Systematic review: are probiotics useful in controlling gastric colonization by *Helicobacter pylori?*

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SUMMARY

Helicobacter pylori is a highly prevalent pathogen considered as an aetiological factor for gastroduodenal ulcers, and a risk factor for gastric adenocarcinoma and lymphoma in humans. Most subjects colonized by this micro-organism are asymptomatic and remain untreated. In symptomatic patients, the antibiotic treatment has a high cost and is not 100% effective because of resistance to antibiotics and to moderate patient compliance. This review discusses the role of probiotics as alternative solutions to assist in the control of *H. pylori* colonization in at-risk populations.

The evidence that some strains of *Lactobacillus* and *Bifidobacterium* are able to inhibit *H. pylori* growth through the release of bacteriocins or organic acids, and may also decrease its adhesion to epithelial cells, is reviewed. In addition, probiotics have a possible role in the stabilization of the gastric barrier function and the decrease of mucosal inflammation. Other aspects that are considered are the contribution of probiotics to the healing of the gastric mucosa linked to their antioxidant and anti-inflammatory properties.

Clinical trials in colonized adults and children are reviewed, and suggest that probiotics do not eradicate *H. pylori* but maintain lower levels of this pathogen in the stomach; in combination with antibiotics, probiotics may increase eradication rate and/or decrease adverse effects. Papers suggesting similar effects on *H. pylori* by foodstuffs such as berry juice and some milk proteins are quoted. Regular intake of these and other dietary products might constitute a low-cost, large-scale alternative solution applicable for populations at-risk for *H. pylori* colonization.

H. PYLORI: AN IMPORTANT FACTOR FOR GASTRODUODENAL PATHOLOGIES

Helicobacter pylori is a micro-organism that colonizes specifically the surface of the mucosa of the stomach; its role as a gastric pathogen was first described by Marshall and Warren, 22 years ago,[1] in studies that earned the 2005 Nobel Prize for Physiology and Medicine. It is a Gram-negative, microaerophilic, fastidious bacterium that, in the process of colonization, induces a chronic inflammation of the mucosa without invading it. All infectious strains of *H. pylori* release large amounts of a urease that, through the degradation of urea to ammonia, is capable of buffering the gastric acid in their microenvironment. The mode of transmission of *H. pylori* remains incompletely understood but must be very efficient: in the less developed countries, the pathogen is generally acquired during childhood and, if untreated, the infection will persist throughout the life of the individual.[2, 3] *Helicobacter pylori* is one of the most prevalent pathogens as more than 50% of the world population is colonized by this micro-organism, low socio-economic level and bad hygienic conditions being the main risk factors. In consequence, the prevalence of infection is lower and begins later in developed countries where 30–40% of the population is infected in adulthood while in developing countries this rate reaches 80–90% in adulthood and most children are already colonized by 10 years of age.[2–5] Gastric colonization by *H. pylori* results in a chronic gastritis which will remain asymptomatic in most subjects who, consequently, may not be treated with antibiotics to eradicate it.

Gastroduodenal symptoms and pathologies develop in 10–15% of the infected population, with *H. pylori* being recognized as one of the aetiological agents of peptic ulcer and a risk factor for the development of gastric adenocarcinoma and lymphoma.[2–4] The determinants for severe disease outcomes include the host's genetic background, specific virulence factors present in some strains such as the cag pathogenicity island and environmental and dietary factors.[2, 6, 7] The treatment currently available for colonized symptomatic patients includes a combination of antibiotics (amoxicillin, clarythromicin, metronidazol, etc.) and a proton-pump inhibitor (omeprazol, lanzoprazol, pentoprazol, etc.) for 1–2 weeks.[2] However, this treatment does not eradicate *H. pylori* in all patients because of the appearance of antibiotic resistance and moderate patient compliance due to adverse effects such as vomiting, diarrhoea, abdominal pain and taste alterations. The high cost of the treatment, in particular for families from the low socio-economic stratum, where *H. pylori* is more frequent, also limits its adequate application. In addition, children who need to be treated tend to relapse more frequently and rapidly than adults. Therefore, it is important to develop low-cost, large-scale, alternative solutions applicable to the at-risk population to prevent or decrease *H. pylori* colonization.

The aim of the present review was to summarize the published studies using probiotics and other functional foodstuffs as possible gastroprotective agents to control *H. pylori* growth and to decrease gastric inflammation. The search strategy in Medline used the following keywords: *Helicobacter pylori*, probiotics, *Lactobacillus*, *Bifidobacterium*, *Saccharomyces boulardii*, bacteriocin, berries, functional foods, gastric ulcer and gastritis.

THE NORMAL GASTRIC MICROBIOTA AS A POSSIBLE PROTECTIVE FACTOR FOR THE GASTRIC MUCOSA

Lactobacillus species are commensal in the human alimentary tract and their concentrations in the normal stomach vary between 0 and 10^3/mL. Being acid resistant, they persist in the stomach longer than other bacteria: dietary strains of bifidobacteria and lactobacilli survive in high proportions (>80%) in the gastric environment for periods of 2 h.[8] Furthermore, some strains of *Lactobacillus* adhere to gastric epithelial cells *in vitro*[9] and also to the keratinized squamous epithelium of the mouse forestomach, probably through lipoteichoic acid.[10] This has been recently confirmed by Valeur *et al*. who reported that, after administration of 4×10^8 *L. reuteri* ATCC 55730 to healthy volunteers, it was possible to detect this strain adhering to epithelial cells from corpus and antral gastric biopsies using fluorescent *in situ* hybridization.[11]

The possible role of the local microbiota in the protection against gastric lesions is suggested by the study of Elliott *et al.*[12] in which the authors observed that the level of total aerobes in the stomach of healthy rats ranged from 10^3 to 10^4 CFU/g of tissue, with Gram-negative micro-organisms representing only 5% of the population; autochthonous gastric lactobacilli were present in all rats. However, 1 day after the induction of gastric ulcers through the serosal application of acetic acid the total aerobe count

peaked at 10^9–10^{10}/g and remained high for 1 week. At this time, Gram-negative bacteria including *Escherichia coli* represented the majority of the total aerobes while the *Lactobacillus* population disappeared. Colonization by Gram-negative bacteria occurred preferentially at the site of ulcer where a more alkaline pH (>5) was observed. The authors observed smaller ulcers in animals treated with antibiotics, suggesting that the alteration of homeostasis of the resident microbiota contributed to the development and persistence of the lesion. The ulcer healed simultaneously with the normalization of the microbiota including the reappearance of a dominant *Lactobacillus* population, and these processes were accelerated by the oral administration of lactulose, a non-digestible disaccharide used as substrate by the *Lactobacillus* population.

