Chapter 8
Probiotic Dairy Products: Inventions Toward Ultramodern Production

Spiros Paramithiotis and Eleftherios H. Drosinos

Abstract Application of the latest approaches and protocols in probiotic research has resulted in significant advances over the last decade. These refer almost exclusively to the design of probiotic dairy products, mainly through the design of the probiotic culture incorporated. Several protocols have been developed for the assessment of probiotic potential through omic approaches, and many more are currently under development. In addition, through the improvement of our knowledge regarding the mechanisms that lead to infections and disorders, the genetic engineering of probiotic strains aiming at the delivery of bioactive molecules to specific cites was made possible. All these indicate that we are entering an exciting new era with great expectations.

Keywords Probiotics · Starter culture · Selection · Omics · Genetic engineering

Introduction

The probiotic concept is one of the favorites among the researchers due to the width of the topic and the importance for health and well-being. Dairy products are the main vehicle for probiotic delivery worldwide, with several other products considered for this scope including fermented meat products, fruits, and vegetables (Montoro et al. 2016; Park and Jeong 2016; Neffe-Skocinska et al. 2017). A large number of these are already available in the market.

Recent advances, especially in the field of molecular biology, have allowed several improvements to take place. From a production point of view, innovations have occurred in the field of design of a probiotic product. Manufacture of a probiotic product essentially involves three steps: (a) selection of the starter culture, which is

S. Paramithiotis (✉) · E. H. Drosinos
Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
e-mail: sdp@aua.gr

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based on the ability to reach a specific niche within the gastrointestinal track of the host, colonize it, and confer the probiotic action; (b) technological evaluation of the starter culture, which is based on the ability of the starter culture to propagate at industrial level and retain viability and functionality after a series of processing steps; and (c) incorporation into the product, a probiotic culture may be incorporated as a starter or an adjunct culture.

Research in recent years has focused on the design of starter cultures. This can take place either by evaluating the potential of wild isolates or by engineering heavily studied strains. In the first case, the genetic background of many desired properties has been identified allowing the evaluation of the probiotic potential to take place through genetic determinants. On the other hand, improvements in the understanding of the mechanisms leading to infections and disorders have allowed the genetic engineering of probiotic strains for the delivery of specific bioactive molecules with prophylactic and/or therapeutic function. In the succeeding sections, recent advances in the field of probiotic product design are presented and critically discussed.

Probiotic Culture Selection

Culture selection involves the assessment of a culture's safety, efficiency to reach the colonization site, and probiotic potential, considering the possible health benefits exerted to the consumer. These functions are currently predicted almost exclusively through in vitro tests. Recent advances in the field of molecular biology have allowed the prediction of these properties through the application of omic approaches.

Safety was traditionally assessed through hemolytic activity, antibiotic resistance, as well as production of enzymes (e.g., hyaluronidase, gelatinase), toxins (e.g., cytolysin), and biogenic amines. In the latter years, safety assessment is usually performed through the detection of the respective genes (e.g., *cylA/B* encoding cytolysins, *hyl* encoding hyaluronidase, *gelE* encoding gelatinase, *tet*(M), *tet*(K), and *tet*(W) responsible for tetracycline resistance, etc.) along with several virulence-associated ones. Especially regarding the latter, detection of *agg* (aggregation protein), *esp* (enterococcal surface protein), *asa1* (aggregation substance), *ace* (collagen protein adhesin), and $efaA_{fs}$ (cell wall adhesin) is more often (Perumal and Venkatesan 2017; Hwanhlem et al. 2017; Motahari et al. 2017; Ojekunle et al. 2017; Guo et al. 2017; Rzepkowska et al. in press).

Efficiency to reach the colonization site is predicted through the ability to survive the stresses faced within the host and colonization potential. In the case of the former, ability to maintain high populations after exposure to pH values ranging from one to three in the presence of pepsin (simulating gastric juice conditions) and alkaline pH values in the presence of bile salts and pancreatin (simulating intestinal juice conditions) is the criterion applied more often. Colonization potential is predicted mostly through the assessment of cell surface hydrophobicity, cellular

autoaggregation ability, binding to solubilized collagen, human or animal mucus, and adhesion to various cell lines, mostly Caco-2 and HT29. The disadvantages of in vitro testing were addressed with the use of animal models. However, in that case, other restrictions are introduced, including ethical ones, depending on the type of animal model (Yadav et al. 2017). Although the genome of several lactic acid bacteria species has been described, markers of probiotic features have been detected (Abriouel et al. 2017), and survival mechanisms and strategies to adhere to surfaces have been described (Bove et al. 2012; Arena et al. 2017), no omic approach has been utilized yet as an indicator. However, prediction through in silico models has been performed (Lee et al. 2000).

The desired functional properties of a probiotic culture are constantly updated; they include antagonistic activity against potentially pathogenic microorganisms, particularly invasive Gram-negative pathogens, as well as a series of assets that are beneficial to the host. The latter may either have prophylactic or therapeutic character (Varankovich et al. 2015). A wealth of literature is currently available on that subject and includes both tentative and demonstrated positive effects. In general, the health benefits that have been claimed include modulation of immune responses, protection of the function of the mucosal barrier, reduction of cholesterol levels, anticancer activity, as well as activity against gastrointestinal diseases.

