Potential role of probiotics in the management of gastric ulcer (Review)

GHALIA KHODER[1], ASMA A. AL-MENHALI[2], FARAH AL-YASSIR[3] and SHERIF M. KARAM[3]

[1]Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, University of Sharjah, Sharjah 27272; [2]Department of Biology, College of Science; [3]Department of Anatomy, College of Medicine and Health Sciences, United Arab Emirates University, Al-Ain 17666, United Arab Emirates

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Abstract. Gastric ulcer is one of the most common chronic gastrointestinal diseases characterized by a significant defect in the mucosal barrier. *Helicobacter pylori (H. pylori)* infection and the frequent long-term use of non-steroidal anti-inflammatory drugs are major factors involved in gastric ulcer development. Acid inhibitors and antibiotics are commonly used to treat gastric ulcer. However, in the last few decades, the accumulating evidence for resistance to antibiotics and the side effects of antibiotics and acid inhibitors have drawn attention to the possible use of probiotics in the prevention and treatment of gastric ulcer. Probiotics are live microorganisms that when administered in adequate amounts confer health benefits on the host. Currently, the available experimental and clinical studies indicate that probiotics are promising for future applications in the management of gastric ulcers. This review aims to provide an overview of the general health benefits of probiotics on various systemic and gastrointestinal disorders with a special focus on gastric ulcer and the involved cellular and molecular mechanisms: i) Protection of gastric mucosal barrier; ii) upregulation of prostaglandins, mucus, growth factors and anti-inflammatory cytokines; iii) increased cell proliferation to apoptosis ratio; and iv) induction of angiogenesis. Finally, some of the available data on the possible use of probiotics in *H. pylori* eradication are discussed.

Contents

Correspondence to: Professor Sherif M. Karam, Department of Anatomy, College of Medicine and Health Sciences, United Arab Emirates University, Khalifa Street, Al-Ain 17666, United Arab Emirates
E-mail: skaram@uaeu.ac.ae

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1. Introduction

The gastric mucosa is lined by a single layer of epithelial cells that is supported by delicate elements of loose connective tissue underlaid by a thin layer of smooth muscle fibers. In many individuals, the gastric epithelium is exposed not only to its own acidic and enzymatic secretions, but also to duodenal bile, highly prevalent *Helicobacter pylori* (*H. pylori*), frequently used non-steroidal anti-inflammatory drugs (NSAIDs) and alcohol intake (1). Therefore, gastric mucosal damage is very common and may evolve into gastric ulcers in many patients. If not treated adequately, a gastric ulcer may lead to serious complications, such as perforation and bleeding, or may progress toward gastric cancer with substantial morbidity and mortality rates (2-4). Inhibition of acid secretion using proton pump inhibitors and eradication of *H. pylori* by treatment with clarithromycin, amoxicillin and metronidazole, are currently the most widely used therapeutic regimens for gastric ulcer (5). However, with the side effects of these therapeutic agents (6,7), the emerging resistance of *H. pylori* to antibiotics (8,9), and the high recurrence rate of gastric ulcer (10-12), efforts are being directed toward the identification of new therapeutic modalities.

With the increase of their popularity of use in the prevention and treatment of a number of systemic and gastrointestinal diseases (Fig. 1), probiotics have attracted the attention of numerous cell biologists and clinicians who are interested in exploring their effects on gastric ulcers and *H. pylori*. Even though the number of clinical studies investigating the impact of probiotics on gastric ulcer is relatively low, a number of experimental studies have generated promising results. The present review aims to summarize the available data concerning the potential role of probiotics in the prevention and healing of gastric ulcer.

2. Gastric ulcer

Gastric ulcer is one of the most common and serious chronic diseases of the upper gastrointestinal tract. The prevalence of gastric ulcer is 2.4% in the Western population (13) and may be up to 6.1% in Asia (14). Despite advancements in anti-ulcer therapy, the recurrence rate remains high (10-12,15). A gastric ulcer is a localized deep necrotic lesion involving the entire mucosal thickness and the muscularis mucosa (16). It is generally considered that these ulcers develop from an imbalance between mucosal defensive mechanisms and damaging factors at the luminal surface of the stomach (1). In developing countries, the high prevalence of *H. pylori*, long-term frequent use of NSAIDs, and cigarette smoking represent the major risk factors involved in ulcer development (17,18).

Ulcerogenesis starts by disruption of the protective mucous layer formed by the epithelial cells. Enhanced secretion of acid and pepsin by parietal and zymogenic cells may contribute to damage of the mucous layer (1). Smoking contributes to ulcer formation by upregulating the production of the proton pump and, therefore, acid secretion (19). Damage to the mucous layer may lead to peeling of the surface epithelium and exposure of the endothelial cells of capillaries in the underlying connective tissue. Once capillaries are damaged, oxygen and nutrients will be deficient. As a consequence, hypoxic necrosis will occur in deep glandular cells, namely stem/progenitor cells, mucous neck cells, zymogenic cells, enteroendocrine cells and parietal cells. Moreover, damaged macrophages, mast cells and endothelial cells release vasoactive agents and pro-inflammatory mediators that worsen the mucosal microcirculation. Epithelia and connective tissue necrosis eventually lead to the formation of ulcers (1,20).

Healing of gastric ulcer involves an orchestrated array of different mechanisms that work together to correct the imbalance between damaging and defensive factors in the stomach (Fig. 2). Healing occurs by repairing the mucosal defect with epithelial cells and connective tissue elements, which involves the production of extracellular matrix, cell proliferation, migration, differentiation and gland reconstruction. These events are controlled by many factors, including epidermal growth factor, hepatocyte growth factor, insulin-like growth factor 1, trefoil factors, cyclooxygenase 2-generated prostaglandin, and several cytokines in a spatially and temporally coordinated manner (21). Healing also requires angiogenesis, which is triggered by hypoxia and involves vascular endothelial growth factor, fibroblast growth factor and angiopoietins (22). In addition to local mucosal cells from viable tissue at the ulcer edge, a study demonstrated that bone marrow-derived stem and progenitor cells are attracted to the site of injury and contribute to the regeneration of epithelial and connective tissue components (23). It has been proposed that the proliferation of these stem cells is followed by their commitment to different pathways and differentiation into parietal, surface mucous, mucous neck and zymogenic cells (24). Mucous neck cells are thought to be also involved in the healing of gastric ulcer (25,26). They synthesize and secrete trefoil factor 2, which downregulates acid secretion by parietal cells and, therefore, promotes mucosal healing (26).