These findings suggest that the gastroduodenal microbiota, though low numerically, could participate in the protection of the mucosa. In consequence, the intake of exogenous lactic acid bacteria, particularly those with probiotic properties, may reinforce these protective functions in the stomach by maintaining local microbiological homeostasis, interfering with *H. pylori* and/or decreasing inflammatory processes.[13]

Probiotics are generally recognized as safe micro-organisms; they are mostly lactic acid bacteria such as lactobacilli and bifidobacteria, which are present in high concentrations in some foodstuffs; in addition, yeasts such as *Saccharomyces boulardii* are also considered as probiotics. Probiotic micro-organisms survive during their transit along the gastrointestinal tract where they modulate the gut microbiota and exert health-promoting activities beneficial for the host. The most important properties of probiotics are related to the regulation of intestinal microbiota homeostasis, interference with pathogens, modulation of local and systemic immune responses, stabilization of the gastrointestinal barrier function, decrease of procarcinogenic enzymatic activities and production of enzymatic activities of nutritional interest.[14–19] Various *in vitro* studies support the use of probiotics with the aim of interfering *H. pylori* colonization.

INHIBITION OF *H. PYLORI* BY ORGANIC ACID AND BACTERIOCIN-PRODUCING PROBIOTICS

Two main types of substances have been implicated in the inhibition of *H. pylori* by lactic acid bacteria: short chain fatty acids (SCFAs) and bacteriocins. SCFAs such as formic, acetic, propionic, butyric and lactic acids are produced during the metabolism of carbohydrates by probiotics and have an important role in decreasing pH. Bhatia *et al.* were the first to observe an antagonistic effect of a *Lactobacillus* strain against *H. pylori*[20] and to implicate SCFAs in this effect. A dose-dependent inhibition of *H. pylori* growth has been observed with acetic and lactic acid, the later demonstrating the most intense effect.[21] The amounts of lactic acid released by strains of *Lactobacillus*, *Bifidobacterium* and *Pediococcus* (50–156 mM) correlated with the intensity of their inhibitory effect against *H. pylori*. Such antimicrobial activity could be due not only to a direct effect on *Helicobacter* but also to the inhibition of its urease activity, as shown with the high lactic acid producers *L. salivarius* and *L. casei* Shirota.[22, 23] Interestingly these strains, when administered to colonized mice, induced a significant decrease in the counts of *H. pylori* in the body and antral mucosa compared with untreated animals, and resulted in a concomitant reduction in the associated gastric inflammation. A traditional yogurt originating from Asia was recently evaluated for its bactericidal activity against *H. pylori*;[24] both the yeasts (*Kluyveromyces lactis* and *Issatchenkia orientalis*) and lactobacilli (*L. crispatus* and *L. kefiri*) present in the product exert independently an anti-*H. pylori* activity. This yogurt had high levels of lactic acid and formic acid but only the latter was found to have a consistent inhibitory effect against *H. pylori*.

Bacteriocins are compounds with potential anti-*H. pylori* activity. They are small, heat-resistant and dialysable peptidic structures with antimicrobial activities, which are synthesized by several bacterial species including lactic acid bacteria. Many bacteriocins have been characterized and some of them, such as nisin, are added to foodstuffs to decrease the risk of contamination by pathogens.

The release of bacteriocins with anti-*H. pylori* activity has been chiefly studied in *Lactobacillus*, but probiotic strains of *Enterococcus faecium*,[25] *Bacillus subtilis*[26] and *Bifidobacterium*[27] could also produce heat-stable proteinaceous compounds capable of inhibiting the growth of both antibiotic-resistant and -sensitive strains of *H. pylori*. Kim *et al.*[28] evaluated, by microdilution assay, the anti-*H. pylori* activity of seven bacteriocins produced by lactic acid bacteria, including nisin A, pediocin PO2, leucocin K and various types of lacticins. Lacticins A164 and BH5 produced by strains of *L. lactis* had the most potent activity, with minimum inhibitory concentrations varying from 0.097–0.390 mg/L to

12.5–25 mg/L depending on the strains of *H. pylori* tested, indicating a strain-dependent sensitivity of this pathogen. In a co-culture experiment, Lorca *et al.* showed that after 24 h of culture an autolysate of *L. acidophilus* CRL 639 induced the release of a proteinaceous compound and the subsequent mortality of all *H. pylori* after 48 h.[29]

The supernatant of a culture of the probiotic strain, *L. johnsonii* La1 was shown to inhibit both the urease activity and growth of *H. pylori*.[30] This inhibitory activity remained functional even when *H. pylori* was bound to HT-29 epithelial cells; furthermore, this activity was not observed with La10, another strain of *L. johnsonii*, indicating that it is strain-specific, and was independent of the presence of the cag pathogenicty island in *H. pylori* strains. The supernatant inhibitory activity was heat resistant, dialysable and not affected by 10 mM urea, being therefore compatible with the presence of a bacteriocin. Although the molecule responsible for this effect was not purified and characterized by the authors, it could be lactacin F, a two-component class II bacteriocin produced by *L. johnsonii* and composed of LafA and LafX peptides which may combine to form a pore in the membrane of sensitive bacteria, resulting in the efflux of intracellular ions and the eventual death of the pathogen in this case.[31] As discussed later, the administration of this supernatant to adult patients colonized by *H. pylori* significantly decreased the values of ^{13}C-urea breath test (^{13}C-UBT).

