# Therapeutic impact of probiotics in various aspects: a novel prospective strategy

Rehab M. Abdel-Megeed

Department of Therapeutic Chemistry, National Research Centre, Cairo, Egypt

Correspondence to Rehab M. Abdel-Megeed, PhD, Assistant Professor, Department of Therapeutic Chemistry, National Research Centre, El-Buhouth Street, Cairo, Egypt. Tel: +201010075907; fax: +202 33370931; e-mail: rehabzenbaa@gmail.com

**Received:** 22 September 2021
**Revised:** 11 October 2021
**Accepted:** 12 October 2021
**Published:** 7 March 2022

**Egyptian Pharmaceutical Journal** 2022, 21:1–8

Probiotics are viable microorganisms that intentionally provide health benefits when consumed through restoring the gut microflora. Mainly, it is used as a successful approach for the treatment of gastrointestinal upset. Probiotics were initially used as a biotherapeutic regimen around the turn of the century. Probiotics stimulate the immune system that plays an important role in defense response against harmful microflora. Consumption of probiotics improves health against numerous diseases such as colorectal cancer, viral infection, allergies, stress, and different gastrointestinal disorders. This evidence suggests that the functions of commensal bacterial-derived factors are affected by host genetics and the discovery of links between susceptibility gene polymorphisms and protective microbial effects on the host, which might include probiotic-induced host responses. However, while probiotics' prospective health-boosting effects have been explored in many studies, further study is needed to fully understand the processes and agents that promote their beneficial effects. The current review briefly discusses the beneficial effect of probiotics as a promising biotherapeutic approach focusing on the therapeutic properties of probiotics and its role to enhance the treatment of numerous diseases. Furthermore, the current review discusses the challenges and future insights into the development of genetically engineered probiotics and their application for the treatment of various diseases or as a novel nano-drug delivery system that will be a promising regimen for human health and biotherapy.

**Keywords:**
biotherapy, health benefits, *Lactobacillus*, probiotics

Egypt Pharmaceut J 21:1–8

1687-4315

## Introduction

Probiotics are microorganisms that participate in the microbial intestinal balance and play an essential role in health maintenance. Probiotics as a definition came from an assent panel held by the International Scientific Association of Probiotics and Prebiotics (ISAPP), 2001 Food and Agriculture Organization and WHO for the definition of probiotics [1].

These microorganisms include mostly different strains such as *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Pediococcus*, and some yeast. Probiotics are mainly common in dairy products but they can also be located in nondairy products [2].

Probiotics have an important role in the industry that is predominated by food companies, specified probiotic production companies, and nutritional supplement companies. The majority of commercially traded probiotics originated from a limited list of *Lactobacillus spp.* and *Bifidobacterium spp.* [3]. These strains have been previously accepted as generally regarded as safe (GRAS) status in the United States (http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices) and by the European Food Safety Authority [4].

Furthermore, other probiotics are available in the market such as *Saccharomyces*, *Bacillus spp.*, *Escherichia coli*, *Enterococci*, and *Weissella spp.*

Probiotics have gained an increased attention in pharmaceutics for the health of different organs in addition to their benefit in heavy metal detoxification [5,6]. Clinically, the health utility of probiotics has started since the 1990s. It is well known that the microbiota colonies in the healthy body seem to be different from those found in diseased conditions. Moreover, nutritional status is influenced by the role of the gut microbial community [7].

The current review focuses on the therapeutic role of probiotics as a promising biotherapeutic approach, as well as the challenges and future prospects of genetically engineered probiotics and their use for the treatment of a variety of diseases or as a novel




DOI: 10.4103/epj.epj_66_21

nano-drug delivery system that could be a promising health biotherapy regime.

### Probiotics: definition

Probiotic is the Greek words 'pro and bios,' meaning 'of life.' First, Lilly and Stillwell [8] described probiotics as the 'substances produced by any microorganism to promote another to grow.' Accordingly, probiotics are defined as 'substances and microorganisms that collaborate in microbial balance in the gastrointestinal tract.'. Furthermore, probiotics are termed microbial cells that have beneficial effects on the maintenance of health [9].

### History of probiotics

The essential benefit of the gut microbiota was completely unknown until the beginning of the 20th century. Elie Metchnikoff (a Russian scientist, Nobel laureate, and professor at the Pasteur Institute in Paris) detected that lactic acid bacteria (LAB) has health benefits and the ability to promote longevity. He detected a significantly low number of microbiota specified morphologically by a foreign Y-shaped cells that appeared in the stool of children with diarrhea [10]. Tissier discovered a type of bacteria in breastfed infants, who played an important role in establishing the concept that these bacteria take part in the maintenance of children's health. By modulating the flora in infants, those suffered from infections in their intestines were relieved [10]. Elie Metchnikoff also investigated antioxidants that could suppress aging through some modified gut microbiota, which could replace proteolytic microbes as *Clostridium*, which produces toxic substances including phenols, indoles, and ammonia from the digestion of proteins with beneficial microbes. He advanced a diet containing probiotics such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in milk fermentation; this fermented milk played a role in maintaining health [11].