Cell therapy may have some potential applications in gastric ulcer treatment. When bone marrow mesenchymal stem cells were injected (locally or intravenously) in rat models of gastric ulcer, they were found to promote ulcer healing (27,28). However, the involved mechanisms are not known and this stem cell injection method remains to be evaluated. Other studies have demonstrated the possibility of gastric tissue engineering with the formation of all cell lineages or only mucous cells using freshly isolated gastric organoids, isolated gastric stem cells or gastric stem cell line (29-31). These promising studies require further evaluation and testing in animal models of gastric ulcers.

3. Probiotics

Numerous studies have indicated that probiotics can be used for the treatment of gastric ulcers. The idea of using probiotics arose from the study conducted by Elliott *et al* in 1998 (32). In a rat model of acetic acid-induced gastric ulcer, colonization of gram-negative bacteria occurred rapidly at the site of the ulcer and significantly impaired ulcer healing. However, colonization by gram-positive bacteria promoted ulcer healing. Notably, administration of the exogenous probiotic strain *Lactobacillus* accelerated ulcer healing (32).

Historically, the concept of probiotics began around 1900 by the Nobel laureate Elie Metchnikoff who discovered that the consumption of live bacteria (*Lactobacillus bulgaricus*) in yogurt or fermented milk improves the biological features of the gastrointestinal tract (33,34). The Food and Agriculture Organization and the International Scientific Association for Probiotics and Prebiotics define probiotics as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (35).

The gut microbiota includes ~30 species of *Bifidobacterium*, 52 species of *Lactobacillus*, and others, such as *Streptococcus* and *Enterococcus* (36). The most extensively studied probiotics for treating and/or preventing gastrointestinal diseases are lactic acid bacteria, namely *Lactobacillus* and *Bifidobacterium* species. While these species are non-pathogenic, they can resist the harsh luminal environment of the gastrointestinal tract (37).

Several studies have revealed a number of beneficial effects of certain lactobacilli, such as the suppression of pathogenic bacteria in the gut and inhibition of allergic, inflammatory and neoplastic changes (38-41). Furthermore, it has been shown that lactobacilli are particularly useful in promoting gastric ulcer healing in rats, when administered as an individual probiotic strain, such as *Lactobacillus rhamnosus* GG (42), *Lactobacillus gasseri* OLL2716 (43,44), or *Lactobacillus acidophilus* (45,46) or as a probiotic mixture, VSL#3 (47). *Lactobacillus rhamnosus* GG increases the cellular proliferation to apoptosis ratio and therefore promotes regeneration of epithelial cells, particularly at the ulcer margins (42,48). In clinical studies, a probiotic mixture was demonstrated to be better than a single strain for improving the characteristics of indigenous microflora (47,49). In addition to bacteria, certain yeasts, such as *Saccharomyces boulardii*, have been investigated and have shown potential therapeutic effects in a rat model of ibuprofen-induced gastric ulcer (50,51). This yeast has neuraminidase activity, which removes sialic acid residues from the apical membranes of gastric epithelial cells. The loss of sialic acid prevents the adhesin-mediated binding of *H. pylori* to the epithelial cells (52).

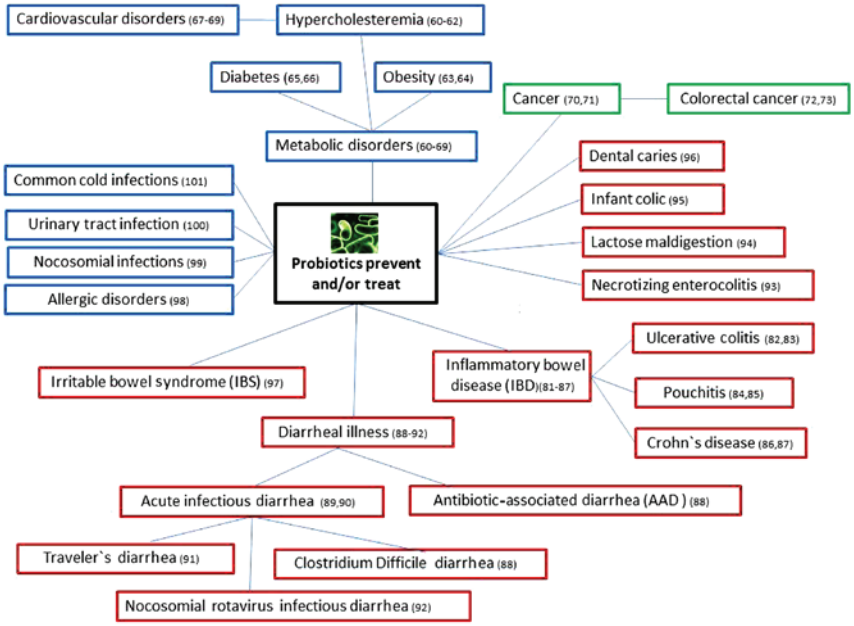

Figure 1. Summary of gastrointestinal (red), non-gastrointestinal (blue) and neoplastic (green) disorders that are currently known to respond to probiotics.