The modulation of immune responses by probiotic cultures refers to the ability of the host to distinguish between the beneficial and pathogenic microorganisms through immune tolerance/hyporesponsiveness and humoral and cell-mediated immune mechanisms, respectively. The mechanisms through which the selective response is activated have been critically presented and discussed by Hardy et al. (2013). The term intestinal or mucosal barrier refers to the physical and immunological barrier that separates the luminal contents and the interstitial tissue and prevents the diffusion of factors that may affect negatively the host (Hardy et al. 2013; Rao and Samak 2013). The epithelial monolayer constitutes the physical barrier, while mucus, protease-resistant IgA, and antimicrobial peptides constitute the immunological. Both barriers are positively affected by the function of probiotics. More accurately, probiotics have been reported to upregulate the expression of the epithelial growth factor (EGF-R) and the pattern recognition receptor (TLR-2) (Resta-Lenert and Barrett 2003; Cario et al. 2004) along with the production of MUC2 and MUC3 intestinal mucins, TGFβ, IL-6 and IL-10 (Rodrigues et al. 2000; Rautava et al. 2006; He et al. 2007; Shang et al. 2008). These functions may be assessed through a variety of phenotypic assays that have been recently reviewed by Papadimitriou et al. (2015).

Interestingly, the interaction with the host may take place even in a probiotic culture viability-independent manner through the activation of responses upon recognition of specific bacterial components or metabolites (Adams 2010). Indeed, inactivated whole cells of *Lactobacillus casei* strain Shirota upregulated IL-12, IL-10, and IL-2 and inhibited the production of IgE, IgG1, IL-4, IL-5, IL-6, and IL-13, while TNFα and TNFγ provided with a mixed response (Matsuzaki and Chin 2000; Cross et al. 2004; Lim et al. 2009). There is currently a wealth of literature on the effect of inactivated whole cells, cell wall components, lipoteichoic acids, and

even genomic DNA of a wide range of probiotic and potential probiotic cultures on the immune response of mouse and human cells, cell lines, and macrophages as measured by indicators such as interleukins (such as IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13), factors (such as TGF-β andTNF-α), and immunoglobulins (such as IgA, IgE, and IgG1) (Lammers et al. 2003; Matsuguchi et al. 2003; Shida et al. 2006; Mastrangeli et al. 2009; Kaji et al. 2010; Jensen et al. 2010; van Hoffen et al. 2010). These studies and many more along with a discussion on the possible mode of action have been comprehensively reviewed by Taverniti and Guglielmetti (2011). These functions indicate the efficacy of ghost probiotics and highlight the need for reassessment of the whole probiotic concept, including nomenclature.

Anticancer activity has been attributed to a series of actions including decomposition of carcinogenic compounds, production of compounds with anticarcinogenic activity, inhibition of cancer cell proliferation, and induction of apoptosis as well as modification of the composition and metabolic activity of intestinal microbiota.

Prevention of colorectal cancer (CRC) through probiotics has been extensively studied (dos Reis et al. 2017). Each of the aforementioned parameters plays its own role in the decrease of the colorectal cancer risk. Maintenance of a healthy intestinal microbiota reduces the risk of CRC both directly and through immumodulation. Moreover, reduction of β-glucuronidase and nitrate reductase activities by the intestinal biota has been proposed as a possible mechanism for the reduction of the production of metabolites that have been associated with the development of CRC (Hatakka et al. 2008; Mohania et al. 2013; Verma and Shukla 2013; Zhu et al. 2013). Increase in the production of anti-inflammatory cytokines, with a parallel decrease in the production of pro-inflammatory ones (Koboziev et al. 2013; Zhu et al. 2013) along with the production of short-chain fatty acids and conjugated linoleic acid, as well as compounds with established carcinogenic activity from probiotics or potential probiotic bacteria have been shown to reduce colorectal cancer risk (Ewaschuk et al. 2006; Hosseini et al. 2011; Bassaganya-Riera et al. 2012; Kumar et al. 2012; Vipperla and O'Keefe 2012; Serban 2014).

The use of probiotics has been proved beneficial against a series of gastrointestinal tract (GIT) disorders including infectious, antibiotic-associated, and travelers'diarrhea (Sullivan and Nord 2005; McFarland 2007; Preidis et al. 2011; Girardin and Seidman 2011; Maziade et al. 2013; Patro-Golab et al. 2015; Szajewska and Kolodziej 2015a, b; Lau and Chamberlain 2016), irritable bowel syndrome (O'Mahony et al. 2005; Whorwell et al. 2006; Lorenzo-Zuniga et al. 2014; Yoon et al. 2014), pouchitis (Turroni et al. 2010; Shen et al. 2014; Tomasz et al. 2014), and *H. pylori* infection (Mukai et al. 2002; Tong et al. 2007; Dore et al. 2015; Holz et al. 2015; Szajewska et al. 2015). Specific effects on each of the aforementioned along with the respective mode of action have been recently presented by Domingo (2017).

Cholesterol-reducing capacity of probiotics has been repeatedly exhibited and recently reviewed by Ishimwe et al. (2015). A series of mechanisms have been proposed including enzymatic deconjugation of bile, coprecipitation of cholesterol with deconjugated bile, binding to probiotic cell surface, and conversion to coprostanolin that is excreted in feces (Daliri and Lee 2015).

The above mentioned functions have been studied in depth and known to influence the choice of probiotic cultures. Despite the fact that several mechanisms of action have been described, no omic approach has been yet applied for their prediction.

Technological Evaluation of Probiotic Cultures

Evaluation of a strain's capacity from a technological point of view is very often neglected. However, the ability to reach high population during industrial production scale, capacity to withstand processing such as drying or freezing, and the ability to remain viable and retain functionality during food processing and storage are crucial and strain-dependent properties.

Industrial-scale biomass production takes place in bioreactors whose capacity may reach several hundred liters. Specific attention should be paid to the nutrient content, pH value, dissolved oxygen, and temperature that very often compromise scale-up of biomass production.