### Types of probiotics and their health benefits

Different types of probiotics have been investigated and listed as helpful microorganisms to human health [2,12]:

(1) *Lactobacilli*: are the common bacteria in the small intestine and essential to initiate digestion and absorption of food. Lactobacilli give numerous benefits to intestinal mucosa health conditions.
*Lactobacilli acidophilus*: it can be found at the end of the small intestine and the colon. It helps in the inhibition of vaginal inflammation occurred by *Candida albicans*. It also helps in the development of natural defenses against harmful bacteria as well as viral infections.
*Lactobacilli rhamnosus*: it is normally common in the intestine and the vagina as it can grow well in acidic media.

(2) *Bifidobacteria*: this type of probiotics is abundant in the colon and the vagina. *Bifidobacteria* synthesize short-chain fatty acids, such as propionic, lactic, acetic, formic, and butyric acids, with acetic acids. These acids have antimicrobial activity against harmful bacteria, molds, and yeasts.
*Bifidobacteria bifidum*: it is mainly found in both the vagina and the lower part of the small intestine with an essential role for vitamin production and protection of candida infection. Furthermore, it is able to inhibit baleful enzymes and modulate the pH level. It has antibacterial activity against *E. coli*, *Shigella*, and *Salmonella*.
*Bifidobacteria breve*: it is the main enhancer for immunity that protects against rotavirus and stabilizes the microflora.
*Bifidobacteria infantis*: mainly found in infants' gastrointestinal tract keeping it very healthy.
*Bifidobacteria longum*: it improves the intestinal environment and defecation frequency.

(3) *Saccharomyces: Saccharomyces* are microorganisms from the yeast family. It is used in the treatment of diarrhea associated with antibiotic administration.

(4) *Streptococcus thermophilus* was defined as LAB, which is mainly found in milk and milk products and used in yogurt production.

(5) *Enterococcus*: they are Gram-positive, facultative anaerobic cocci. It is useful in the treatment of diarrhea.

### Mechanism of action of probiotics and immune response

The main role of probiotics is to stimulate the immune system enabling it to cope with harmful microflora. The immune system may be innate or adaptive systems. The main role of adaptive immunity is dependent on B and T lymphocytes that are specific to a responsible antigen. However, innate immunity responds to pathogen-associated molecular patterns. Toll-like receptors are a good example of immunity [13–16].

Numerous studies have demonstrated the mechanisms by which probiotics can regulate the immune response. Indole derivatives were stimulated by *Lactobacillus reuteri* for the activation of aryl-hydrocarbon receptor leading to the reduction of gene expression of Thpok in CD4 and intraepithelial lymphocytes and

**Table 1 Health beneficial effects attributed to lactic acid bacteria**

| Health benefit | Proposed mechanism | Reference |
|---|---|---|
| Stimulation of helpful microflora | Lactobacilli stimulate growth microflora which decreases toxic metabolites | [12] |
| Urinary infections | Adhesion to urinary and vaginal tract cells | [12] |
| Improvement of immunity | Modulation of cytokines expression | [18] |
| | Prevent antigen translocation into the blood | |
| Lactose intolerance reduction | By bacterial β-galactosidase | [21] |
| Prevention of infections in the intestine | Stimulation of immune response | [22] |
| | Alteration in intestinal conditions such as pH | |
| | Regulation of binding sites of toxins | |
| Colon cancer protection | Carcinogen suppression | [23] |
| | Improvement microbiota in the colon | |
| | Increment of bile salt concentrations | |
| Heart disorders and lipid profile in blood | Regulation of cholesterol concentration | [24] |
| | Antioxidative impact | |

reprogramming CD4+ IELs into CD4+CD8aa+ IELs [17].

Probiotics enhance gastrointestinal immune response during infection [18]. Probiotics are reported to prevent colitis induced by *Citrobacter rodentium* in mice by the downregulation of interferon-γ and tumor necrosis factor-α. Moreover, upregulation of interleukin (IL)-10 and FOXP3 transcription increases follicular T-regulatory cells [19]. Immunological role of bifidobacterium has been investigated through Foxp3 and IL-10 secreting T cells, which requires toll-like receptor signaling pathways [20] (Table 1).

## Probiotics as a promising biotherapeutic regimen

### *Intolerance of lactose*

Nearly 70% of people suffer from an intolerance of lactose and it is most predominant in kids. Lactose intolerance is associated with several gastrointestinal symptoms such as diarrhea, flatulence, vomiting, and abdominal pain resulting from colon fermentation of undigested lactose [25].

Previous investigations declared the superiority of yogurt to improve lactose absorption [26]. Furthermore, living bacteria that contain β-galactosidase improves lactose digestion [21].

Various studies have demonstrated the impact of probiotic administration including *Lactobacillus spp.* such as *L. acidophilus*, *L. bulgaricus*, *B. longum*, *L. reuteri*, *L. rhamnosus*, and *Streptococcus spp.* such as *S. thermophilus* to attenuate the pathological effect of lactose intolerance both *in vivo* and *in vitro*. These bacteria species are characterized by powerful mucus adhesion properties, inhibiting pathogens, and evolving barrier function, as well as improving lactose absorption [27].