Similar findings were observed by Coconnier *et al.* incubating *H. pylori* with the culture supernatant of *L. acidophilus* LB; the viability of the pathogen as well as its urease activity and its binding to the HT29-MTX cell line decreased in a dose-dependent manner.[32] This effect was independent of the presence of lactic acid and the pH and was not suppressed by heating at 100 °C for 1 h, supporting the presence of at least one bacteriocin. Interestingly, *H. pylori* was morphologically affected by the incubation with supernatant of the LB culture, changing from its characteristic curved form with polar flagella to U-shaped and decreased size, considered as pre-coccoid. No effects on *H. pylori* were observed by these authors using the culture supernatant of another well-known probiotic strain, *L. rhamnosus* GG (LGG). On the other hand, oral treatment of conventional mice with a supernatant of *Lactobacillus* LB culture protected the animals from infection with *H. felis* by decreasing its urease activity, inhibiting gastric colonization and preventing the development of gastric inflammation.

ANTI-INFLAMMATORY PROPERTIES OF PROBIOTIC STRAINS

As described previously, *L. acidophilus* LB and *L. johnsonii* La1 decrease gastric inflammation in colonized animals.[30, 32] This was also observed with other probiotic strains: *L. salivarius* WB1004 (10^8 CFU/mL) was able to displace *H. pylori* adhering to the MKN45 cell line and to exert an anti-inflammatory effect by decreasing dose dependently the release by these cells of IL-8.[33] Therefore, this same probiotic strain was used to evaluate its preventive effect in gnotobiotic BALB/c mice mono-colonized by *H. pylori*. Administration of *L. salivarius* prevented *H. pylori* colonization and the development of gastritis; this effect was specific of this probiotic as it was not observed with other micro-organisms such as *E. faecalis* and *S. aureus*. Administration of *L. salivarius* after infection eradicated *H. pylori* and reversed gastric inflammation. Similar observations were reported with *L. rhamnosus* R0011 and *L. acidophilus* R0052[34] and with *L. gasseri* OLL2716.[35] Furthermore, the intake of yogurt containing this latter strain protected rats in a dose-dependent manner against acute gastric lesions induced by oral administration of HCl, compared with the administration of non-fermented milk.[36] The size of the gastric lesions was decreased by yogurt and this was associated with significantly increased levels of PGE2 in the gastric mucosa. Such protective activity was inhibited when indomethacin was injected, confirming the importance of prostaglandins in this effect.

Increased levels of 6-ketoprostaglandin F1-α, EGF and bFGF have also been implicated in the protective effect displayed by strains of *B. breve* and *B. bifidum* against gastric ulceration induced by acetic acid or ethanol in rats.[37] Interestingly, the oral administration of the polysaccharide fractions of these micro-organisms exerted a similar anti-ulcer effect. The intensity of this activity correlated with the rhamnose content of the polysaccharides, those with more than 60% of rhamnose being the most effective in inducing healing of the gastric mucosa.

OTHER POSSIBLE MECHANISMS OF PROTECTION INDUCED BY PROBIOTICS

Alterations in gastrointestinal permeability are considered as an initial step in the development of lesions such as ulcers. Probiotics stabilized the intestinal barrier function in *in vitro* and animal models and in clinical

studies.[17] In healthy volunteers we determined that intake of LGG protected the gastric mucosa against alterations of permeability induced by the acute administration of non-steroidal anti-inflammatory drugs (NSAIDs), an important cause of gastroduodenal ulcer.[38] Four permeability tests were randomly carried out in every subject using sucrose as a non-invasive marker of gastric permeability: at baseline, after indomethacin administration, after 5 days of live LGG intake before indomethacin administration, and after 5 days of intake of heat-killed LGG before indomethacin administration. Excretion of sucrose in urine was significantly increased by indomethacin while administration of live LGG significantly reduced alteration in gastric permeability induced by the NSAID; heat-killed LGG had no protective effect. These findings suggest that some strains of probiotics may protect the integrity of the gastric mucosal barrier function against the deleterious effects of NSAIDs; they also show that this probiotic effect is only associated with live bacteria. Many factors may explain this protective activity: the stimulation of the expression of gastric mucins,[39] decreases in bacterial overgrowth,[12] stimulation of local immune responses[16] and release of antioxidant substances;[40] all these factors are altered in NSAID-associated gastroenteropathy and can be regulated by probiotic ingestion.

CLINICAL TRIALS WITH PROBIOTICS IN *H. PYLORI*–COLONIZED SUBJECTS

Clinical trials evaluating the use of different probiotic strains in colonized subjects have been recently reviewed by Hamilton-Miller.[41] In some of these studies, probiotics were used alone while in others they were used as adjunctive agents in the classical treatment of *H. pylori* infection. The main outcomes of these studies are the rate or the intensity of colonization, changes in the adverse effects associated with antibiotic administration and the intensity of gastric inflammation.