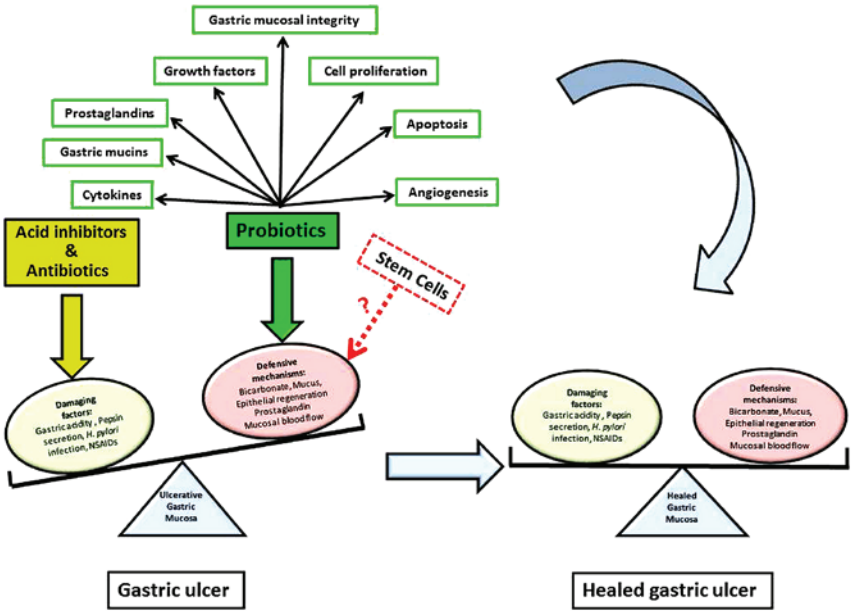

Figure 2. Summary of gastric ulcer etiology and different treatment options. Gastric ulcer results from the imbalance between damaging (gastric acidity, pepsin secretion, *H. pylori* infection and NSAIDS) and defensive factors (bicarbonate and mucus secretion, prostaglandin production, epithelial regeneration, and mucosal blood flow) of the mucosa. Acid inhibitors (e.g., proton pump inhibitors) and antibiotics specific for *H. pylori* (clarithromycin, amoxicillin/metronidazole) are used routinely for the treatment of gastric ulcer. Experimental studies suggest that probiotics could contribute to the prevention and therapeutic modalities of gastric ulcer by enhancing: i) Production of prostaglandin, mucins, growth factors and anti-inflammatory cytokines, ii) the cellular proliferation-to-apoptosis ratio, iii) gastric mucosal integrity, iv) trans-mucosal resistance and v) angiogenesis. Transplantation of bone marrow mesenchymal stem cells or possibly gastric epithelial stem cells is also a proposed modality for the treatment of gastric ulcers that requires further investigation. *H. pylori*, *Helicobacter pylori*; NSAIDs, non-steroidal anti-inflammatory drugs.

To date, >13,438 research articles on probiotics have appeared in PubMed and ~1,422 articles were published during 2015 alone. Many of these articles report invaluable results demonstrating the effects of probiotics on the gastrointestinal tract using *in vitro* studies, animal models and healthy/unhealthy volunteers. The main gastrointestinal disorder targeted by probiotic research is irritable bowel syndrome (53-55). However, studies assessing the effects of probiotics on gastric ulcers are relatively limited. This could be due to the adverse physiological conditions of the host, such as an acidic environment, digestive enzymes, bile acids and mechanical stress that attenuate the survival and growth of certain probiotics. To overcome these conditions, a high dose of multiple probiotics has been administered (47,56,57), and probiotics packaged into a suitable delivery system have been developed (45,46).

The beneficial effects of probiotics depend mainly on their ability to survive the acidic conditions and the hydrolytic enzymes and bile content in the stomach and duodenum (37). Several studies have shown that the strength of acidity, length of exposure and strain of probiotic are major factors affecting their survival (58-60). Among probiotic strains, lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium* exhibit a great ability to survive gastric transit and, therefore, are extensively used in many pharmaceutical and dairy probiotic products.

Screening of different probiotics has revealed that *Lactobacillus acidophilus* and *Bifidobacterium longum* can survive and adhere better to the gastric mucosa than *Streptococcus thermophilus* and *Bifidobacterium infantis/adolescentis/bifidum* (61,62). Studies have shown that *Lactobacillus acidophilus* survive at pH ≥3 after a 3-h incubation (60) and *Lactobacillus rhamnosus* survive a 4-h incubation at pH 2.5 (63). Also, the viability of several strains of *Lactobacillus acidophilus* and *Bifidobacterium* was maintained for ~3 h in the pH range of 1.5-3.0 (60). While the viability of a *Bifidobacterium* strain remains unchanged at a pH of 3 for 3 h, which even declines slowly to pH of 2 or 1 after 1 h (59), *Lactobacillus delbrueckii* and *Streptococcus thermophilus* do not readily survive stomach acidity (64).

The reason underlying the survival of some probiotic strains in the stomach has been attributed to F-type ATPase. This bacterial membrane-bound ATP synthase is responsible for generating a constant gradient between extra- and intracellular pH for protection against acidic conditions (65). So, in an acidic environment, the F_0F_1-ATPase is upregulated and generates a proton motive force via proton expulsion and, therefore, increases the intracellular pH (66). It has been reported that *Lactobacillus acidophilus* has a high cytoplasmic buffering capacity, which allows changes in cytoplasmic pH and stability in acidic conditions (67). Glucose enhances the survival of lactobacilli in acidic conditions because glycolysis provides ATP to F_0F_1-ATPase, and thereby enables proton exclusion (68,69).

To overcome the inability of some probiotics to survive, microencapsulated or coated probiotic strains have been developed (70-72). Recently, Villena and coworkers designed gastro-resistant tablets containing *Lactobacillus fermentum* CECT5716 using sodium alginate (73). Calcium alginate beads have also been proposed to protect the delivery of viable probiotic strains in the gastrointestinal tract (74,75) and have even been used to treat cold restraint-induced gastric ulcers (46).

In addition to microencapsulation, coating and food supplements, the use of non-living probiotic strains could also contribute to overcoming the problem of acid-sensitive probiotic strains not surviving in the stomach. Even though some viable probiotic strains do not survive gastric transit, their dead forms remain beneficial (76). Substantial evidence from *in vitro* and animal studies has shown that both live and dead probiotics can act as biological response modifiers (76-78). Nonviable probiotics are now known as 'paraprobiotics' or 'ghost probiotics' (79).

Studies have shown that heat-killed *Enterococcus faecalis* fraction stimulates the gastrointestinal immune system against vancomycin-resistant enterococci (80) while heat-killed bifidobacteria induce significant increases in tumor necrosis factor (TNF)-α and interleukin (IL)-6 production (81). Using fractions of heat-killed *Lactobacillus acidophilus* and *Lactobacillus casei*, it is possible to protect immunodeficient mice against *Candida albicans* (82). Further studies have shown that even non-viable gamma ray-irradiated probiotic mixtures or their DNA can ameliorate the anti-inflammatory response in rats with experimental colitis (83). Also, it has been shown that viable and nonviable probiotic *Lactobacillus paracasei* IMPC2.1 and *Lactobacillus rhamnosus* GG can exert the same antiproliferative and proapoptotic effects on cancer cells *in vitro* (84).

4. Impacts of probiotics on systemic and gastrointestinal diseases

The prophylactic and therapeutic effects of probiotics in some gastrointestinal and non-gastrointestinal diseases are summarized in Fig. 1. In addition to their conventional benefits for gastrointestinal functions, probiotics have shown potential therapeutic effects in some metabolic diseases, such as hyperlipidemia or hypercholesterolemia (85-89), obesity (90,91) and diabetes (92,93). Therefore, the use of probiotics may contribute to a reduced risk of atherosclerosis (94) and hypertension (95,96).