### *Antibiotic-associated diarrhea*

Some antibiotic-treated patients suffered from antibiotic-associated diarrhea (5–39%). Antibiotics such as cephalosporins and aminopenicillins are associated with a high risk of antibiotic-associated diarrhea [28]. Severe diarrhea is associated with *Clostridium difficile* infections due to antibiotic consumption. Reports suggested that inappropriate antibiotic use should be reduced and replaced by target antibiotics. However, if antibiotic treatment is deemed urgent, it is useful to create a safe method to prevent side effects associated with the issued antibiotic. Probiotics in marketing could be important and effective drugs for antibiotic-associated diarrhea treatment [29]. Different species of probiotics such as as *Lactobacillus* genus, *Bifidobacterium* genus, and *Saccharomyces* genus are recorded to be useful in the prevention of antibiotic-associated diarrhea [30].

### *Gastroenteritis*

Gastroenteritis or acute diarrhea is a disease that results from viral, bacterial, or parasitic infection causing dehydration. Rehydration strategy can reduce the incidence of mortality and morbidity but does not shorten diarrhea duration or normalization of gastrointestinal microbiota [22].

Administration of some probiotic strains (as *L. rhamnosus*, *L. acidophilus*, *Bifidobacterium lactis*, *B. infantis*, and *S. thermophilus*) in combination with the standard oral rehydration solution treatment was recorded to improve acute diarrhea in children [31].

### *Viral infections and rotavirus gastroenteritis in children*

Probiotics are effective in confronting viral infections by stimulating the immune system that has different mechanisms of action. Lactobacilli can increase serum IgA and promotion of phagocytosis in leukocytes.

Furthermore, it was clear that probiotics have a vital role in stimulating the immune response of vaccines against rotavirus and *Salmonella typhi* [32].

Rotavirus is the most recorded virus causing gastrointestinal infections and is responsible for 453 000 deaths of children worldwide [33].

The essential role of CD4+ cells was previously recorded for the promotion of protective response and rotavirus-specific IgA development [34]. Moreover, CD8+ T cells are associated with the protection of rotavirus reinfection [35].

Prevention and treatment of rotavirus diarrhea were effective using *L. rhamnosus*. *Enterococcus faecium* SF68 were effective in rotavirus treatment. This strain is mainly found in yogurt [36].

#### *Bacterial overgrowth*

Bacterial overgrowth in the small intestine is defined as an abnormal growth of colonized bacteria in the small intestine associated with different gastrointestinal syndromes. Abdominal pain, diarrhea, and flatulence are the main abounding characters in the digestion process in bacterial overgrowth. This abnormality of intestinal colonies seems to be more prevalent in older individuals and women [37].

Recently, probiotics therapy has been reported to enhance clearance of bacterial overgrowth as compared with nonprobiotic therapy, although probiotics were not efficient in bacterial overgrowth prevention [38]. However, it has been observed that treatment of bacterial overgrowth and chronic diarrhea can occur with *Lactobacillus* bacteria [39].

#### *Inflammatory bowel disease and irritable bowel syndrome*

A combination of environmental, genetic, and intestinal microbial factors can cause inflammatory bowel disease [40]. Cocktails of probiotic intake may be important to improve inflammatory bowel disease.

Administration of a combination of eight strains of *Lactobacillus* bacteria (*Lactobacillus plantarum*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus casei*, *L. acidophilus*, *Bifidobacteria breve*, *B. longum*, *B. infantis*, and *Streptococcus salivarius*) was commonly applied in inflammatory bowel disease therapy [41]. This formulation increased the levels of regulatory cytokines and downregulated proinflammatory cytokines and toll-like receptor gene expression [42]. Furthermore, tumor necrosis factor-α and NF-κB gene expression was modulated in the presence of *Lactobacillus* and *Bifidobacterium* of affected individuals [43].

Irritable bowel syndrome is a gastrointestinal disorder recording 20% of the population suffered from it due to increasing abnormal microflora such as *Klebsiella spp.* and *Enterococci* in addition to decreasing numbers of *Lactobacilli* and *Bifidobacteria* [44]. Furthermore, probiotic supplementation has demonstrated clinical improvements in up to 80% of the treated patients [45].

#### *Reduction of allergy*

Gut microbiota plays a remarkable role in the development of mucosal immune system and environmental factors in the development of allergic diseases [46]. Infants who suffered from pathogenic bacteria such as *C. difficile* and *Staphylococcus aureus* in the gastrointestinal tract are at more risk of developing the allergy. On the other hand, more colonies by different species of Lactobacillus bacteria and other *Bifidobacteria* were seen in gastrointestinal tracts of nonallergic children. This finding suggested that the presence of microbiota of the described species in the gastrointestinal tract might protect against allergic diseases [47].

Newborns with allergies depend on immune response due to placental immune regulation across feto-paternal antigens [48]. After a neonate is born, this response is reduced due to the activation of some microbiota [49]. Moreover, gastrointestinal probiotics regulate immune response due to their impact on the development of gut-associated lymphoid tissue. Therefore, probiotic supplementation during the pregnancy period decreases risk factors whereas probiotics may be a good modulator of cytokine balance [50].

#### *Colon cancer*

Colorectal cancer (CRC) estimates nearly 9.2% of all cancers recording 1.096 million cases and is the second leading cause of cancer morbidity and mortality worldwide [51].