Utilization of probiotics in association with antibiotics in the treatment of *H. pylori*

Of the clinical trials evaluating the association of the classical antibiotic and proton pump inhibitor therapy with probiotics for *H. pylori* eradication in patients with gastroduodenal pathologies, three were randomized but not placebo controlled (Table 1). This is a critical aspect in the analysis of results because one of the main outcomes evaluated in these studies is the intensity of the adverse effects of the antibiotics and proton pump inhibitors, which has a strong subjective component. Three different probiotics were evaluated in these studies: heat-inactivated *L. acidophilus* LB,[42] *Lactobacillus* GG[43] and a yogurt (AB yogurt) containing *L. acidophilus* La5 and *B. lactis* Bb12.[44] Results showed that the administration of *L. acidophilus* LB was associated with a significant increase in the eradication of *H. pylori* from 72% to 87% ($P < 0.02$) without decreases in adverse effects. The study using LGG showed a lower incidence of side effects, especially diarrhoea, bloating and taste disturbances without an improved eradication rate. In the trial with the AB yogurt, side effects were less frequent and a higher proportion of patients completed the full week of antibiotic treatment. In the intention-to-treat analysis *H. pylori* eradication was also higher in the yogurt group (91% vs. 78%, $P < 0.05$) but this difference disappeared in the per protocol analysis. Fecal bifidobacteria decreased during antibiotic treatment in both groups of patients but in the yogurt group its numbers returned to basal values more rapidly.

Three randomized, double-blind, placebo-controlled studies were carried out with the same objective. Decreases in the side effects of the triple therapy were observed by Cremonini *et al.*[45] in a study in 85 asymptomatic subjects in whom the effects of a single probiotic (LGG or *S. boulardii*), or a combination of *B. lactis* and *L. acidophilus* administered contemporaneously with the triple therapy for 1 week and continued afterwards for an additional week were tested; similar to previous studies no effect on the clearing of *H. pylori* was observed. In another study in 53 asymptomatic volunteers, the administration of 180 mL of a *L. johnsonii* La1-acidified milk (LC-1) twice daily for 3 weeks significantly decreased the density of *H. pylori* in the gastric mucosa and the intensity and activity of gastric inflammation. Clarithromycin eradicated *H. pylori* in 26% of the subjects and LC-1 did not improve the effect of the antibiotic.[46] Side effects decreased during supplementation with *L. casei* DG in the course of quadruple therapy, resulting in a slight improvement of *H. pylori* eradication.[47]

The results of these studies suggest that probiotic supplementation during anti-*H. pylori* therapy decreases adverse side effects, resulting in better compliance and, in some cases, improved rates of eradication.

Utilization of probiotics alone

Results about the use of probiotics alone in *H. pylori*-colonized subjects are summarized in Table 2. The

Table 1. Clinical trials using probiotics in association with antibiotics in the treatment of *H. pylori* colonization

Study design	Subjects (*n*)	Eradication therapy	Probiotic	Results	References
R, O	Dyspeptic adults (120)	Rabeprazole, clarithromycin, amoxicillin	*L. acidophilus* LB for 10 days	E.R.: ↗ A.E.: no effect	Canducci *et al.*[42]
R, O	Asymptomatic adults (120)	Pantoprazole, clarithromycin, tinidazole	*L. rhamnosus* GG, 1.2×10^{10}/day for 10 days	E.R.: no effect A:E.: ↘	Armuzzi *et al.*[43]
R, O	Dyspeptic subjects (160)	Lansoprazole, clarithromycin, amoxicillin	*L. acidophilus* LA5 + *B. lactis* Bb12, 10^{10}/day for 4 weeks	E.R.: ↗	Sheu *et al.*[44]
DB, P, R	Asymptomatic adults (85)	Rabeprazole, clarithromycin, tinidazole	*L. rhamnosus* GG *S. boulardii* *Lactobacillus* LA5 + *B. lactis* Bb12, for 2 weeks	E.R.: no effect A:E.: ↘	Cremonini *et al.*[45]
DB, R, P	Asymptomatic adults (52)	clarithromycin	*L. johnsonii* LA1 acidified milk, 180 mL/day for 3 weeks	E.R.: no effect Lower gastric density of Hp Lower gastric inflammation	Felley *et al.*[46]
R	Dyspeptic patients with resistant *H. pylori* infection (70)	Esomeprazole or pantoprazole, ranitidine bismuth citrate, amoxicillin and tinidazole	*L. casei* DG, 1.6×10^{10}/day for 10 days	E.R.: no effect A:E.: ↘	Tursi *et al.*[47]

DB, double-blind; R, randomized; P, placebo-controlled; O, open; E.R., eradication rate; A.E., adverse effects.

supernatant of a culture of *L. johnsonii* La1 was tested in double-blind fashion in 20 asymptomatic volunteers who also received omeprazole or a placebo; a decrease in the δ Over the Baseline value (DOB) in the ^{13}C-UBT in comparison with the initial determinations was observed; however, *H. pylori* was not eradicated from the stomach of the affected individuals.[30]

The effect of *L. johnsonii* La1 was further explored in asymptomatic infected school children by Cruchet *et al.* in Chile.[48] In a double blind, randomized, controlled clinical trial, 252 of 326 children (77.3%) 5–16 years of age and *H. pylori*-positive by ^{13}C-UBT were allocated to one of five groups. Two groups received a product containing live *L. johnsonii* La1 or *L. paracasei* ST11 at concentrations $>10^7$ CFU/mL every day for 4 weeks; two other groups received the product but with heat-killed La1 or ST11 while the fifth group received the yogurt vehicle as a negative control. A moderate but significant decrease in the results of the ^{13}C-UBT after the treatment compared with basal DOB values was observed in children receiving live La1. The magnitude of the decrease induced by La1 was greater the higher the basal DOB values, suggesting that *L. johnsonii* La1 exerted a modulating effect on *H. pylori* colonization by restricting the size of its population in the gastric mucosa. To test whether more frequent intakes exerted a more intense effect, 80 mL of the same commercial preparation was administered to 12 adult asymptomatic volunteers every 2 h for 14 h for 2 weeks.[49] This resulted in higher decreases (40%) in DOB values; as in the previous study, higher initial DOB values correlated with greater decreases in DOB after treatment.