In the last few decades, several studies have suggested a potential role for probiotics in cancer prevention and therapy (97). Data have shown specific alterations of the gut microbial composition (dysbiosis) in patients with colon cancer (98). Induction of colon cancer in rats using 1,2-dimethylhydrazine is associated with significant dysbiosis, which could be inhibited by the oral administration of *Lactobacillus salivarius* Ren, leading to effective suppression of colon carcinogenesis (99). In mice, treatment with the probiotics *Clostridium butyricum* and *Bacillus subtilis* has been found to inhibit the development of 1,2-dimethylhydrazine-induced colorectal cancer (100). As for gastric cancer, little is known about the possible association between probiotics and carcinogenesis. However, some *in vitro* studies have demonstrated very promising anti-proliferative and pro-apoptotic effects of probiotics on gastric cancer cells (84,101-104). Moreover, clinical studies have provided evidence for the possible effects of probiotics in preventing the toxic effects of chemotherapy and radiation therapy in cancer patients (105,106).

The possible use of probiotics as supplements or even alternatives to oral antibiotic therapy has been suggested, especially with increasing cases of resistance to antibiotics. When frequently and unspecifically used, antibiotics may not only induce resistance, but also harm the gastrointestinal microflora. In these cases, the administration of probiotics may restore the normal microflora, compete with the pathogenic resistant bacteria and, therefore, help patients to recover (107,108).

Novel approaches have been used to design some genetically modified probiotic strains with specific capabilities for the delivery of anti-inflammatory cytokines, vaccines and anti-pathogenic molecules (109-111). Engineered *Lactococcus lactis* strains were produced as live mucosal vaccines for a large number of antigens derived from bacteria, viruses and parasites (112). In addition, recombinant strains of *Lactococcus lactis* were used to produce the rotavirus spike-protein subunit VP8 that can prevent rotavirus infection (113). The future use

of probiotics as vectors targeted to gastrointestinal mucosal lesions is promising. This new targeted drug delivery approach using probiotics is known as 'pharmabiotics' (35).

There are data suggesting that probiotics could be useful for gastrointestinal colic, acute infectious diarrhea, inflammatory bowel syndrome, antibiotic-associated diarrhea, travelers' diarrhea, lactose malabsorption and inflammatory bowel diseases (85,86). However, the data available regarding the possible association between probiotic administration and gastric ulcer healing and prevention are limited.

5. Prophylactic and therapeutic effects of probiotics in gastric ulcer

Over the last two decades, the use of probiotics in the management of gastric ulcer has been investigated in a number of studies. Promising results for studies exploring both prophylactic (Table I) and therapeutic (Table II) effects of probiotics have been obtained. The studies concerning the roles of probiotics in gastric ulcer healing reported in the literature were mainly conducted in rats. These studies were based on the use of either individual probiotic strains, such as *Lactobacillus rhamnosus* GG (42,48), *Lactobacillus gasseri* OLL2716 (44), *Lactobacillus acidophilus* (45,46), *Escherichia coli* Nissle 1917 (114), *Bifidobacterium animalis* VKL/VKB (115), *Bifidobacterium bifidum/brevis* (116) and *Saccharomyces boulardii* (51), or a mixture of probiotic strains, such as VSL#3 (47). A number of studies have reported that probiotics not only inhibit the development of acute gastric mucosal lesions, but also accelerate the process of healing of induced gastric ulcers (42,44,47). The effects of probiotics on gastric ulcer are attributed to several cellular and molecular mechanisms (Fig. 3).

Protection of gastric mucosal barrier. In a normal stomach, the mucosal integrity is maintained by three main barriers (117,118). i) The preepithelial barrier is made of a mucus-bicarbonate-phospholipid layer located between the gastric lumen and the epithelium. ii) The epithelial barrier characterized by a) a continuous sheet of surface epithelial cells connected by tight junctions and generating different secretory products including trefoil factors, prostaglandins, and heat shock proteins, and b) continuous cell renewal accomplished by proliferation of stem/progenitor cells and regulated by different mechanisms involving growth factors, prostaglandins, gastrin and the anti-apoptotic protein survivin. iii) The subepithelial barrier composed of a) microcirculation through capillaries maintained by the continuous generation of prostaglandins, nitric oxide and hydrogen sulfide that protect endothelial cells from injury and prevent aggregation of platelets and leukocytes, and b) sensory innervations that regulate the mucosal blood flow (117,118).

When one or more of the above listed defensive mechanisms is altered, the gastric mucosal barrier is disrupted and a gastric ulcer may develop. The beneficial effects for probiotics on the gastrointestinal mucosa may occur via two main mechanisms (119-121). i) Antagonistic action achieved through lactic acid or antimicrobial compounds that inhibit the growth of pathogenic bacteria (122,123) or by competing for the available nutrients and growth factors and, therefore, inhibit the growth of pathogens or block their adhesion to gastric epithelial cells (124,125). ii) Immunomodulatory activity which involves the induction of phagocytosis, secretion of immunoglobulin A (IgA), activation of natural killer cells, stimulation of protective cytokines, downregulation of proinflammatory cytokines, and modulation of T cell responses (Th1 induction and Th2 attenuation) (126-129).

Probiotics can also protect the integrity of the gastric mucosal barrier by upregulating prostaglandin, mucous secretion, tight junction protein expression and cell proliferation, and by inhibiting apoptosis (43,48,130-132). In rats, the administration of *Bifidobacterium bifidum* BF-1 or *Bifidobacterium animalis* VKL and VKB has been found to protect the gastric mucosa through either preventing the mucous barrier from degradation (115) or increasing gastric mucous production (133). The probiotic mixture VSL#3 protects the epithelial barrier and upregulates the expression of tight junction proteins (occludin and zonula occludens-1) *in vivo* and *in vitro* via the activation of p38 or mitogen-activated protein (MAP) kinase and extracellular signal-regulated kinase (ERK) signaling pathways (134). Mennigen *et al* demonstrated that probiotics can strengthen the gastric mucosal barrier by inhibiting the redistribution and expression of tight junction proteins and blocking apoptosis (135). The probiotic strains *Lactobacillus gasseri* OLL2716, *Lactobacillus rhamnosus* GG and *Escherichia coli* Nissle 1917 are able to protect the altered gastric mucosal barrier (43,48,114). In humans, Gotteland *et al* found that pretreatment with *Lactobacillus* GG protected against indomethacin-induced disruption of the gastric mucosal barrier (131).