The gut microbiome plays a crucial role in host health [23]; meanwhile, an imbalance in the gut microbiome can influence bacterial toxin secretions leading to carcinogenic metabolites such as hydrogen sulfide in addition to lowering beneficial metabolites as butyrate. These metabolic disorders cause dysregulation of the immune system and lead to CRC [52].

Numerous studies have assessed the gut microbiome of CRC patients, revealing several patterns [53]. CRC patients revealed an increment in the abundances of the genus *Fusobacterium* in their gut microbiomes [54–59]. On the other hand, tissue and stool specimens of CRC revealed several harmful bacterial species such as *Streptococcus* [58], *Peptostreptococcus* [54–56], *Selenomonas* [56,57], and *Porphyromonas* [56–59]. Microbial composition of CRC patients as compared with healthy individuals based on the analysis of 16 S rRNA amplicon (V3) declared an increment in the above-mentioned genera besides a remarkable decrease in butyrate-producing bacteria. Another study investigated similar results using compositional 16 S rDNA sequencing of CRC specimens [54]. The relative abundances of *Fusobacterium* bacteria in carcinogenic colon tissue observed a significant difference as compared with noncancerous mucosa, with 8.5 and 4.13%, respectively [55].

Exposure to probiotics could activate caspase-3 besides the inhibition of p21 gene expression. This result suggested that probiotic supplementation improves cell apoptosis [60].

Furthermore, *L. casei* and *L. rhamnosus* recorded an ability to reduce the invasion of human CRC cell line (HCT-116) [61] in addition to an observed downregulation of matrix metalloproteinase-9 gene expression.

The therapeutic impact of probiotics was investigated *in vivo* and *in vitro.* Gao *et al.* [57] proved the effect of probiotic supplementation by regulating microRNA in CRC-induced mice. The result declared that miR-135b, miR-155, and KRAS expression have been downregulated; meanwhile, there was an obvious upregulation of miR-26b, miR-18a, APC, PU.1, and PTEN in the probiotic-treated group as compared with the nontreated group [62]. Furthermore, the prophylactic effect of probiotics was demonstrated along with the anti-inflammatory standard drug celecoxib in the CRC rat model. Daily supplementation of probiotic strains for 18 weeks reduced the tumor burden, downregulate Bcl-2 and K-ras and anti-apoptotic genes and upregulate p53 and Bax tumor-suppressor genes [63,64].

Clinically, the protective effect of probiotics and dietary fiber was conducted on some volunteers with CRC who had colorectal removal surgery. Atypia of colorectal tumors has been prevented [65,66]. Another study investigated the changes in the microbiota, DNA methylation, epithelial proliferation, and biomarkers of CRC in probiotic supplementation [67]. Gianotti *et al.* [68] studied the impact of *B. longum* and *Lactobacilli johnsonii* with two different doses when supplemented perioperatively to CRC patients. High-dose supplementation significantly modulated the expression of CD3, CD4, CD8, and lymphocytes. This study suggested that probiotic supplementation could modulate the health status of CRC patients.

#### *Cholesterol-lowering effects*

The relationship between consuming Lactobacilli and cholesterol level was previously investigated. Moreover, *L. acidophilus* can remove cholesterol from tissue culture media.

Cholesterol concentration decreased upon intake of a large quantity of yogurt [24]. In the animal model, hypercholesterolemia declared a significant decrease in serum cholesterol levels after Lactobacilli administration [69].

#### *Heavy meatal detoxification*

Probiotics are a promising generation of microorganisms that have the capacity to mitigate toxicity of heavy metals. As previously reported, probiotics could modulate oxidative stress, modify, and adjust pH levels [7,70]. Furthermore, they modulate the expression of enzymes and proteins that are related to heavy metal toxicity [71]. Moreover, it has a vigorous antioxidative and immune modulatory ability, sustain fluid of the gut, and suppress microflora overgrowth [72]. *L. plantarum* was recorded to modulate cadmium, lead, and chromium toxicity [73]. Moreover, *Lactobacillus brevis* (23017) could modulate mercury toxicity. Probiotic strains are able to get rid of heavy metals through fecal excretion [74]. *Lactobacillus* and *Acidobacillus* strains were used in our previously published article to detoxify cadmium chloride in mice [6].

### Therapeutic application of genetically engineered probiotics

However, host responses to the harmful microbes are carefully studied; the major attention is to discover novel and safe therapies using the helpful bacteria. Novel production of bioengineered probiotics is established through genetic modulation to improve the efficiency of conventional probiotics in addition to reduce the pathogenic potential of target strains. These strains are used for different applications such as a drug delivery system vaccine or to simulate surface receptors, targeting specified pathogens or toxins, and to improve the host immune response [75].