The effect of an La1-acidified milk (LC-1) administration was studied in 50 *H. pylori*-positive healthy volunteers who did not receive any antibiotic treatment in a randomized, controlled, double-blind study; the histology and *H. pylori* density from gastric biopsies obtained before and after 3 and 16 weeks of treatment were evaluated.[50] LC1 decreased the severity and activity of the gastritis ($P = 0.04$) as well as the density of *H. pylori* colonization. Interestingly, the thickness of the mucus layer increased during LC1 consumption. Altogether, these results suggest that *L. johnsonii* La1 may reduce the risk of disorders associated with *H. pylori* and the high risk of gastric inflammation.

Wang *et al.* observed that of the two probiotic strains present in AB yogurt, Bb12 and La5, only Bb12

Table 2. Clinical trials using probiotics alone in *H. pylori*-colonized subjects

Study design	Subjects (*n*)	Probiotic	Results	References
R, DB, P	Asymptomatic adults (20)	*L. johnsonii* La1 culture supernatant + omeprazole	^{13}C-UBT results ↘	Michetti *et al.*[30]
R, DB, P	Asymptomatic children (236)	*L. johnsonii* La1 *L. paracasei* ST11 1.8×10^9/day for 4 weeks	^{13}C-UBT results ↘	Cruchet *et al.*[48]
	Asymptomatic adults (12)	*L. johnsonii* La1 8×10^8 every 2 h during 14 h for 2 weeks	^{13}C-UBT results: ↘	Gotteland and Cruchet[49]
R, DB, P	Asymptomatic adults (50)	*L. johnsonii* La1 acidified milk for 3 and 16 weeks	↘ gastritis ↘ gastric density of *H. pylori* ↗ gastric mucus layer	Pantoflickova *et al.*[50]
P	Dyspeptic adults (70)	AB-yogurt with *L. acidophilus* LA5 + *B. lactis* Bb12, 10^{10}/day for 4 weeks	^{13}C-UBT results ↘	Wang *et al.*[51]
R	Asymptomatic children (254)	*S. boulardii* (500 mg/day) + inulin (10 g/day) *L. acidophilus* LB (heat inactivated), 10^{10}/day for 8 weeks	Eradication of 12% with Sb, 6.5% with LB	Gotteland *et al.*[52]
O	Asymptomatic adults (27)	Yogurt with *L. casei* 03, *L. acidophilus* 2412, *L. acidophilus* ACD1 + starter strains, 175 mL/day for 30 days	No effect	Wendakoon *et al.*[53]
R, P	Asymptomatic adults (14)	*L. casei* Shirota, 1.95×10^{10}, for 3 weeks	No clear effect	Cats *et al.*[66]
P	Asymptomatic adults (31)	*L. gasseri* OLL 2716, 2.2×10^9/day, for 8 weeks	^{13}C-UBT results: ↘ Pepsinogen1/2 : ↘	Sakamoto *et al.*[54]
O	Symptomatic children (12)	*L. gasseri* OLL 2716 for 8 weeks	^{13}C-UBT results: no effect *H. pylori* stool antigen: ↘ Pepsinogen1/2: ↘	Shimizu *et al.*[55]

DB, double-blind; R, randomized; P, placebo-controlled; O, open.

exerted an inhibitory effect *in vitro* against *H. pylori*.[51] They subsequently administered this yogurt (containing $>10^7$ CFU/mL of each strain) to 59 *H. pylori*-positive individuals twice daily for 6 weeks, while 11 subjects were given a placebo. The authors reported significantly lower DOB values in those receiving the AB yogurt. Moreover, a steeper decrease was observed in those individuals whose initial readings had been the highest, similar to observations by Cruchet *et al.*[48] and Gotteland and Cruchet[49] with *L. johnsonii* La1 in asymptomatic volunteers.

Gotteland *et al.* evaluated with ^{13}C-UBT the effects of *S. boulardii* plus inulin and of heat-killed *L. acidophilus* LB in comparison with triple therapy in colonized schoolchildren;[52] the probiotics were administered twice daily for 2 months. Triple therapy eradicated the micro-organism in 66% of the children compared with 12% in those receiving *S. boulardii* and 6.5% of those receiving *L. acidophilus* LB. The group receiving *S. boulardii* also showed a significant decrease in DOB values. To evaluate whether the eradication associated with probiotic intake was a spontaneous process, 81 asymptomatic colonized children were followed with ^{13}C-UBT and without any treatments for 2 months; none of them cleared spontaneously this micro-organism.

Other clinical trials have been carried out with probiotics alone, but with small numbers of subjects and without placebo control. A yogurt containing five

strains of lactic acid bacteria with strong *in vitro* inhibitory activity against *H. pylori* was investigated by Wendakoon *et al.*; the intake of 175 mL yogurt thrice daily for 30 days by 27 asymptomatic women resulted in eradication in only one case.[53] Similarly, the administration of a product with *L. casei* Shirota with anti-*H. pylori* activity thrice daily for 3 weeks to 14 subjects[54] produced a slight non-significant suppressive effect.

The effect of 90 g of yogurt with *L. gasseri* OLL2716 (LG21) administered twice daily for 8 weeks in 29 asymptomatic adults was evaluated by means of the ^{13}C-UBT and the pepsinogen I/ II ratio as a marker of gastric inflammation.[55] LG21 decreased significantly DOB values in comparison with baseline measurements and reduced the intensity of gastric inflammation as indicated by the lower pepsinogen I/II ratio. Similar results were obtained with LG21 in children.[56]

UTILIZATION OF OTHER FUNCTIONAL DIETARY COMPONENTS WITH GASTROPROTECTIVE PROPERTIES

Many foodstuffs, generally of plant origin and some milk products, have been evaluated in relation to their protective effects on the gastric mucosa and anti-*H. pylori* properties. For example 16 Chilean red wines and their extracts were tested *in vitro* against *H. pylori* and showed an inhibitory activity dependent mainly on the presence of resveratrol, a recognized antioxidant.[57] Garlic extracts have also been extensively studied, showing antimicrobial and anti-inflammatory effects in *H. pylori*-infected Mongolian gerbils.[58] Unfortunately, the extensive use of garlic in humans is limited for obvious reasons.