Recently, three mouse models of induced gastric ulcers using alcohol, restraint cold stress and pyloric ligation were investigated. Pretreatment of these mice with the probiotic bacterial strain *Clostridium butyricum* alleviated the histopathological changes, specifically, the infiltration of inflammatory cells and gastric mucosal damage (136). Moreover, the same study showed that this bacterium alleviated oxidative stress damage by inhibiting the activity of superoxide dismutase and catalases and decreasing malondialdehyde levels. These results were similar to those obtained with omeprazole pretreatment (136).

Production of prostaglandins, growth factors and anti-inflammatory cytokines. Prostaglandins are involved in the ulcer healing process by inhibiting acid secretion, stimulating the production of mucus, bicarbonate and phospholipids, increasing blood flow and accelerating epithelial restitution (119). Therefore, prostaglandins are also thought to be a target for the prophylactic effect of probiotics in gastric ulcers (43,48,114). Ethanol-induced gastric mucosal lesions in rats were prevented by pretreatment with the probiotic strain *Lactobacillus rhamnosus* GG through the upregulation of prostaglandin E2 (48). The effectiveness of the probiotic strain *Escherichia coli* Nissle 1917 in preventing stress-induced ulcers in rats has also been reported. This effect was achieved through induction of mucosal anti-inflammatory cytokines, synthesis of gastric mucosal protective factors (ghrelin and heat shock protein 70), enhancement of gastric microcirculation, and involvement of prostaglandins and nitric oxide (114).

Uchida and Karakazu demonstrated that pretreatment of rats with LG21 yogurt containing *Lactobacillus gasseri*

Table I. Summary of studies on the prophylactic effects of probiotics against gastric mucosal lesions.

Authors, year	Probiotic strain	Modeling method	Lesions	Effects of probiotics	Refs.
Uchida and Kurakazu, 2004	*Lactobacillus gasseri* OLL 2716	Acetic acid	Gastric lesions and antral ulcer	Inhibit antral ulcer formation, and prevent gastric ulcers by increasing prostaglandin E2	(43)
Lam *et al*, 2007	*Lactobacillus rhamnosus* GG	Ethanol	Gastric mucosal lesions	Protect gastric mucosal lesions by upregulating prostaglandin E2, mucus secretion and transmural resistance, and downregulating apoptosis	(48)
Konturek *et al*, 2009	*Escherichia coli* Nissle 1917	Stress	Acute gastric lesions	Attenuate lesions through anti-inflammatory actions, induce ghrelin and HSP70 synthesis, enhance gastric microcirculation, and upregulate prostaglandin, nitric oxide and neuropeptides	(114)
Spivak *et al*, 2013	Probiotic mixture (2 bacterial strains)	Stress	Gastric erosions and ulcer	Protect the gastric mucosal barrier	(115)
Gomi *et al*, 2013	*Bifidobacterium bifidum* BF-1	Acid-ethanol	Acute gastric injury	Protect and alleviate acute gastric injury by enhancing the production of gastric mucus	(133)
Wang *et al*, 2015	*Clostridium butyricum*	Alcohol, stress and pyloric ligation in mice	Gastric ulcer	Reduce gastric mucosal injury severity, oxidative stress and inflammatory responses.	(136)
Senol *et al*, 2011	Probiotic mixture (13 bacterial strains)	Aspirin	Gastric mucosal lesions	Inhibit mucosal lipid peroxidation, stimulate sIgA secretion, and stabilize mucosal mast cell degranulation	(142)
Senol *et al*, 2011	Probiotic mixture (13 bacterial strains)	Ethanol	Gastric erosions	Inhibit mucosal lipid peroxidation, inhibit tumor necrosis factor-α, interferon-γ, interleukin-2, interleukin-4 and malondialdehyde, and upregulate IgA secretion	(143)
Taketani *et al*, 2014	Thioredoxin from *Saccharomyes cerevisiae*	Stress and acid-ethanol	Gastric mucosal lesions	Upregulate 385 genes and downregulate 65 genes associated with healing in damaged mucosa	(164)

HSP70, heat-shock protein 70; IgA, immunoglobulin A; sIgA, secretory immunoglobulin A.

Table II. Summary of studies on the therapeutic effects of probiotics against gastric mucosal lesions.

Authors, year	Probiotic strain(s)	Modeling method	Lesions	Effects of probiotics	Refs.
Elliott *et al*, 1998	*Lactobacillus* spp.	Acetic acid	Gastric ulcer	Enhance healing of a pre-existing gastric ulcer	(32)
Lam *et al*, 2007	*Lactobacillus rhamnosus* GG	Acetic acid	Gastric ulcer	Inhibit cell apoptosis to proliferation ratio, and induce angiogenesis	(42)
Uchida *et al*, 2010	*Lactobacillus gasseri* OLL 2716	Acetic acid	Gastric ulcer	Accelerate healing by enhancing generation of gastric mucosal prostaglandin E2	(44)
Singh and Kaur, 2012	*Lactobacillus acilidophilus* encapsulated in ginger extract	Stress	Gastric ulcer	Improve healing by restoring all biochemical, physiological and histological changes	(45)
Singh *et al*, 2012	*Lactobacillus acidophilus* and alginate floating beads	Stress	Gastric ulcer	Improve healing by restoring all biochemical, physiological and histological changes	(46)
Dharmani *et al*, 2013	Probiotic mixture (VSL#3) (8 probiotic strains)	Acetic acid	Gastric ulcer	Enhance healing by promoting angiogenesis via upregulation of vascular endothelial growth factor	(47)
Girard *et al*, 2010	*Saccharomyces boulardii*	Ibuprofen	Gastric ulcer	Potential treatment or prevention	(51)
Nagaoka *et al*, 1994	Polysaccharides fractions (PSFs) of *Bifidobacterium breve* and *bifidum*	Acetic acid and ethanol	Gastric erosion and ulcer	Repair and protect gastric mucosa by increasing expression of epidermal and fibroblast growth factors and 6-ketoprostaglandin F1	(116)
Virchenko *et al*, 2015	Probiotic mixture (2 bacterial strains) and composite probiotic (3 bacterial strains)	Stress	Gastric erosion and ulcer	Reduce lesions and intensity of bleeding through the restoration of pro- and antioxidant balance	(137)
Virchenko *et al*, 2015	Probiotic mixture (14 bacterial strains)	Stress	Gastric mucosal lesions	Enhance recovery of stress hormones, downregulate pro-inflammatory cytokines and upregulate anti-inflammatory cytokines	(138)

