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Chapter 18

Probiotics, Prebiotics, and Biogenics for the Stomach

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62828

Abstract

Recently, many studies concerning probiotics, prebiotics, and biogenics have been performed, whereas only a few are related to the stomach (about 2% as publication number). In this chapter, we focus on recent studies on probiotics, prebiotics, and biogenics for the stomach and also describe our recent research on a novel strain of lactobacillus beneficial to stomach, *Lactobacillus johnsonii* No.1088 (LJ88). As probiotics for the stomach, some beneficial strains were summarized, and underlying mechanisms of anti-*Helicobacter pylori* activity were discussed. Prebiotics for the stomach were considered as a future potential target, since no indigenous bacteria beneficial to the stomach have been found to date. As biogenics, some plant-derived candidates were discussed. In this context, recent results on LJ88 lactobacillus were presented. Orally administered LJ88 inhibited *H. pylori* growth and the increase in the number of gastrin-producing cells, which side effect is caused by triple therapy for *H. pylori*. LJ88 had no resistance to typical antibiotics, and both living and heat-killed forms of it increased the number of bifidobacteria among human intestinal-microbiota in mice. These results suggest that LJ88 is a lactobacillus beneficial to both stomach and intestine as a probiotic and biogenic.

Keywords: Probiotics, Prebiotics, Biogenics, Stomach, *Helicobacter pylori*

1. Introduction

Historically, probiotics have been thought as agents beneficial to improve the microbial environment in the intestines, but some strains of lactic acid bacteria have been used as probiotics, with the claim of providing health benefits to the stomach.

Nestlé's *Lactobacillus L. johnsonii* La1 (LC1) [1–3] and Meiji's *Lactobacillus gasseri* OLL2716 [4–6] are typical strains said to be useful to reduce the number of *Helicobacter pylori* in stomach

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infections. Recently, we found a novel strain of lactic acid bacteria, *L. johnsonii* No.1088 (LJ88), which is extremely acid resistant and also has the ability to significantly reduce the number of infective *H.pylori* in the stomach [7]. Furthermore, LJ88 not only has anti-pylori activity but also reduces excessive gastric acid production [7]. So we are very interested in the beneficial effects of probiotics on stomach health. Likewise, those effects of "Prebiotics" are also of great interest.

In addition to living bacteria, i.e., "Probiotics", heat-killed "dead" bacteria retain some beneficial properties of probiotic bacteria. For example, the ability of heat-killed LJ88 to reduce excessive gastric acid production can be thought as having this property [7]. Such food ingredients that beneficially affect the host by "direct" stimulation, suppression, etc., were defined by Mitsuoka as "Biogenics" [8]. So we added this category to this chapter. So the title of the chapter was chosen to be "Probiotics, Prebiotics, and Biogenics for the Stomach".

In this chapter, we review the current status of probiotics, prebiotics, and biogenics for the stomach, and also discuss novel aspects of our lactic acid bacterium, LJ88, which is beneficial to the stomach.

2. Number of publications

Figure 1 depicts yearly changes up to 2014 in the number of publications related to "probiotics OR prebiotics OR biogenics" as a whole (A) and those related to the stomach (B), based on a PubMed search. The total number of publications shown in Figure 1A was 14,417, of which those including the word "stomach" (Figure 1B) were only 290 (about 2% of the total publications). As shown in Figure 1A, the number of publications in this area increased almost linearly from year 2000, reaching 1936 publications in 2014; whereas the subset related to the stomach hit its ceiling at about 30 publications/year (Figure 1B).