More recently, the investigation in this field has focused on the possible use of cranberries. Cranberry juice is traditionally used in North America to decrease risk relapses of urinary infection; the urine of people drinking cranberry juice inhibits the adhesion of uropathogens to urinary epithelial cells. It has been recently shown that high molecular weight compounds in cranberry and other berries, probably proanthocyanidins, may also interfere with the adhesion of *H. pylori* to human gastric mucus and to gastric epithelial cells.[59, 60] Furthermore, various extracts of berries, alone or in combination, have been shown to inhibit the growth of *H. pylori*.[61] Recently Zhang *et al.* administered 500 mL of cranberry juice daily for 90 days to 189 subjects in a randomized, double-blind, placebo-controlled trial.[62] ^{13}C-UBT measurements were obtained at baseline and repeated after 35 and 90 days. ^{13}C-UBT was negative in 14 of the 97 subjects (14.3%) who consumed cranberry juice. The majority (11/14) of the negative subjects receiving cranberry juice were negative at both 35 and 90 days; two control subjects also became negative during follow-up. These results are promising and efforts are currently being made to evaluate mixes of different vegetal extracts to obtain additional or synergistic effects vis-à-vis *H. pylori*.

On the other hand, Horie *et al.* have elaborated a yogurt containing antibodies against *H. pylori* urease isolated from the yolk of eggs from immunized hens. The egg yolk was present in a concentration of 1% and this provided 45 mg of the antibody. Intake of this product was associated with a statistically significant decrease in DOB values at 2 and 4 weeks and represents another avenue for the control of *H. pylori* in the mucosa of the stomach.[63]

Bovine lactoferrin was used in an open randomized clinical trial in 150 dyspeptic patients as adjunctive agent during a 1-week triple therapy for *H. pylori* infection.[64] The efficacy of triple therapy plus lactoferrin was significantly higher in the treated group than in the control group ($P = 0.01$). Other milk components such as α-lactalbumin or glycomacropeptide also exert gastroprotective properties.[65, 66]

CONCLUSIONS

The use of probiotics and foodstuffs with bioactive components in *H. pylori*-colonized subjects with gastric inflammation is supported by many observations. Specific strains of *Lactobacillus* and *Bifidobacterium* exert *in vitro* bactericidal effects against *H. pylori* through the release of bacteriocins or production of organic acids, and/or inhibit its adhesion to epithelial cells. Such protective effects have been confirmed in animal models. Some clinical trials have evaluated the effect of probiotics in colonized adults and children. Their results indicate that probiotics generally do not eradicate *H. pylori* but decrease the density of colonization, thereby maintaining lower levels of this pathogen in the stomach; in association with antibiotic treatments, some probiotics increased eradication rates and/or decreased adverse effects due to the antibiotics. On the other hand, the antioxidant and anti-inflammatory properties exerted by probiotics may stabilize the gastric barrier function and decrease mucosal inflammation. These findings confirm that, as suggested by

the 2000-Maastricht Consensus Conference on *H. pylori*, probiotic micro-organisms may be used as a 'possible' tool for the management of *H. pylori* infection and its associated gastric inflammation.