In view of the removal of beneficial microbiota associated with antibiotic treatment, unique

**Table 2 Therapeutic effect of various probiotic strains**

| Probiotic strain | Therapeutic effect against numerous diseases | Reference |
|---|---|---|
| *Lactobacilli acidophilus, Lactobacillus bulgaricus*, *Bifidobacteria longum,* Lactobacillus *reuteri, Lactobacilli rhamnosus*, and *Streptococcus thermophilus* | Intolerance of lactose | [21] |
| *Lactobacillus plantarum*, *Lactobacillus acidophilus* | Antibiotic-associated diarrhea | [28] |
| *Lactobacillus rhamnosus* and *Enterococcus faecium* SF68 | Viral infections and rota virus | [32] |
| *Streptococcus boulardii and Streptococcus cerevisiae* isolated from fruit juice | *Helicobacter pylori* infection | [37] |
| *Lactobacillus* spp, *Bifidobacterium*, *Enterococcus*, *Streptococcus L. salivarius*, *Lactobacilli acidophilus, Bifidobacteria bifidum* of BGN4 strain | Inflammatory bowel disease | [40] |
| *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Lactobacillus crispatus* | Colorectal cancer | [51] |
| *Lactobacilli acidophilus* | Cholesterol-lowering effects | [69] |

antimicrobial drugs that are environment-friendly and seem riskless have been developed [76]. Genetic-engineered probiotics have numerous advantages, as they are more stable and lower in cost.

Genetically modified LAB was previously used to improve its therapeutic potential against CRC. The modified strain of *Pediococcus pentosaceus*, SL4 has a significant antitumor effect after oral administration in mice [77].

Furthermore, cotreatment of IL-10 and genetically engineered *Lactococcus lactis* declared a significant reduction (50%) in colitis incidence inflammatory bowel disease in a mouse model [78]. Moreover, another study suggested that IL-10 efficacy can be improved through the integration of recombinant *L. lactis* [79].

LAB is the best-characterized model organism used for the production and delivery of antigens and cytokines [80] and DNA delivery system [81]. In a previous study, DNA delivery into mammalian cells with LAB through invading target genes such as fibronectin-binding protein A is derived from *S. aureus* and was expressed in *L. lactis* by vector transformation to increase target gene expression [82,83].

### Probiotica as a drug delivery system

It was indicated that probiotics can intermediate the synthesis of selenium nanoparticles biologically. However, biogenic synthesis of selenium nanoparticles by probiotics is usually observed to be eco-friendly, of low-cost, safe, and nontoxic. Therefore, selenium-enriched probiotica approach attracted wide interests and attention due to the above advantages. Selenium loaded in *L. casei* can synthesize lactomicroselenium particles (85–200 nm) [84].

The therapeutic effect of probiotic strains is summarized in Table 2.

## Conclusion

Probiotics have opened a new drift on the correlation between nutrition and health. Different studies prove that the intake of probiotics is effective in the treatment of numerous diseases and stimulates the immune system. Furthermore, it could be a promising generation of biotherapy. Gastrointestinal microbiota eventually manipulate all health directions including drug metabolism, toxicology, and improvement of creative therapies. Their combined benefits include antagonistic response against microflora in addition to their immunomodulatory impact against numerous diseases, such as pathogenic bacteria, cancers, and metabolic disorders, can be assessed. In particular, the combined therapeutic approach including immunomodulatory, antimicrobial, and anti-inflammatory role of genetically engineered probiotics could be beneficial to overcome the progression of different diseases and metabolic disorders Furthermore, probiotics are used for various applications such as the nano-drug delivery system to target specific pathogens or toxins and for improving host immune response. However, up to now, the mechanism of probiotic-mediated synthesis of nanoprticles is still unclear. Moreover, the potential biological activity and application effect of different promising drug-enriched probiotics need further investigation.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

**1** Food and Agriculture Organization/World Health Organization (FAO/WHO), Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria, Córdoba, Argentina 2001. Available at: http://www.who.int/foodsafety/

publications/fs_management/en/probiotics.pdf. [Accessed 20 August 2020].

2 Phad A. Probiotics: biotherapeutic agents in the human health. Adv Drug Deliv Rev 2014; 1:9–29.

3 Nataraj BH, Ali SA, Behare PV, Yadav H. Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. Microb Cell Fact 2020; 19:168.

4 European Food Safety Authority. Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. 2: suitability of taxonomic units notified to EFSA until March 2015. EFSA J 2015; 13:4138.

5 Kadry MO, Abdel-Megeed RM. Probiotics as a complementary therapy in the model of cadmium chloride toxicity: crosstalk of β-Catenin, BDNF, and StAR signaling pathways. Biol Trace Elem Res 2018; 185:404–413.

6 Abdel-Megeed RM. Probiotics: a promising generation of heavy metal detoxification. Biol Trace Elem Res 2020; 199:2406–2413.

7 Lilly DM, Stillwell RH. Probiotics: growth-promoting factors produced by microorganisms. Science 1965; 147:747–748.

8 Marteau PR, Vrese M, de Cellier CJ, Schrezenmeir J. Protection from gastrointestinal diseases with the use of probiotics, Am J Clin Nutr 2001; 73:430–436.

9 Tissier H. The treatment of intestinal infections by the method of transformation of bacterial intestinal flora. C R Soc Biol 1906; 60:359–361.

10 Guarner F, Khan AG, Garisch J, Eliakim R, Gangl A, Thomson A, *et al.* World Gastroenterology Organization Practice Guideline Probiotics and prebiotics. S. Afr Gastroenterol Rev 2008; 14–25.

11 Oyetayo VO, Oyetayo FL. Potential of probiotics as biotherapeutic agents targeting the innate immune system. Afr J Biotechnol 2005; 4:123–127.