Preservation of Probiotic Cultures

Freezing or drying is the processes most commonly applied for the preservation of probiotic cultures. Regarding the former, the rate of temperature decrease is the most crucial factor. High rate creates small ice crystals evenly distributed that minimizes the damage caused by mechanical or osmotic stresses during both freezing and thawing. However, drying is the most commonly process of choice for culture preservation because it may facilitate stability and shelf-life of the culture and on the other hand reduce the logistics costs. Among the drying techniques, spray-drying is most commonly applied in the case of dairy products (Huang et al. 2017). The factors that affect culture viability include the inlet temperature that may be as high as 200 °C (Silva et al. 2002, 2005), the dehydration itself, and the subsequent storage conditions. The strategy most commonly applied to improve viability is the use of appropriate growth conditions before drying and the addition of protective molecules, such as skim milk, polydextrose, inulin, etc. These factors along with many more have been critically discussed by Silva et al. (2011). In general, resistance to the stresses inflicted by spray-drying, namely, heat and osmotic, seems to be a strain-dependent property. However, there are reports stating that *Propionibacterium* spp. are usually more resistant than *Bifidobacterium* spp., *Lactobacillus* spp., and *Lactococcus* spp. (Schuck et al. 2013; Huang et al. 2016), *Streptococcus* spp. than *Lactobacillus* spp. (Bielecka and Majkowska 2000; Kumar and Mishra 2004; Wang et al. 2004), and *Bifidobacterium longum* than *B. infantis* (Lian et al. 2002). Omic approaches for the prediction of tolerance are possible to occur in the near future since a series of genes involved in the adaptation to these

conditions, such as the *clp* and *opu* genes, have been described, and their effect on survival is already known (Zotta et al. 2017).

Survival of Probiotic Cultures During Storage

Another aspect that has been extensively studied is the survival of the probiotic cultures during storage of the product in which they have been incorporated as a starter or an adjunct culture. It has been reported that factors such as pH value, concentration of organic acids, type and concentration of other ingredients, and storage temperatures may significantly affect the viability of the probiotic cultures (Donkor et al. 2007). Viability during storage of dairy products has been given much consideration. Enrichment of yoghurt with whey proteins (Marafon et al. 2011), flavoring agents (Vinderola et al. 2002), fruit pulps (Kailasapathy et al. 2008; El-Nagga and Abd El–tawab 2012), cereals (Coda et al. 2012; Zare et al. 2012), lactulose (Oliveira et al. 2011), or inulin (Bozanic et al. 2001; Donkor et al. 2007) either had no negative effect or resulted in the enhancement of the survival of the respective probiotic culture that has been incorporated. However, there are also several reports in the literature that claim the exact opposite, i.e., the decrease of the viability (Ranadheera et al. 2012; Bedani et al. 2014) leading to the conclusion that this property is strain-dependent. The viability during production and storage of a variety of cheeses including Feta (Mazinani et al. 2016), soft goat (Radulovic et al. in press), Italico (Blaiotta et al. 2017), Pecorino Siciliano (Pino et al. 2017), white brined (Liu et al. 2017), Minas (Buriti et al. 2007), and Cheddar (Phillips et al. 2006) has also been assessed confirming that cheese is the best product for probiotic delivery and leading to the basic conclusion that this property is strain-dependent. However, due to the complexity of the microecosystem, and the number of genes involved, an omic approach to predict such a virtue is not expected to occur soon.

Genetic Engineering of Probiotic Strains

An alternative approach to the selection procedure is to provide the probiotic culture with the desired properties through bioengineering. These properties may extend from enhanced tolerance to the GIT or technologically relevant conditions to the improvement of the functionality within the host. The rationale behind the use of bioengineering is to address the limitations of the cultures currently characterized as probiotics or potential probiotics. However, there are certain concerns regarding the use of genetically modified organisms in general that are discussed at the end of this paragraph. In the succeeding paragraphs, the most characteristic studies involving genetic engineering of probiotics or potential probiotics are presented aiming to depict the possibilities offered by genetic engineering.

otsB, the gene encoding for trehalose-6-phosphate phosphatase originating from *Propionibacterium freudenreichii* strain B365, was expressed in *Lactococcus lactis* strain MG1363 in order to provide with trehalose synthesis capacity (Carvalho et al. 2011). Then, strains with the ability to produce trehalose exhibited improved tolerance to acid, cold and heat shocks. On the contrary, no improvement in the viability upon exposure to freeze-drying was observed. However, a nearly 100% viability after freeze-drying was reported for the same strain containing *atsBA* genes originating from *E. coli* DH5a (Termont et al. 2006). In addition, improved tolerance to gastric juice as well as resistance to bile was reported. This enhanced tolerance did not interfere with IL-10 secretion by the same strain (Steidler et al. 2000). *Lc. lactis* strain MG1363 was also used by Bermudez-Humaran et al. (2015) to construct recombinant strains able to secrete cytokines (IL-10 or TGF-β1) and serine protease inhibitors (Elafin or Secretory Leukocyte Protease Inhibitor, SLPI). Then, a DSS-induced murine colitis model (C57BL/6 mice) was used to evaluate the effect after oral administration of the recombinant strains. Significant reduction of the inflammation was observed as an effect of the serine protease inhibitors. On the contrary, only moderate anti-inflammatory effect was recorded when IL-10 or TGF-β1 expressing recombinant strains were administered. In addition, overproduction of Elafin obtained by inactivation of HtrA resulted in enhanced reduction of the inflammation indicating dose dependence.