REFERENCES

1 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **8390**: 1311–5.
2 Taylor DN, Parsonnet J. The epidemiology and natural history of *Helicobacter pylori* infection. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, eds. *Infections of the Gastrointestinal Tract*. New York: Raven Press, 1995: 551–64.
3 Marshall BJ, Windsor HM. The relation of *Helicobacter pylori* to gastric adenocarcinoma and lymphoma: pathophysiology, epidemiology, screening, clinical presentation, treatment, and prevention. *Med Clin North Am* 2005; **89**: 313–44.
4 Perez-Perez GI, Rothenbacher D, Brenner H. Epidemiology of *Helicobacter pylori* infection. *Helicobacter* 2004; **9**(Suppl. 1): 1–6.
5 Vera JF, Gotteland M, Chavez E, *et al*. Sucrose permeability in children with gastric damage and *Helicobacter pylori* infection. *J Pediatr Gastroenterol Nutr* 1997; **24**: 506–11.
6 Crowe SE. *Helicobacter* infection, chronic inflammation, and the development of malignancy. *Curr Opin Gastroenterol* 2005; **21**: 32–8.
7 Gotteland M, Corvalan A, Sarmiento F, *et al*. Gastric permeability is not increased in children colonized by CagA-positive strains of *Helicobacter pylori*. *Dig Liver Dis* 2001; **33**: 750–4.
8 Marteau P, Minekus M, Havenaar R, Huis in't Veld JH. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. *J Dairy Sci* 1997; **80**: 1031–7.
9 Conway PL, Gorbach SL, Goldin BR. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J Dairy Sci* 1987; **70**: 1–12.
10 Sherman LA, Savage DC. Lipoteichoic acids in *Lactobacillus* strains that colonize the mouse gastric epithelium. *Appl Environ Microbiol* 1986; **52**: 302–4.
11 Valeur N, Engel P, Carbajal N, Connolly N, Ladefoged K. Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Appl Environ Microbiol* 2004; **70**: 1176–81.
12 Elliott SN, Buret A, McKnight W, Miller MJ, Wallace JL. Bacteria rapidly colonize and modulate healing of gastric ulcers in rats. *Am J Physiol* 1998; **275**(Pt 1): G425–32.
13 Alsahli M, Michetti P. Lactobacilli for the management of *Helicobacter pylori*. *Nutrition* 2001; **17**: 268–9.
14 Garrido D, Suau A, Pochart P, Cruchet S, Gotteland M. Modulation of the fecal microbiota by the intake of a *Lactobacillus johnsonii* La1-containing product in human volunteers. *FEMS Microbiol Lett* 2005; **248**: 249–56.
15 Servin AL, Coconnier MH. Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. *Best Pract Res Clin Gastroenterol* 2003; **17**: 741–54.
16 Sartor RB. Probiotic therapy of intestinal inflammation and infections. *Curr Opin Gastroenterol* 2005; **21**: 44–50.
17 Salminen S, Isolauri E, Salminen E. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. *Antonie Van Leeuwenhoek* 1996; **70**: 347–58.
18 Ling WH, Korpela R, Mykkanen H, Salminen S, Hanninen O. *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. *J Nutr* 1994; **124**: 18–23.
19 Marteau P, Flourie B, Pochart P, Chastang C, Desjeux JF, Rambaud JC. Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an *in vivo* study in lactase-deficient humans. *Br J Nutr* 1990; **64**: 71–9.
20 Bhatia SJ, Kochar N, Abraham P, Nair NG, Mehta AP. *Lactobacillus acidophilus* inhibits growth of *Campylobacter pylori in vitro*. *J Clin Microbiol* 1989; **27**: 2328–30.
21 Midolo PD, Lambert JR, Hull R, Luo F, Grayson ML. *In vitro* inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. *J Appl Bacteriol* 1995; **79**: 475–9.
22 Aiba Y, Suzuki N, Kabir AM, Takagi A, Koga Y. Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotic model. *Am J Gastroenterol* 1998; **93**: 2097–101.
23 Sgouras D, Maragkoudakis P, Petraki K, *et al*. *In vitro* and *in vivo* inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain Shirota. *Appl Environ Microbiol* 2004; **70**: 518–26.
24 Oh Y, Osato MS, Han X, Bennett G, Hong WK. Folk yoghurt kills *Helicobacter pylori*. *J Appl Microbiol* 2002; **93**: 1083–8.
25 Tsai CC, Huang LF, Lin CC, Tsen HY. Antagonistic activity against *Helicobacter pylori* infection in vitro by a strain of *Enterococcus faecium* TM39. *Int J Food Microbiol* 2004; **96**: 1–12.
26 Pinchuk IV, Bressollier P, Verneuil B, *et al*. *In vitro* anti-*Helicobacter pylori* activity of the probiotic strain *Bacillus subtilis* 3 is due to secretion of antibiotics. *Antimicrob Agents Chemother* 2001; **45**: 3156–61.
27 Collado MC, Gonzalez A, Gonzalez R, Hernandez M, Ferrus MA, Sanz Y. Antimicrobial peptides are among the antagonistic metabolites produced by *Bifidobacterium* against *Helicobacter pylori*. *Int J Antimicrob Agents* 2005; **25**: 385–91.
28 Kim TS, Hur JW, Yu MA, *et al*. Antagonism of *Helicobacter pylori* by bacteriocins of lactic acid bacteria. *J Food Prot* 2003; **66**: 3–12.
29 Lorca GL, Wadstrom T, Valdez GF, Ljungh A. *Lactobacillus acidophilus* autolysins inhibit *Helicobacter pylori* in vitro. *Curr Microbiol* 2001; **42**: 39–44.
30 Michetti P, Dorta G, Wiesel PH, *et al*. Effect of whey-based culture supernatant of *Lactobacillus acidophilus* (johnsonii) La1 on *Helicobacter pylori* infection in humans. *Digestion* 1999; **60**: 203–9.
31 Dalmau M, Maier E, Mulet N, Vinas M, Benz R. Bacterial membrane injuries induced by lactacin F and nisin. *Int Microbiol* 2002; **5**: 73–80.
32 Coconnier MH, Lievin V, Hemery E, Servin AL. Antagonistic activity against *Helicobacter* infection in vitro and in vivo by the human *Lactobacillus acidophilus* strain LB. *Appl Environ Microbiol* 1998; **64**: 4573–80.

33 Kabir AM, Aiba Y, Takagi A, Kamiya S, Miwa T, Koga Y. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. *Gut* 1997; **41**: 49–55.

34 Johnson-Henry KC, Mitchell DJ, Avistzur Y, Galindo-Mata E, Jones NL, Sherman PM. Probiotics reduce bacterial colonization and gastric inflammation in *H. pylori*-infected mice. *Dig Dis Sci* 2004; **49**: 1095–102.

35 Ushiyama A, Tanaka K, Aiba Y, *et al*. *Lactobacillus gasseri* OLL2716 as a probiotic in clarythromycin-resistant *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2003; **18**: 986–91.

36 Uchida M, Kurakazu K. Yogurt containing *Lactobacillus gasseri* OLL2716 exerts gastroprotective action against acute gastric lesion and antral ulcer in rats. *J Pharmacol Sci* 2004; **96**: 84–90.

37 Nagaoka M, Hasimoto S, Watanabe T, Yokokura T, Mori Y. Anti-ulcer effects of lactic acid bacteria and their cell wall polysaccharides. *Biol Pharm Bull* 1994; **17**: 1012–7.

38 Gotteland M, Cruchet S, Verbeke S. Effect of *Lactobacillus* ingestion on the gastrointestinal mucosal barrier alterations induced by indomethacin in humans. *Aliment Pharmacol Ther* 2001; **15**: 11–7.

39 Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA. Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. *Am J Physiol* 1999; **276**: G941–50.

40 Songisepp E, Kullisaar T, Hutt P, *et al*. A new probiotic cheese with antioxidative and antimicrobial activity. *J Dairy Sci* 2004; **87**: 2017–23.

41 Hamilton-Miller JMT. The role of probiotics in the treatment and prevention of *Helicobacter pylori* infection. *Int J Antimicrob Agents* 2003; **22**: 360–6.

42 Canducci F, Armuzzi A, Cremonini F, *et al*. A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. *Aliment Pharmacol Ther* 2000; **14**: 1625–9.

43 Armuzzi A, Cremonini F, Ojetti V, *et al*. Effect of *Lactobacillus* GG supplementation on antibiotic-associated gastrointestinal side-effects during *Helicobacter pylori* eradication therapy: a pilot study. *Digestion* 2001; **63**: 1–7.