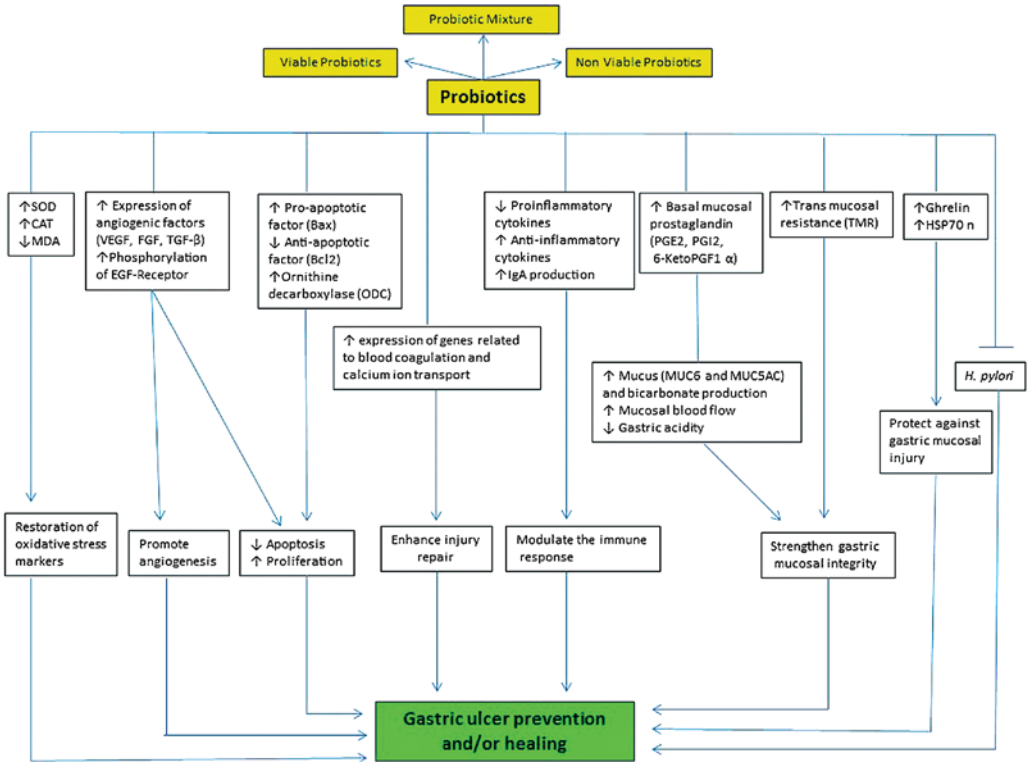

Figure 3. Summary of the proposed main cellular and molecular events involved in the effects of probiotics on gastric ulcer.SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; TGF, transforming growth factor; EGF, epidermal growth factor; Bax, Bcl-2-associated X protein; Bcl2, B cell lymphoma 2; IgA, immunoglobulin A; PGE2, prostaglandin E2; PGI2, prostaglandin I2; PGF1α, prostaglandin F1α; HSP70, heat-shock protein 70; MUC6, mucin 6; MUC5AC, mucin 5AC.

OLL2716 significantly inhibited the formation of acetic acid-induced gastric ulcers in a dose dependent manner. This effect was mediated by increasing the production of mucosal prostaglandin E2/I2. Notably, the gastro-protective effect of prostaglandin was attenuated by pretreatment of the rats with indomethacin, which is known to inhibit prostaglandin (43). A few years later, the same authors demonstrated that the administration of the same *Lactobacillus gasseri* OLL2716 yogurt for 10 days significantly accelerated the healing of chronic gastric ulcers through the stimulation of prostaglandin production (44). However, yogurt containing gamma-ray-exposed *Lactobacillus gasseri* OLL2716 increased the generation of prostaglandin without affecting the healing of the acetic acid-induced gastric ulcers. These findings indicate that the increased production of prostaglandin does not necessarily explain the therapeutic effect of LG21 yogurt on ulcer healing (44). Recently, pretreatment with the probiotic *Clostridium butyricum* in mouse models of induced gastric ulcer caused a reduction in the level of 6-keto-prostaglandin F1α, the stable metabolite of prostaglandin I2 (136).

Aside from the probiotic bacteria itself, the polysaccharide fractions can also exert a gastroprotective effect against gastric ulcers. Nagaoka *et al* demonstrated that polysaccharides of *Bifidobacterium breve* YIT4014 and 4043I and *Bifidobacterium bifidum* YIT4007 were able to repair and protect the mucosa of rats against acetic acid- and ethanol-induced gastric ulcers and erosions. The polysaccharides of these probiotic mixtures were found to increase the expression of growth factors such as fibroblast growth factor and epidermal growth factor in addition to 6-ketoprostaglandin F1 (116).

Recent studies on stress-induced gastric mucosal lesions demonstrated that using a mixture of probiotics (*Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Propionibacterium* and *Acetobacter*) enhanced ulcer healing by restoring the balance between pro- and anti-oxidants in the gastric mucosa (137). In addition, probiotic mixtures (comprising *Bifidobacterium animalis* VKL and VKB with or without *Lactobacillus casei* IMVB-7280) enhanced the recovery of stress hormones (adrenocorticotropin and corticosterone), decreased proinflammatory cytokines and increased anti-inflammatory cytokines (138). Moreover, pretreatment with *Clostridium byturicum* attenuated the elevation of proinflammatory factors (IL-1β, TNF-α and leukotriene B4) in mice with induced gastric ulcer (136).