12 Bermudez-Brito M, Plaza-Diaz J, Munoz-Quezada S. Probiotic mechanisms of action. Ann Nutr Metab 2012; 61:160–174.

13 Lebeer S, Vanderleyden J, De Keersmaecker CJ. Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. Nat Rev Microbiol 2010; 8:171–184.

14 Wells JM. Immunomodulatory mechanisms of lactabacilli. Microb Cell Fact 2011; 10:S17.

15 Mez-Llorente GC, M'oz S, Gil A. Role of Toll-like receptors in the development of immune-tolerance mediated by probiotics. Proc Nutr Soc 2010; 69:381–389.

16 Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010; 11:373–384.

17 Cervantes-Barragan L, Chai JN, Tianero MD, DiLuccia B, Ahern PP, Merriman J, *et al.* Lactobacillus reuteri induces gut intraepithelial CD4 +CD8aa+ T cells. Science 2017; 357:806–810.

18 Klatt NR, Canary LA, Sun X, Vinton CL, Funderburg NT, Morcock DR, *et al.* Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques. J Clin Invest 2013; 123:903–907.

19 Rodrigues DM, Sousa AJ, Johnson-Henry KC, Sherman PM, Gareau MG. Probiotics are effective for the prevention and treatment of *Citrobacter rodentium*-induced colitis in mice. J Infect Dis 2012; 206:99–109.

20 Zhong Y, Huang CY, He T, Harmsen HM. Effect of probiotics and yogurt on colonic microflora in subjects with lactose intolerance. Wei Sheng Yan Jiu 2006; 35:587–591.

21 Marteau P, Messing B, Arrigoni E, Briet F, Flourie B, Morin XX, Rambaud MC. Do patients with short-bowel syndrome need a lactose-free diet? J C Nutrition 1997; 13:13–16.

22 Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. Cochrane Database Syst Rev 2010 2010:CD003048.

23 Gagnière J, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, *et al.* Gut microbiota imbalance and colorectal cancer. World J Gastroenterol 2016; 22:501–518.

24 Mei L, Tang Y, Li M, Yang P, Liu Z, Yuan J, *et al.* Co-administration of cholesterol-lowering probiotics and anthraquinone from *Cassia obtusifolia* L. ameliorate non-alcoholic fatty liver. PLoS one. 2015; 10:e0138078.

25 Klein G, Pack A, Bonnaparte C, Reuter G. Taxonomy and physiology of lactic acid bacteria. Int J Food Microbiol 1998; 41:103–125.

26 Leclercq R, Courvalin P. Resistance to glycopeptides in enterococci. Clin Infect Dis 1997; 24:545–554.

27 Wistrom J, Norrby SR, Myhre EB, Eriksson S, Granstrom G, Lagergren L, *et al.* Frequency of antibiotic-associated diarrhoea in 2462 antibiotic treated hospitalized patients: a prospective study. J Antimicrob Chemother 2001; 47:43–50.

28 Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, *et al.* European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. The use of probiotics for the management of acute gastroenteritis. a position paper by The Espghan Working Group For Probiotics. J Pediatr Gastroenterol Nutr 2014; 58:531–539.

29 Van Wietmarschen HA, Busch M, van Oostveen A, Pot G, Jong MC. Probiotics use for antibiotic-associated diarrhea: a pragmatic participatory evaluation in nursing homes. BMC Gastroenterol 2020; 20:151.

30 Kluijfhout S, Trieu T, Vandenplas Y. Efficacy of the probiotic probiotical confirmed in acute gastroenteritis. J Pediatr Gastroenterol Hepatol Nutr 2020; 23:464–471.

31 Underwood MA. Probiotics and innate and adaptive immune responses in premature infants. Immunopathol Dis Therap 2016; 7:1–15.

32 Chhabra P, Payne DC, Szilagyi PG, Edwards KM, Staat MA, Shirley SH, *et al.* Etiology of viral gastroenteritis in children <5 years of age in the United States, 2008–2009. J Infect Dis 2013; 208:790–800.

33 Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis 2012; 12:136–141.

34 Kuklin NA, Rott L, Feng N, Conner ME, Wagner N, Müller W, *et al.* Protective intestinal anti-rotavirus B cell immunity is dependent on α4β7 integrin expression but does not require IgA antibody production. J Immunol 2001; 166:1894–1902.

35 Jiang JQ, He X-S., Feng N, Greenberg HB. Qualitative and quantitative characteristics of rotavirus-specific CD8 T cells vary depending on the route of infection. J Virol 2008; 82:6812–6819.

36 Parker EPK, Praharaj I, Zekavati A, Lazarus RP, Giri S, Operario DJ, *et al.* Influence of the intestinal microbiota on the immunogenicity of oral rotavirus vaccine given to infants in south India. Vaccine 2018; 36:264–272.

37 Lazarus RP, John J, Shanmugasundaram E, Rajan AK, Thiagarajan S, Giri S. The effect of probiotics and zinc supplementation on the immune response to oral rotavirus vaccine: a randomized, factorial design, placebo-controlled study among Indian infant. Vaccine 2018; 36:273–279.

38 Choung RS, Ruff KC, Malhotra A, Herrick L, Locke GR, Harmsen WS, *et al.* Clinical predictors of small intestinal bacterial overgrowth by duodenal aspirate culture. Aliment Pharmacol Ther 2011; 33:1059–1067.