44 Sheu BS, Wu JJ, Lo CY, *et al*. Impact of supplement with *Lactobacillus*- and *Bifidobacterium*-containing yogurt on triple therapy for *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 2002; **16**: 1669–75.

45 Cremonini F, Di Caro S, Covino M, *et al*. Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study. *Am J Gastroenterol* 2002; **97**: 2744–9.

46 Felley CP, Corthesy-Theulaz I, Rivero JL, *et al*. Favourable effect of an acidified milk (LC-1) on *Helicobacter pylori* gastritis in man. *Eur J Gastroenterol Hepatol* 2001; **13**: 25–9.

47 Tursi A, Brandimarte G, Giorgetti GM, Modeo ME. Effect of *Lactobacillus casei* supplementation on the effectiveness and tolerability of a new second-line 10-day quadruple therapy after failure of a first attempt to cure *Helicobacter pylori* infection. *Med Sci Monit* 2004; **10**: CR662–6.

48 Cruchet S, Obregon MC, Salazar G, Diaz E, Gotteland M. Effect of the ingestion of a dietary product containing *Lactobacillus johnsonii* La1 on *Helicobacter pylori* colonization in children. *Nutrition* 2003; **19**: 716–21.

49 Gotteland M, Cruchet S. Suppressive effect of frequent ingestion of *Lactobacillus johnsonii* La1 on *Helicobacter pylori* colonization in asymptomatic volunteers. *J Antimicrob Chemother* 2003; **51**: 1317–9.

50 Pantoflickova D, Corthesy-Theulaz I, Dorta G, *et al*. Favourable effect of regular intake of fermented milk containing *Lactobacillus johnsonii* on *Helicobacter pylori* associated gastritis. *Aliment Pharmacol Ther* 2003; **18**: 805–13.

51 Wang K-J, Li S-L, Liu C-S, *et al*. Effects of ingesting *Lactobacillus*- and *Bifidobacterium*-containing yogurt in subjects with colonized *Helicobacter pylori*. *Am J Clin Nutr* 2004; **80**: 737–41.

52 Gotteland M, Poliak L, Cruchet S, Brunser O. Effect of regular ingestion of *Saccharomyces boulardii* plus inulin or *Lactobacillus acidophilus* in children colonised by *Helicobacter pylori*. *Acta Paediatr* 2005; **94**: 1747–51.

53 Wendakoon CN, Thomson ABR, Ozimek L. Lack of therapeutic effect of a specially designed yogurt for the eradication of *Helicobacter pylori*. *Digestion* 2002; **65**: 16–20.

54 Cats A, Kuipers EJ, Boschaert MA, Pot RG, Vandenbroucke-Grauls CM, Custers JG. Effect of frequent consumption of a *Lactobacillus casei*-containing milk drink in *Helicobacter pylori*-colonized subjects. *Aliment Pharmacol Ther* 2003; **17**: 429–35.

55 Sakamoto I, Igarashi M, Kimura K, Takagi A, Miwa T, Koga Y. Suppressive effect of *Lactobacillus gasseri* OLL 2716 (LG21) on *Helicobacter pylori* infection in humans. *J Antimicrob Chemother* 2001; **47**: 709–10.

56 Shimizu T, Haruna H, Hisada K, Yamashiro Y. Effects of *Lactobacillus gasseri* OLL 2716 (LG21) on *Helicobacter pylori* infection in children. *J Antimicrobial Chemother* 2002; **50**: 617–8.

57 Daroch F, Hoeneisen M, Gonzalez CL, *et al*. *In vitro* antibacterial activity of Chilean red wines against *Helicobacter pylori*. *Microbios* 2001; **104**: 79–85.

58 Limuro M, Shibata H, Kawamori T, *et al*. Suppressive effects of garlic extract on *Helicobacter pylori*-induced gastritis in Mongolian gerbils. *Cancer Lett* 2002; **187**: 61–8.

59 Burger O, Weiss E, Sharon N, Tabak M, Neeman I, Ofek I. Inhibition of *Helicobacter pylori* adhesion to human gastric mucus by a high-molecular weight constituent of cranberry juice. *Crit Rev Food Sci Nutr* 2002; **42**(Suppl.): 279–84.

60 Lengsfeld C, Deters A, Faller G, Hensel A. High molecular weight polysaccharides from black currant seeds inhibit adhesion of *Helicobacter pylori* to human gastric mucosa. *Planta Med* 2004; **70**: 620–6.

61 Chatterjee A, Yasmin T, Bagchi D, Stohs S. Inhibition of *Helicobacter pylori in vitro* by various berry extracts, with enhanced susceptibility to clarythromycin. *Mol Cell Biochem* 2004; **265**: 19–26.

62 Zhang L, Ma J, Pan K, Go VLW, Chen J, You W. Efficacy of cranberry juice on *Helicobacter pylori* infection: a double-blind, randomized placebo-controlled trial. *Helicobacter* 2005; **10**: 139–45.

63 Horie K, Horie N, Abdou AM, *et al*. Suppressive effect of functional drinking yogurt containing specific egg yolk immunoglobulin on *Helicobacter pylori* in humans. *J Dairy Sci* 2004; **87**: 4073–9.

64 Di Mario F, Aragona G, Dal Bo N, *et al*. Use of bovine lactoferrin for *Helicobacter pylori* eradication. *Dig Liver Dis* 2003; **35**: 706–10.

65 Ushida Y, Shimokawa Y, Matsumoto H, Toida T, Hayasawa H. Effects of bovine alpha-lactalbumin on gastric defense mechanisms in naive rats. *Biosci Biotechnol Biochem* 2003; **67**: 577–83.

66 Brody EP. Biological activities of bovine glycomacropeptide. *Br J Nutr* 2000; **84**(Suppl. 1): S39–46.