A recombinant strain based on a *Lb. paracasei* strain able to produce *Listeria* adhesion protein was constructed by Koo et al. (2012) in order to outcompete *Listeria monocytogenes* in adhesion, transepithelial translocation, and cytotoxicity in Caco-2 cells. In addition, several wild-type bacteria with probiotic potential were also examined for the same capacity. The latter failed to prevent *L. monocytogenes* infection. On the contrary, the recombinant strain managed to reduce *L. monocytogenes* translocation by 46% after 24 h and cytotoxicity by 99.8% after 1 h.

Focareta et al. (2006) engineered *E. coli* strain DH5a to express glycosyltransferase genes from *Neisseria gonorrhoeae* and *Campylobacter jejuni*. The aim was to produce a mimic of the ganglioside GM_1 receptor and inactivate in situ the cholera toxin. Indeed, administration of the construct significantly protected 3-day-old Swiss mice against fatal challenge with *Vibrio cholerae*. Another approach was employed by Duan and March (2008, 2010). They constructed a strain based on *E. coli* Nissle 1917 able to express cholera autoinducer 1 and studied the effect on *V. cholerae* colonization and virulence gene expression. Regarding the latter, downregulation of virulence gene expression in Caco-2 cells was reported. Furthermore, pretreatment of 2–3-day-old CD-1 mice with the recombinant strain resulted in 69% reduction of the *V. cholerae* intestinal population after 40 h and 80% reduction of the cholera toxin intestinal binding after 8 h.

Volzing et al. (2013) constructed a recombinant *Lc. lactis* strain able to produce Alyteserin-1a and A3APO, two peptides with antimicrobial activity against both Gram-positive and Gram-negative species. The recombinant strains effectively inhibited growth of *E. coli* and *Salmonella* strains in vitro providing with promising results, necessitating further in situ study. *Lc. lactis* was also used to create a recombinant strain expressing Tcd-AC and Tcd-BC, two fragments of the cytotoxins

A (TdcA) and B (TcdB) produced by *Clostridium difficile* (Guo et al. 2015). Purified fragments or the recombinant strain was orally administered to 5–6-week-old pathogen-free C57BL6 mice that were subsequently challenged with *Cl. difficile*. The vaccinated mice exhibited significantly lower mortalities due to the higher IgG and IgA titers.

The construction of a recombinant *Lb. acidophilus* strain able to produce K99 fimbrial protein was reported by Chu et al. (2005). The strain effectively inhibited binding of enterotoxigenic *E. coli* to intestinal epithelium of pigs exhibiting dose-dependence. Similarly, a recombinant *Lc. lactis* strain able to produce a surface-associated flagellin after induction with nisin for 6 h was reported by Sanchez et al. (2011). The recombinant strain was able to outcompete *E. coli* and *Salmonella enterica* strains to adhesion to mucin-coated polystyrene plates.

Apart from the above mentioned approaches, probiotics have been extensively studied as a vehicle for the targeted delivery of bioactive molecules for prevention and/or treatment of various diseases. Among the most characteristic studies performed so far are the following. Ma et al. (2014) reported the construction of a recombinant *Lc. lactis* strain expressing HSP65 with tandem repeats of P277 that was able to combat the onset of diabetes mellitus type 1. Oral administration of the recombinant strain in non obese diabetic mice resulted in reduced insulitis, improved glucose tolerance, and ultimately prevented hyperglycemia.

Antitumor activity of probiotic bacteria has also been considered to some extend with promising results. The study of Wei et al. (2016) is characteristic of the potential applications. In that study, a *B. longum* strain was engineered to produce tumstatin, an effective angiostatin that inhibits proliferation and induces apoptosis of tumorous endothelial cells. The in situ effectiveness of this approach was examined after intragastric administration of the recombinant strain to tumor-bearing mice. The antitumor effects recorded were significant and very promising for further study.

Finally, Chamcha et al. (2015) reported the construction of a *Lc. lactis*-based strain able to produce HIV-1 Gag-p24 antigen. The aim was to induce HIV-specific immune responses in BALB/c mice and achieve immunity. Indeed, a strong humoral and cellular immunity against HIV was obtained through oral administration of the recombinant strain in which the antigen was expressed on the tip of Group A *Streptococcus* pilus.

The aforementioned approach, although promising, still requires the use of genetically modified organisms (GMOs) for which there are certain concerns. These concerns may result from predicted (Stemke 2004) or unpredicted functions (Hill Jr. et al. 1993). However, there are approaches that may lead to the improvement of the potential probiotic cultures without the need of genetic modification. Such an improvement may occur mainly through directed evolution and dominant selection (Derkx et al. 2014). These approaches may not lead to the development of strains with the improved or targeted health benefits described above, but may result in enhanced resistance to certain stresses and out-competition of pathogens. Such phenotypes may be obtained through adaptation to specific adverse conditions. However, adaptation is only temporary and is still under debate whether it is possible to inflict permanent changes without any change in the genetic material. Epigenetics

may provide with a solution, but further study is still necessary to detect and understand the mechanisms involved. Moreover, such an approach requires high level of expertise, time, and effort with safety assessment being still a prerequisite for industrial use.

Conclusions and Future Perspectives

A series of exciting advances in the design of probiotic products have taken place over the last decade. The majority of them refer to the selection of the most appropriate strains, regarding the desired properties or the approach used for their assessment. In parallel, strains with probiotic properties, referring at least to their ability to reach the colonization niche within the host, have been used in genetic engineering studies for targeted delivery of bioactive molecules aiming at the treatment and/or prevention of infections and diseases. The number of such studies is expected to increase within the next few years enriching the selection criteria for the characterization of potential probiotic strains. Moreover, it is very likely that meta-transcriptomic approaches will find their way into the in situ assessment of the GIT microecology and the effect of the probiotic strains.