Probiotics are not only effective against gastric ulcers induced by acetic acid, ethanol or stress, but also play important roles in the prevention or treatment of ulcers induced by NSAIDs, such as aspirin or indomethacin (139). In aspirin-treated rats, TNF upregulates neutrophil-derived superoxide leading to oxygen radical-mediated tissue damage (140,141). Thus, this pro-inflammatory cytokine is an ideal target for protection against gastric ulcer. In this context, using a rat model of asprin-or ethanol-induced gastric mucosal damage, it was found that using a probiotic mixture of 13 bacterial strains composed of four strains of *Lactobacillus fermentum* (BB16-75, AK2-8, AK5-22, AK6-26), three strains of *Lactobacillus plantarum* (AA17-73, AK7-28, AK8-31B) and six strains of *Enterococcus faecium* (AB6-21, AB16-68, AK4-120, AK7-31, BK9-40, BK13-54) caused a reduction in mucosal damage scores, lipid peroxidation, malondialdehyde

content and pro-inflammatory cytokine levels. In addition, these probiotics also induced an increase in mucosal secretory IgA production and the stabilization of mucosal mast cells (142,143).

Production of gastric mucus. Mucus is a cohesive mixture of ~95% water, 5% mucin glycoprotein molecules, salts, immunoglobulins, cellular and serum macromolecules, and trefoil peptides (144,145). Mucus on the luminal surface of gastric mucosa forms two main layers: The outer loosely adherent mucus and the inner firmly adherent mucus. The former plays a role in binding luminal noxious agents, absorbing nitrite and releasing nitric oxide. The latter is important for protection against digestive enzymes and corrosive acid (146).

There are several mucin genes encoding secreted and transmembrane mucins, such as MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6-8, MUC11, MUC12 and MUC16. The stomach has two distinct cell types secreting different mucins: Surface mucus cells secreting MUC5AC and mucus neck cells secreting MUC6 (147). Transmembrane mucins (MUC1, MUC4 and MUC16) are mainly involved in signal transduction and cell adhesion phenomena (148). Another noteworthy class of secretory proteins is the trefoil peptides. These are produced and secreted together with mucins, and thus are present in fairly high concentrations in the mucous gel layer and in the mucosal epithelial cells (145). They are intimately associated with mucus to improve protection against noxious agents. They are upregulated during mucosal injury and have been implicated in promoting cell migration and the repair process (149-151).

The mucus layer protects the gastric mucosa by different mechanisms (152,153): i) Acting as a physical barrier, ii) binding to bacterial adhesins, iii) maintaining high concentrations of secreted IgA and lysozyme at the epithelial surface, iv) acting as a free radical scavenger, and v) delaying proton permeation from the luminal acid into gastric surface cells to enable its neutralization by secreted bicarbonate. Therefore, while the gastric mucus protects the gastric epithelial cells, it also helps in the protection and survival of microflora.

Some studies have shown that probiotics promote mucous secretion. Treatment of colonic epithelial Caco-2 cells or colorectal HT29 cells with probiotics increased the expression of mucins (154-156). Administration of VSL#3 to rats for 7 days was enough to induce a 60-fold increase in MUC2 expression and its concomitant secretion (157). Probiotics can also adhere to mucus via specific binding proteins and eventually modulate the immune system for protection against pathogens (158,159). In the stomach, the available studies on the effects of probiotics on mucous production have demonstrated different results. Pretreatment with *Bifidobacterium* BF-1 upregulates MUC5AC gene expression and enhances the production of mucus by surface mucous cells in rats with acute gastric lesions induced by acid/ethanol (133). However, the expression of MUC5AC was moderately upregulated or unchanged, respectively, in VSL#3- or *Lactobacillus* rhamnosus GG-treated rats with ethanol-induced gastric mucosal lesions (47,48). Even though the MUC5AC gene is responsible for the most abundantly produced mucin in the normal mucosa, Lam and colleagues reported that pretreatment of rats with *Lactobacillus rhamnosus* GG caused upregulation of MUC6 mRNA expression (specific for mucous neck cells) in gastric mucosal lesions induced by ethanol (48). Moreover, it was demonstrated that pretreatment with a probiotic mixture (*Bifidobacterium animalis* VKL and VKB) in rats with stress-induced gastric mucosal erosion and ulcer, prevented degradation of the mucous layer (115).

Cell proliferation and apoptosis. Perpetual cell renewal is an important epithelial factor required for the maintenance of the gastric mucosal barrier. The dynamics and cells involved in this physiological phenomenon have been defined in rodents and humans (160,161). Several factors and cell types in the corpus region of the stomach are involved in the regulation of this renewal process including enteroendocrine cells (Karam and Al-Menhali, unpublished data) and parietal cells (162). Some studies have also explored the effects of probiotics on cellular proliferation and apoptosis. Pretreatment of rats with *Lactobacillus rhamnosus* GG significantly reduced the number of apoptotic cells in ethanol-induced gastric mucosal lesions (48). The reduction of apoptosis is controlled by upregulation of the anti-apoptotic protein, B cell lymphoma 2 (42). Further investigations revealed that the same *Lactobacillus* probiotic strain not only inhibits the apoptosis of gastric mucosal cells, but also stimulates gastric cell proliferation, which is mediated by ornithine decarboxylase (42).

Angiogenesis. Induction of angiogenesis is one of the most important effects of probiotics on gastric ulcers (42,47). Vascular endothelial growth factor is required to stimulate the formation of granulation tissue and development of new microvessels (163). Administration of the probiotic mixture VSL#3, composed of eight probiotic strains: four *Lactobacilli* (*acidophilus, bulgaricus, casei* and *plantarum*), three *Bifidobacteria* (*breve, infantis* and *longum*) and *Streptococcus* accelerates gastric ulcer healing in a rat model by upregulating the expression and production of vascular endothelial growth factor. This ulcer-healing effect was confirmed using neutralizing antibodies (47). In another study, administration of the probiotic strain *Lactobacillus rhamnosus* GG to rats with acetic acid-induced gastric ulcers led to a significant increase in the number of blood microvessels (42). Notably, this angiogenic effect was observed only at the edge of damaged gastric mucosa and not in the surrounding normal tissues. Therefore, *Lactobacillus rhamnosus* GG is a potential therapeutic agent for promoting vascularization and gastric ulcer healing and requires further clinical investigation.

Since the harsh physiological conditions in the stomach may interfere with the colonization of some probiotic strains, efforts have been directed toward packing probiotics into a suitable delivery system. A novel synbiotic approach using *Lactobacillus acidophilus* encapsulated with ginger extract (45) or loaded in alginate floating beads (46), significantly enhanced the healing of gastric stress-induced ulcers in rats. This was evidenced by the restoration of various biochemical (lipid peroxidation, catalase and superoxide dismutase), physiological (mucous content) and histological (ulcer index and hemorrhagic streaks) changes. Moreover, histopathological studies have indicated that the administration of *Lactobacillus acidophilus* encapsulated with ginger extract leads to complete recovery from gastric ulcer with no

signs of inflammation or mucosal damage visible at the ulcer edge (45,46).