39 Zhong C, Qu C, Wang B, Liang S, Zeng B. Probiotics for preventing and treating small intestinal bacterial overgrowth: a meta-analysis and systematic review of current evidence. J Clin Gastroenterol 2017; 51:300–311.

40 Rao SSC, Bhagatwala J. Small intestinal bacterial overgrowth: clinical features and therapeutic management. Clin Transl Gastroenterol 2019; 10:e00078.

41 Coqueiro AY, Raizel R, Bonvini A, Tirapegui J, Rogero MM. Probiotics for inflammatory bowel diseases: a promising adjuvant treatment. Int J Food Sci Nutr 2018; 28:1–10.

42 Mora D, Filardi R, Arioli S, Boeren S, Aalvink S, de Vos WM. Development of omics-based protocols for the microbiological characterization of multi-strain formulations marketed as probiotics: the case of VSL#3. Microb Biotechnol 2019; 12:1371–1386.

43 Silva NOE, de Brito BB, da Silva FAF, Santos MLC, de Melo FF. Probiotics in inflammatory bowel disease: does it work? World J Meta-Anal 2020; 8:54–66.

44 Shadnoush M, Hosseini RS, Khalilnezhad A, Navai L, Goudarzi H, Vaezjalali M. Effects of probiotics on gut microbiota in patients with inflammatory bowel disease: a double-blind, placebo-controlled clinical trial. Korean J Gastroenterol 2015; 65:215–221.

45 Dale HF, Rasmussen SH, Asiller ÖÖ, Lied GA. Probiotics in irritable bowel syndrome: an up-to-date systematic review. Nutrients 2019; 11:2048.

46 Wong RK, Yang C, Song GH, Wong J, Ho KY. Melatonin regulation as a possible mechanism for probiotic (VSL#3) in irritable bowel syndrome: a randomized double-blinded placebo study. Dig Dis Sci 2015; 60:186–194.

47 Özdemir Ö. Gut flora development in infancy and its effect on immune system. J Pediatr Inf 2009; 3:202–203.

48 Özdemir O. Various effects of different probiotic strains in allergic disorders: an update from laboratory and clinical data. Clin Exp Immunol 2010; 160:295–304.

49 Jones CA, Holloway JA, Warner JO. Does atopic disease start in fetal life? Allergy 2000; 55:2–10.

**50** Mennini M, Dahdah L, Artesani MC, Fiocchi A, Martelli A. Probiotics in asthma and allergy prevention. Front Pediatr 2017; 5:165.

**51** Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68:394–424.

**52** Sun J, Kato I. Gut microbiota, inflammation and colorectal cancer. Genes Dis 2016; 3:130–143.

**53** De Almeida CV, de Camargo MR, Russo E, Amedei A. Role of diet and gut microbiota on colorectal cancer immune-modulation. World J Gastroenterol 2019; 25:151–162.

**54** Sivamaruth BS, Kesika P, Chaiyasut C. The role of probiotics in colorectal cancer management. Evid-Based Compl Alternat Med 2020; 2020:1–17.

**55** Jahani-Sherafat S, Alebouyeh M, Moghim S, Ahmadi Amoli H, Ghasemian-Safaei H. Role of gut microbiota in the pathogenesis of colorectal cancer; a review article. Gastroenterol Hepatol Bed Bench 2018; 11:101–109.

**56** Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, *et al.* Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. ISME J 2012; 6:320–329.

**57** Gao R, Kong C, Huang L, Li H, Qu X, Liu Z, *et al.* Mucosa-associated microbiota signature in colorectal cancer. Eur J Clin Microbiol Infect Dis 2017; 36:2073–2083.

**58** Hibberd AA, Lyra A, Ouwehand AC, Rolny P, Lindegren H, Cedgård L, *et al.* Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. BMJ Open Gastroenterol 2017; 4:e000145.

**59** Sinha R, Ahn J, Sampson JN, Shi J, Yu G, Xiong X, *et al.* Fecal microbiota, fecal metabolome, and colorectal cancer interrelations. PLoS ONE 2016; 11:e0152126.

**60** Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, *et al.* Comparison of human gut microbiota in control subjects and patients with colorectal carcinoma in adenoma: terminal restriction fragment length polymorphism and next-generation sequencing analyses. Oncol Rep 2016; 35:325–333.

**61** Gao Z, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. Front Microbiol 2015; 6:20.

**62** Escamilla MA, Lane V, Maitin V. Cell-free supernatants from probiotic *Lactobacillus casei* and *Lactobacillus rhamnosus* GG decrease colon cancer cell invasion in vitro. Nutr Cancer 2012; 64:871–878.

**63** Heydari Z, Rahaie MA, Alizadeh M, Agah S, Khalighfard S, Bahmani S. Effects of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* probiotics on the expression of microRNAs 135b, 26b, 18a and 155, and their involving genes in mice colon cancer. Probiotics Antimicro 2018; 11:1155–1162.

**64** Sharaf LK, Sharma M, Chandel D, Shukla G. Prophylactic intervention of probiotics (*L. acidophilus*,L. rhamnosus GG) and celecoxib modulate Bax-mediated apoptosis in 1,2-dimethylhydrazine-induced experimental colon carcinogenesis. BMC Cancer 2018; 18:1111.