References

Abriouel H, Perez Montoro B, Casimiro-Soriguer CS et al (2017) Insight into potential probiotic markers predicted in *Lactobacillus pentosus* MP-10 genome sequence. Front Microbiol 8:891

Adams CA (2010) The probiotic paradox: live and dead cells are biological response modifiers. Nutr Res Rev 23:37–46

Arena MP, Capozzi V, Spano G et al (2017) The potential of lactic acid bacteria to colonize biotic and abiotic surfaces and the investigation of their interactions and mechanisms. Appl Microbiol Biotechnol 101:2641–2657

Bassaganya-Riera J, Viladomiu M, Pedragosa M, Simone C, Hontecillas R (2012) Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria. PLoS ONE 7:1–8

Bedani R, Vieira ADS, Rossi EA et al (2014) Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in symbiotic soy yoghurt with okara during storage. LWT-Food Sci Technol 55:436–443

Bermudez-Humaran LG, Motta J-P, Aubry C et al (2015) Serine protease inhibitors protect better than IL-10 and TGF-β anti-inflammatory cytokines against mouse colitis when delivered by recombinant lactococci. Microb Cell Factories 14:26

Bielecka M, Majkowska A (2000) Effect of spray drying temperature of yoghurt on the survival of starter cultures, moisture content and sensoric properties of yoghurt powder. Nahrung/Food 44:257–260

Blaiotta G, Murru N, Di Cerbo A et al (2017) Commercially standardized process for probiotic "Italico" cheese production. LWT – Food Sci Technol 79:601–608

Bove P, Fiocco D, Gallone A et al (2012) Abiotic stress responses in lactic acid bacteria. In: Wong HC (ed) Stress response of foodborne microorganisms. Nova Publishers, New York, pp 355–403

Bozanic R, Rogelj I, Tratni IJ (2001) Fermented acidophilus goat's milk supplemented with inulin: comparison with cow's milk. Milchwissenschaft 56:618–622
Buriti FCA, Okazaki TY, Alegro JHA et al (2007) Effect of a probiotic mixed culture on texture profile and sensory performance of Minas fresh-cheeses in comparison with the traditional products. Arch Latinoam Nutr 57:179–185
Cario E, Gerken G, Podolsky DK (2004) Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. Gastroenterology 127:224–238
Carvalho AL, Cardoso FS, Bohn A (2011) Engineering trehalose synthesis in *Lactococcus lactis* for improved stress tolerance. Appl Environ Microbiol 77:4189–4199
Chamcha V, Jones A, Quigley BR et al (2015) Oral immunization with a recombinant *Lactococcus lactis*–expressing HIV-1 antigen on group A *Streptococcus* pilus induces strong mucosal immunity in the gut. J Immunol 195:5025–5034
Chu H, Kang S, Ha S et al (2005) *Lactobacillus acidophilus* expressing recombinant K99 adhesive fimbriae has an inhibitory effect on adhesion of enterotoxigenic *Escherichia coli*. Microbiol Immunol 49:941–948
Coda R, Laner A, Trani A et al (2012) Yogurt-like beverages made of a mixture of cereals, soy and grape must: microbiology, texture, nutritional and sensory properties. Int J Food Microbiol 155:120–127
Cross ML, Ganner A, Teilab D et al (2004) Patterns of cytokine induction by gram-positive and gram-negative probiotic bacteria. FEMS Immunol Med Microbiol 42:173–180
Daliri EB-M, Lee BH (2015) New perspectives on probiotics in health and disease. Food Sci Human Wellness 4:56–65
Derkx PMF, Janzen T, Sorensen KI et al (2014) The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology. Microb Cell Factories 13:S5
Domingo JJS (2017) Review of the role of probiotics in gastrointestinal diseases in adults. Gastroenterol Hepatol 40:417–429
Donkor ON, Tsangalis D, Shah NP (2007) Viability of probiotic bacteria and concentrations of organic acids in commercial yoghurts during refrigerated storage. Food Aust 59:121–126
Dore MP, Goni E, di Mario F (2015) Is there a role for probiotics in *Helicobacter pylori* therapy? Gastroenterol Clin N Am 44:565–575
dos Reis SA, da Conceicao LL, Siqueira NP et al (2017) Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. Nutr Res 37:1–19
Duan F, March JC (2008) Interrupting *Vibrio cholerae* infection of human epithelial cells with engineered commensal bacterial signaling. Biotechnol Bioeng 101:128–134
Duan F, March JC (2010) Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model. PNAS 107:11260–11264
El-Nagga EA, Abd El–tawab YA (2012) Compositional characteristics of date syrup extracted by different methods in some fermented dairy products. Ann Agric Sci 57:29–36
Ewaschuk JB, Walker JW, Diaz H et al (2006) Bioproduction of conjugated linoleic acid by probiotic bacteria occurs in vitro and in vivo in mice. J Nutr 136:1483–1487
Focareta A, Paton JC, Morona R et al (2006) A recombinant probiotic for treatment and prevention of cholera. Gastroenterology 130:1688–1695
Girardin M, Seidman EG (2011) Indications for the use of probiotics in gastrointestinal diseases. Dig Dis 29:574–587
Guo S, Yan W, McDonough SP et al (2015) The recombinant *Lactococcus lactis* oral vaccine induces protection against *C. difficile* spore challenge in a mouse model. Vaccine 33:1586–1595
Guo H, Pan L, Li L et al (2017) Characterization of antibiotic resistance genes from *Lactobacillus* isolated from traditional dairy products. J Food Sci 82:724–730
Hardy H, Harris J, Lyon E et al (2013) Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Forum Nutr 5:1869–1912
Hatakka K, Holma R, El-Nezami H et al (2008) The influence of *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp. *shermanii* JS on potentially carcinogenic bacterial activity in human colon. Int J Food Microbiol 128:406–410