The effects of probiotics on angiogenesis are not restricted to bacterial strains. Yeast, such as *Saccharomyces boulardii,* has been reported to have potential in the treatment and prevention of gastric ulcer induced by ibuprofen in rats (51). More recently, it was demonstrated using DNA microarray that thioredoxin derived from edible yeast, *Saccharomyces cerevisiae*, can protect the gastric mucosa by up- or downregulating hundreds of genes involved in the healing of the ulcerative mucosa induced by stress or HCl/ethanol in rats (164).

6. Effects of probiotics on *H. pylori*

For a long time, gastric ulcers were considered to be a result of stress, improper diet and NSAID usage. However, the discovery of *H. pylori* and its association with gastric ulcers has changed the gastroenterological practice worldwide (165). *H. pylori* can uniquely survive and colonize in the harsh acidic environment of the stomach for decades, leading to progressive inflammatory, ulcerative and neoplastic changes (166). Among patients infected by *H. pylori*, 10-20% may ultimately develop ulcers (16). Recent regression of ulcer incidence is highly dependent on the worldwide eradication of *H. pylori* (167).

Eradication of *H. pylori* and associated gastric mucosal changes remain a challenge for gastroenterologists. This could be due to the fact that *H. pylori* infection may start early during childhood where developing gastric glands are characterized by prominent dividing stem cells (Karam and Bharwani, unpublished data). No antibiotic is effective enough to eliminate *H. pylori* when given as a monotherapy. The gold standard triple regimen (clarithromycin and amoxicillin/metronidazole combined with a proton pump inhibitor) represents the worldwide accepted protocol used in the eradication of *H. pylori*. Studies using this triple therapy have demonstrated an eradication rate of 90% (168). However, none of the studies reported 100% eradication of *H. pylori*.

In some countries, the marked rise in resistance to clarithromycin has caused a steady decline in the efficiency of the standard triple therapy (169,170). To overcome this problem, new regimens including quadruple, sequential, concomitant, dual and rescue therapies have been introduced (168). However, the development of resistance to antibiotics and their side effects has caused poor patient compliance and, therefore, has limited their applications (171).

During the last decade, numerous studies have explored the possible use of probiotics to improve the protocol of *H. pylori* eradication and to prevent its side effects (172-176). The use of probiotics has also been tested in asymptomatic *H. pylori*-infected patients and found to lower the risk of gastric ulcer development (177). Kabir and co-workers were one of the first groups to report that probiotic strain *Lactobacillus salivarus* can prevent and eliminate *H. pylori* colonization in the stomach of gnotobiotic BALB/c mice (178).

On the basis of *in vitro* studies using gastric epithelial cells and different probiotic strains, several effects for probiotics against *H. pylori* infection have been identified (179). Probiotics can inhibit *H. pylori* infection by non-immunological and immunological mechanisms (179-181). The non-immunological effects of probiotics include: i) Production of antimicrobials and antioxidants that could inhibit either the growth or urease activity of *H. pylori* (182,183), ii) competing with *H. pylori* for binding to the surface of gastric epithelial cells and blocking their specific membrane receptors (125,184-186), and iii) stabilizing the gastric mucosal barrier by stimulating mucus production by surface epithelial cells (187).

The immunological effects of probiotics include: i) Maintaining the balance between pro- and anti-inflammatory cytokines, which leads to recovery from gastritis (188), ii) downregulating the production of IL-8 and TNF-α by producing conjugated linoleic acid that targets the nuclear factor κB pathway (189,190), iii) upregulating the anti-inflammatory suppressor of cytokine signaling through activation of signal transducer and activator of transcription (STAT)-1/STAT-3 transcription factors and inactivation of Janus kinase 2 (191), and iv) enhancing gastric mucosal barrier by stimulating IgA secretion and transport (181).

In animal models of *H. pylori* infection and also in humans, the use of different probiotic strains has demonstrated different favorable effects, specifically, prophylaxis against *H. pylori,* inhibition of *H. pylori* colonization, and alleviation of *H. pylori*-associated gastric inflammation (173,181,192,193). Therefore, some clinical and laboratory-based studies have demonstrated an improvement in *H. pylori* eradication by using probiotics (194,195).

The most frequently used probiotic strains for *H. pylori* infection are *Lactobacillus johnsonii* La1 (177,196,197). Lactobacilli, the predominant gut bacteria, inhibit adhesion of *H. pylori* to gastric epithelial cells *in vitro*. Thus, using lactobacilli exogenously can help in the eradication of *H. pylori*. Other probiotic strains such as *Saccharomyces boulardii*, *Lactobacillus acidophilus* and *Bifidobacterium lactis*, have been used either alone or combined with antibiotics specific to *H. pylori*. Meta-analytic studies have recommended the use of either *Saccharomyces boulardii* or *Lactobacillus* species supplementation in combination with the standard triple therapy (198,199).

Some *in vitro* studies demonstrating the inhibition or even killing of *H. pylori* by probiotics have been followed by preclinical and clinical applications (200,201). These studies indicated only a partial efficacy of probiotics against *H. pylori* when administered alone. Increase of efficacy and/or reduction of side effects was demonstrated when probiotics were administered in combination with the standard triple treatment of *H. pylori* (201). However, to date, there is no study that demonstrates complete eradication of *H. pylori* infection by probiotic treatment.

7. Conclusions

Gastric ulcers develop due to an imbalance between damaging factors and the defense mechanisms of the gastric mucosa (Fig. 2). The available studies in the literature indicate that probiotics can accelerate the healing of gastric ulcers via multiple mechanisms that involve both damaging and defensive factors (Fig. 2). Even though only limited *in vivo* studies have explored the impact of probiotics in gastric ulcer, some cellular and molecular findings have suggested their protective and therapeutic effects (Fig. 2). Several studies also identified

probiotic strains effective in *H. pylori* eradication via immunological and non-immunological mechanisms. Therefore, the use of probiotics in the management of gastric ulcer appears promising and further studies are required. Taking in consideration the probiotic strains, dosage, commercial preparations and the heterogeneity of patients, a combined clinical and basic science experimental approach is likely to yield important strategies to optimize the use of probiotics in health and disease (202).

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