**65** Sharaf KL, Shukla G. Probiotics (*Lactobacillus acidophilus* and *Lactobacillus rhamnosus* GG) in conjunction with celecoxib (selective COX-2 inhibitor) modulated DMH induced early experimental colon carcinogenesis. Nutr Cancer 2018; 70:946–955.

**66** Ishikawa H, Akedo I, Otani T, Suzuki T, Nakamura T, Takeyama I, *et al.* Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors. Int J Cancer 2005; 116:762–767.

**67** Worthley DL, Le Leu RK, Whitehall VL, Conlon M, Christophersen C, Belobrajdic D, *et al.* A human, double-blind, placebo-controlled, crossover trial of prebiotic, probiotic, and synbiotic supplementation: effects on luminal, inflammatory, epigenetic, and epithelial biomarkers of colorectal cancer. Am J Clin Nutr 2009; 90:578–586.

**68** Gianotti L, Morelli L, Galbiati F, Rocchetti S, Coppola S, Beneduce A, *et al.* A randomized double-blind trial on perioperative administration of probiotics in colorectal cancer patients. World J Gastroenterol 2010; 16:167–175.

**69** Nazir Y, Hussain SA, Abdul Hamid A, Song Y. Probiotics and their potential preventive and therapeutic role for cancer, high serum cholesterol, and allergic and hiv diseases. Biomed Res Int 2018; 2018:3428437.

**70** Claus SP, Ellero SL, Berger B, Krause L, Bruttin A, Molina J, *et al.* Host-gut microbial metabolic interaction. MBio 2011; 2:e00271–e00310.

**71** Breton J, Daniel C, Dewulf J, Pothion S, Froux N, Sauty M, *et al.* Gut microbiota limits heavy metals burden caused by chronic oral exposure. Toxicol Lett 2013; 222:132–138.

**72** Zhai Q, Cen S, Jiang J, Zhao J, Zhang H, Chen W. Disturbance of trace element and gut microbiota profiles as indicators of autism spectrum disorder: a pilot study of Chinese children. Environ Res 2019a; 171:501–509.

**73** Yu H, Wu B, Zhang XX, Liu S, Yu J, Cheng S, *et al.* Arsenic metabolism and toxicity influenced by ferric iron in simulated gastrointestinal tract and the roles of gut microbiota. Environ Sci Technol 2016; 50:7189–7197.

**74** Zhai Q, Tian F, Zhao J, Zhang H, Narbad A, Chen W. Oral administration of probiotics inhibits absorption of the heavy metal cadmium by protecting the intestinal barrier. Appl Environ Microbiol 2016a; 82:4429–4440.

**75** Mazhar SF, Afzal M, Almatroudi A, Munir S, Ashfaq UA, Rasool M, *et al.* The prospects for the therapeutic implications of genetically engineered probiotics. J Food Qual 2020; 2020:1–11.

**76** An BC, Ryu Y, Choi O, Hong S, Heo JY, Chung MJ. Genetic engineering of a probiotic-based drug delivery system for colorectal cancer therapy. Cancer Rep Rev 2020; 4:1–3.

**77** An BC, Ryu Y, Yoon YS, Choi O, Park HJ, Kim TY, *et al.* Colorectal cancer therapy using a *Pediococcus pentosaceus* SL4 drug delivery system secreting lactic acid bacteriaderived protein p8. Mol Cells 2019; 30:755–762.

**78** Steidler L, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, *et al.* Treatment of murine colitis by *Lactococcus lactis* secreting minterleukin-10. Science 2000; 25:1352–1355.

**79** Steidler L, Neirynck S, Huyghebaert N, Snoeck V, Vermeire A, Goddeeris B, *et al.* Biological containment of genetically modified *Lactococcus lactis* for intestinal delivery of human interleukin 10. Nat Biotechnol 2003; 2:785–789.

**80** Robert S, Steidler L. Recombinant *Lactococcus lactis* can make the difference in antigen-specific immune tolerance induction, the type 1 diabetes case. Microb Cell Fact 2014; 13:S1–S11.

**81** Wells JM, Mercenier A. Mucosal delivery of therapeutic and prophylactic molecules using lactic acid bacteria. Nat Rev Microbiol 2008; 6:349–362.

**82** Innocentin S, Guimaraes V, Miyoshi A, Azevedo V, Langella P, Chatel J-M, *et al.* *Lactococcus lactis* expressing either *Staphylococcus aureus*fibronectin-binding protein A or Listeria monocytogenes internalin A can efficiently internalize and deliver DNA in human epithelial cells. Appl Environ Microbiol 2009; 75:4870–4878.

**83** Mancha-Agresti P, de Castro CP, dos Santos JSC, Araujo MA, Pereira VB, LeBlanc JG, *et al.* Recombinant invasive *Lactococcus lactis* carrying a DNA vaccine coding the Ag85A antigen increases INF-$\gamma$, IL-6, and TNF-$\alpha$ cytokines after intranasal immunization. Front Microbiol 2017; 8:1263.

**84** Cui YH, Li LL, Zhou NQ, Liu JH, Huang Q, Wang HJ, *et al.* In vivo synthesis of nano-selenium by *Tetrahymena thermophila* SB210. Emzyme Microb Technol 2016; 95:185–191.