He B, Xu W, Santini PA et al (2007) Intestinal bacteria trigger T cell-independent immunoglobulin A2 class switching by inducing epithelial-cell secretion of the cytokine APRIL. Immunology 26:812–826

Hill RH Jr, Caudill SP, Philen RM et al (1993) Contaminants in L-tryptophan associated with eosinophilia myalgia syndrome. Arch Environ Contam Toxicol 25:134–142

Holz C, Busjahn A, Mehling H et al (2015) Significant reduction in *Helicobacter pylori* load in humans with non-viable *Lactobacillus reuteri* DSM17648: a pilot study. Probiotics Antimicrob Proteins 7:91–100

Hosseini E, Grootaert C, Verstraete W et al (2011) Propionate as a health-promoting microbial metabolite in the human gut. Nutr Rev 69:245–258

Huang S, Cauty C, Dolivet A et al (2016) Double use of highly concentrated sweet whey to improve the biomass production and viability of spray-dried probiotic bacteria. J Funct Foods 23:453–463

Huang S, Vignolles M-L, Chen XD et al (2017) Spray drying of probiotics and other food-grade bacteria: a review. Trends Food Sci Technol 63:1–17

Hwanhlem N, Ivanova T, Biscola V et al (2017) Bacteriocin producing *Enterococcus faecalis* isolated from chicken gastrointestinal tract originating from Phitsanulok, Thailand: isolation, screening, safety evaluation and probiotic properties. Food Control 78:187–195

Ishimwe N, Daliri E, Lee B et al (2015) The perspective on cholesterol-lowering mechanisms of probiotics. Mol Nutr Food Res 59:94–105

Jensen GS, Benson KF, Carter SG et al (2010) GanedenBC30 cell wall and metabolites: anti-inflammatory and immune modulating effects in vitro. BMC Immunol 11:1–15

Kailasapathy K, Harmstorf I, Phillips M (2008) Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in stirred fruit yogurts. LWT-Food Sci Technol 41:1317–1322

Kaji R, Kiyoshima-Shibata J, Nagaoka M et al (2010) Bacterial teichoic acids reverse predominant IL-12 production induced by certain lactobacillus strains into predominant IL-10 production via TLR2-dependent ERK activation in macrophages. J Immunol 184:3505–3513

Koboziev I, Webb CR, Furr KL et al (2013) Role of the enteric microbiota in intestinal homeostasis and inflammation. Free Radic Biol Med 68:122–133

Koo OK, Amalaradjou MAR, Bhunia AK (2012) Recombinant probiotic expressing Listeria adhesion protein attenuates *Listeria monocytogenes* virulence in vitro. PLoS ONE 7:e29277

Kumar P, Mishra HN (2004) Yoghurt powder-a review of process technology, storage and utilization. Food Bioprod Process 82:133–142

Kumar M, Nagpal R, Verma V et al (2012) Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutr Rev 71:23–34

Lammers KM, Brigidi P, Vitali B et al (2003) Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. FEMS Immunol Med Microbiol 38:165–172

Lau CS, Chamberlain RS (2016) Probiotics are effective at preventing *Clostridium difficile*-associated diarrhea: a systematic review and meta-analysis. Int J Gen Med 9:27–37

Lee YK, Lim CY, Teng WL et al (2000) Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. Appl Environ Microbiol 66:3692–3697

Lian WC, Hsiao HC, Chou CC (2002) Survival of bifidobacteria after spray drying. Int J Food Microbiol 74:79–86

Lim LH, Li HY, Huang CH et al (2009) The effects of heat-killed wild-type *Lactobacillus casei* Shirota on allergic immune responses in an allergy mouse model. Int Arch Allergy Immunol 148:297–304

Liu L, Li X, Zhu Y et al (2017) Effect of microencapsulation with the Maillard reaction products of whey proteins and isomaltooligosaccharide on the survival rate of *Lactobacillus rhamnosus* in white brined cheese. Food Control 79:44–49

