation - EPMA position paper 2016. EPMA J. 2016;7:23.
73. Pamer EG. Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. Science. 2016;352:535–8.
74. Darouiche RO, Thornby JI, Stewart CC, Donovan WH, Hull RA. Bacterial interference for prevention of urinary tract infection: a prospective, randomized, placebo-controlled, double-blind pilot trial. Clin Infect Dis. 2005;41:1531–4.
75. Hilton E. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for *Candidal vaginitis*. Ann Intern Med. 1992;116:353–7.