Lorenzo-Zuniga V, Llop E, Suarez C et al (2014) I.31, a new combination of probiotics, improves irritable bowel syndrome-related quality of life. World J Gastroenterol 20:8709–8716
Ma Y, Liu J, Hou J et al (2014) Oral administration of recombinant *Lactococcus lactis* expressing HSP65 and tandemly repeated P277 reduces the incidence of type I diabetes in non-obese diabetic mice. PLoS ONE 9(8):e105701
Marafon AP, Sumi A, Alcantara MR et al (2011) Optimization of the rheological properties of probiotic yoghurts supplemented with milk proteins. LWT-Food Sci Technol 44:511–519
Mastrangeli G, Corinti S, Butteroni C et al (2009) Effects of live and inactivated VSL#3 probiotic preparations in the modulation of in vitro and in vivo allergen-induced Th2 responses. Int Arch Allergy Immunol 150:133–143
Matsuguchi T, Takagi A, Matsuzaki T et al (2003) Lipoteichoic acids from *Lactobacillus* strains elicit strong tumor necrosis factor alpha inducing activities in macrophages through toll-like receptor 2. Clin Diagn Lab Immunol 10:259–266
Matsuzaki T, Chin J (2000) Modulating immune responses with probiotic bacteria. Immunol Cell Biol 78:67–73
Maziade PJ, Andriessen JA, Pereira P et al (2013) Impact of adding prophylactic probiotics to a bundle of standard preventative measures for *Clostridium difficile* infections: enhanced and sustained decrease in the incidence and severity of infection at a community hospital. Curr Med Res Opin 29:1341–1347
Mazinani S, Fadaei V, Khosravi-Darani K (2016) Impact of *Spirulina platensis* on physicochemical properties and viability of *Lactobacillus acidophilus* of probiotic UF feta cheese. J Food Process Preserv 40:1318–1324
McFarland LV (2007) Meta-analysis of probiotics for the prevention of traveler's diarrhea. Travel Med Infect Dis 5:97–105
Mohania D, Kansal VK, Sagwal R et al (2013) Anticarcinogenic effect of probiotic Dahi and piroxicam on DMH-induced colorectal carcinogenesis in Wistar rats. Am J Cancer Ther Pharmacol 1:1–17
Montoro BP, Benomar N, Lerma LL et al (2016) Fermented Alorena table olives as a source of potential probiotic *Lactobacillus pentosus* strains. Front Microbiol 7:1583
Motahari P, Mirdamadi S, Kianirad M (2017) Safety evaluation and antimicrobial properties of *Lactobacillus pentosus* 22C isolated from traditional yogurt. Food Measure 11:972–978
Mukai T, Asasaka T, Sato E et al (2002) Inhibition of binding of *Helicobacter pylori* to the glycolipid receptors by probiotic *Lactobacillus reuteri*. FEMS Immunol Med Microbiol 32:105–110
Neffe-Skocinska K, Okon A, Kolozyn-Krajewska et al (2017) Amino acid profile and sensory characteristics of dry fermented pork loins produced with a mixture of probiotic starter cultures. J Sci Food Agric 97:2953–2960
O'Mahony L, McCarthy J, Kelly P et al (2005) *Lactobacillus* and *Bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 128:541–551
Ojekunle O, Banwo K, Sanni AI (2017) In vitro and in vivo evaluation of *Weissella cibaria* and *Lactobacillus plantarum* for their protective effect against cadmium and lead toxicities. Lett Appl Microbiol 64:379–385
Oliveira RPS, Florence ACR, Perego P et al (2011) Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. Int J Food Microbiol 145:22–27
Papadimitriou K, Zoumpopoulou G, Foligne B et al (2015) Discovering probiotic microorganisms: in vitro, in vivo, genetic and omics approaches. Front Microbiol 6:58
Park K-Y, Jeong J-K (2016) Kimchi (Korean fermented vegetables) as a probiotic food. In: Watson RR, Preedy VR (eds) Probiotic, prebiotics and synbiotics. Bioactive foods in health promotion. Academic, London, pp 391–408
Patro-Golab B, Shamir R, Szajewska H (2015) Yogurt for treating antibiotic-associated diarrhea: systematic review and meta-analysis. Nutrition 31:796–800

Perumal V, Venkatesan A (2017) Antimicrobial, cytotoxic effect and purification of bacteriocin from vancomycin susceptible *Enterococcus faecalis* and its safety evaluation for probiotization LWT – Food Sci Technol 78:303–310

Phillips M, Kailasapathy K, Tran L (2006) Viability of commercial probiotic cultures (*L. acidophilus*, *Bifidobacterium* sp., *L. casei*, *L. paracasei* and *L. rhamnosus*) in cheddar cheese. Int J Food Microbiol 108:276–280

Pino A, Van Hoorde K, Pitino I et al (2017) Survival of potential probiotic lactobacilli used as adjunct cultures on Pecorino Siciliano cheese ripening and passage through the gastrointestinal tract of healthy volunteers. Int J Food Microbiol 252:42–52

Preidis GA, Hill C, Guerrant RL et al (2011) Probiotics, enteric and diarrheal diseases, and global health. Gastroenterology 140:8–14

Radulovic Z, Miocinovic J, Mirkovic N et al (in press) Survival of spray-dried and free-cells of potential probiotic *Lactobacillus plantarum* 564 in soft goat cheese. Anim Sci J. https://doi.org/10.1111/asj.12802

Ranadheera CS, Evans CA, Adams MC et al (2012) In vitro analysis of gastrointestinal tolerance and intestinal cell adhesion of probiotics in goat's milk ice cream and yogurt. Food Res Int 49:619–625

Rao RK, Samak G (2013) Protection and restitution of gut barrier by probiotics: nutritional and clinical implications. Curr Nutr Food Sci 9:99–107

Rautava S, Arvilommi H, Isolaur E (2006) Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. Pediatr Res 60:221–224

Resta-Lenert S, Barrett KE (2003) Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut 52:988–997

Rodrigues AC, Cara DC, Fretez SH et al (2000) *Saccharomyces boulardii* stimulates sIgA production and the phagocytic system of gnotobiotic mice. J Appl Microbiol 89:404–414

Rzepkowska A, Zielińska D, Ołdak A et al (in press) Safety assessment and antimicrobial properties of the lactic acid bacteria strains isolated from polish raw fermented meat products. Int J Food Prop in press https://doi.org/10.1080/10942912.2016.1250098

Sanchez B, Lopez P, Gonzalez-Rodrıguez I et al (2011) A flagellin-producing *Lactococcus* strain: interactions with mucin and enteropathogens. FEMS Microbiol Lett 318:101–107

Schuck P, Dolivet A, Mejean S et al (2013) Spray drying of dairy bacteria: new opportunities to improve the viability of bacteria powders. Int Dairy J 31:12–17

Serban DE (2014) Gastrointestinal cancers: influence of gut microbiota, probiotics and prebiotics. Cancer Lett 345:258–270

Shang L, Fukata M, Thirunarayanan N et al (2008) Toll-like receptor signaling in small intestinal epithelium promotes B-cell recruitment and IgA production in lamina propria. Gastroenterology 135:529–538

Shen J, Zuo ZX, Mao AP (2014) Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. Inflamm Bowel Dis 20:21–35

Shida K, Kiyoshima-Shibata J, Nagaoka M et al (2006) Induction of interleukin-12 by lactobacillus strains having a rigid cell wall resistant to intracellular digestion. J Dairy Sci 89:3306–3317

Silva J, Carvalho AS, Teixeira P et al (2002) Bacteriocin production by spray-dried lactic acid bacteria. Lett Appl Microbiol 34:77–81

Silva J, Carvalho AS, Ferreira R et al (2005) Effect of the pH of growth on the survival of *Lactobacillus delbrueckii* subsp. *bulgaricus* to stress conditions during spray-drying. J Appl Microbiol 98:775–782

Silva J, Freixo R, Gibbs P et al (2011) Spray-drying for the production of dried cultures. Int J Dairy Technol 64:321–335

Steidler L, Hans W, Schotte L et al (2000) Treatment of murine colitis by *Lactococcus lactis* secreting Interleukin-10. Science 289:1352–1355

Stemke DJ (2004) Geneticallymodified microorganisms biosafety and ethical issues. In: Parekh SR (ed) The GMO handbook. Genetically modified animals, microbes, and plants in biotechnology. Humana Press, Totowa, pp 85–132

Sullivan A, Nord CE (2005) Probiotics and gastrointestinal diseases. J Intern Med 257:78–92

Szajewska H, Kolodziej M (2015a) Systematic review with meta-analysis: *Lactobacillus rhamnosus* GG in the prevention of antibiotic-associated diarrhoea in children and adults. Aliment Pharmacol Ther 42:1149–1157

Szajewska H, Kolodziej M (2015b) Systematic review with meta-analysis: *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea. Aliment Pharmacol Ther 42:793–801

Szajewska H, Horvath A, Kolodziej M (2015) Systematic review with meta-analysis: *Saccharomyces boulardii* supplementation and eradication of *Helicobacter pylori* infection. Aliment Pharmacol Ther 41:1237–1245

Taverniti V, Guglielmetti S (2011) The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). Genes Nutr 6:261–274

Termont S, Vandenbroucke K, Iserentant D et al (2006) Intracellular accumulation of trehalose protects *Lactococcus lactis* from freeze-drying damage and bile toxicity and increases gastric acid resistance. Appl Environ Microbiol 72:7694–7700

Tomasz B, Zoran S, Jaroslaw W et al (2014) Long-term use of probiotics *Lactobacillus* and *Bifidobacterium* has a prophylactic effect on the occurrence and severity of pouchitis: a randomized prospective study. Biomed Res Int 2014:208064

Tong JL, Ran ZH, Shen J et al (2007) Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during *Helicobacter pylori* eradication therapy. Aliment Pharmacol Ther 25:155–168

Turroni S, Vitali B, Candela M et al (2010) Antibiotics and probiotics in chronic pouchitis: a comparative proteomic approach. World J Gastroenterol 16:30–41

van Hoffen E, Korthagen NM, de Kivit S et al (2010) Exposure of intestinal epithelial cells to UV-killed *Lactobacillus* GG but not *Bifidobacterium breve* enhances the effector immune response in vitro. Int Arch Allergy Immunol 152:159–168

Varankovich NV, Nickerson MT, Korber DR (2015) Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. Front Microbiol 6:685

Verma A, Shukla G (2013) Probiotics *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* suppresses DMH-induced procarcinogenic fecal enzymes and preneoplastic aberrant crypt foci in early colon carcinogenesis in Sprague Dawley rats. Nutr Cancer 65:84–91

Vinderola CG, Costa GA, Regenhardt S et al (2002) Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. Int Dairy J 12:579–589

Vipperla K, O'Keefe SJ (2012) The microbiota and its metabolites in colonic mucosal health and cancer risk. Nutr Clin Pract 27:624–635

Volzing K, Borrero J, Sadowsky MJ et al (2013) Antimicrobial peptides targeting gram-negative pathogens, produced and delivered by lactic acid bacteria. ACS Synth Biol 2:643–650

Wang YC, Yu RC, Chou CC (2004) Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. Int J Food Microbiol 93:209–217

Wei C, Xun AY, Wei XX et al (2016) Bifidobacteria expressing tumstatin protein for antitumor therapy in tumor-bearing mice. Technol Cancer Res Treat 15:498–508

Whorwell PJ, Altringer L, Morel J et al (2006) Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. Am J Gastroenterol 101:1581–1590

Yadav AK, Tyagi A, Kumar A et al (2017) Adhesion of lactobacilli and their anti-infectivity potential. Crit Rev Food Sci Nutr 57:2042–2056

Yoon JS, Sohn W, Lee OY et al (2014) Effect of multispecies probiotics on irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. J Gastroenterol Hepatol 29:52–59

Zare F, Champagne CP, Simpson BK et al (2012) Effect of the addition of pulse ingredients to milk on acid production by probiotic and yoghurt starter cultures. LWT-Food Sci Technol 45:155–160

Zhu Q, Gao R, Wu W et al (2013) The role of gut microbiota in the pathogenesis of colorectal cancer. Tumor Biol 34:1285–1300

Zotta T, Parente E, Ricciardi A (2017) Aerobic metabolism in the genus *Lactobacillus*: impact on stress response and potential applications in the food industry. J Appl Microbiol 122:857–869