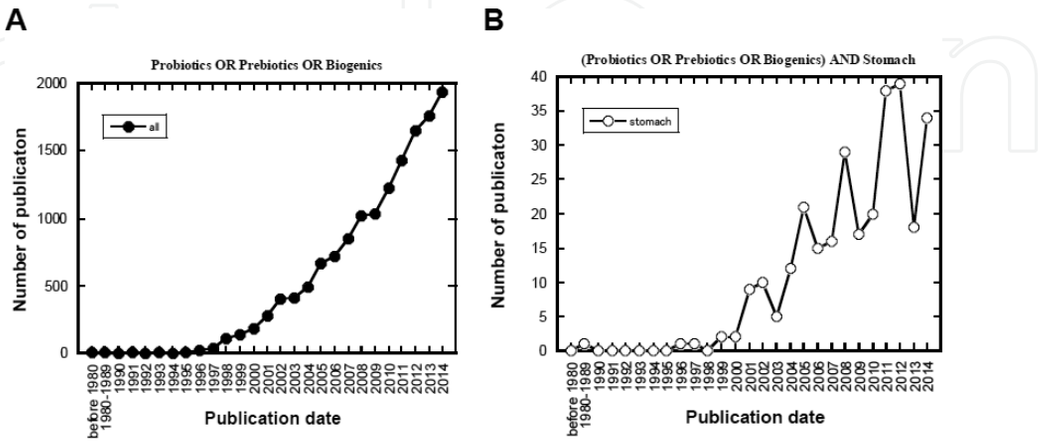

Figure 1. Yearly change in the number of publications related to probiotics/prebiotics/biogenics (A) and the subset of "A" related to the stomach (B).

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As shown above, probiotics/prebiotics/biogenics involving the stomach is not a major area of this research field. However, since a variety of bacteria have been detected not only from feces or saliva but also from gastric fluid, although mainly as dead forms [9], it is thought that this area will expand in the future.

2.1. Anti-H. pylori activity of probiotics

2.1.1. Probiotics and virulent bacteria

Although a very recent definition of probiotics is "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host" [10], probiotics have been thought as agents that improve the balance of microbiota mainly in the intestines. Typically, the ingestion of probiotics brings about an increase in the number of so-called "beneficial bacteria", e.g., bifidobacteria, and a decrease in the number of so-called "bad" bacteria, e.g., clostridia. Moreover, some probiotic strains have been reported to inhibit the growth of some virulent bacteria, resulting in prevention of and recovery from diarrhea.

As regards the stomach, *H. pylori* is the main virulent bacteria residing in the gastric mucosa, causing chronic gastritis and peptic ulcer. Also, *H. pylori* is now thought to be responsible for almost all cases of gastric cancer [11]. Some strains of probiotic bacteria have been reported to be effective in reducing the number of *H. pylori*, and also decreasing the extent of inflammation caused by infection by this bacterium.

2.1.2. Probiotic strains useful to reduce symptoms related to H. pylori infection

One of the well-known probiotic strains beneficial for the treatment of *H. pylori* infections is *L. johnsonii* La1, which was found and developed by a Swiss company, Nestlé, and has been widely used in fermented milk worldwide [1–3]. Another strain beneficial to *H. pylori*-infected subjects is *L. gasseri* OLL2716, found by Meiji, a Japanese company [4–6]. This strain is now used mainly in fermented milk in Japan as LG21 and promoted as "lactic acid bacteria combating risk" (a catchy tag from Meiji). In addition to these two strains of probiotic bacteria, some other strains have been reported to be effective in ameliorating symptoms derived from *H. pylori* infection, e.g., *Lactobacillus acidophilus* Strain LB [12], *Bacillus subtilis* 3 [13], *Weissella confusa* Strain PL9001 [14], *Lactobacillus delbrueckii subsp. bulgaricus* [15], and *Lactobacillus reuteri* [16].

2.1.3. L. johnsonii No. 1088 (LJ88) as a probiotic

Recently, we found a novel strain of lactobacillus, LJ88, in the gastric juice of a healthy human volunteer. When administered as a living form, LJ88 reduced the number of *H. pylori* in the stomach of human intestinal microbiota-bearing mice, as shown in **Figure 2** [7]. This anti-*H. pylori* effect of LJ88 can be brought not only by proliferating bacteria (Figure 2A) but also by its lyophilized form (Figure 2B), suggesting that this strain is useful both as fermented milk and also as the lyophilized form of a dietary supplement.

From Aiba et al. [7] with permission.

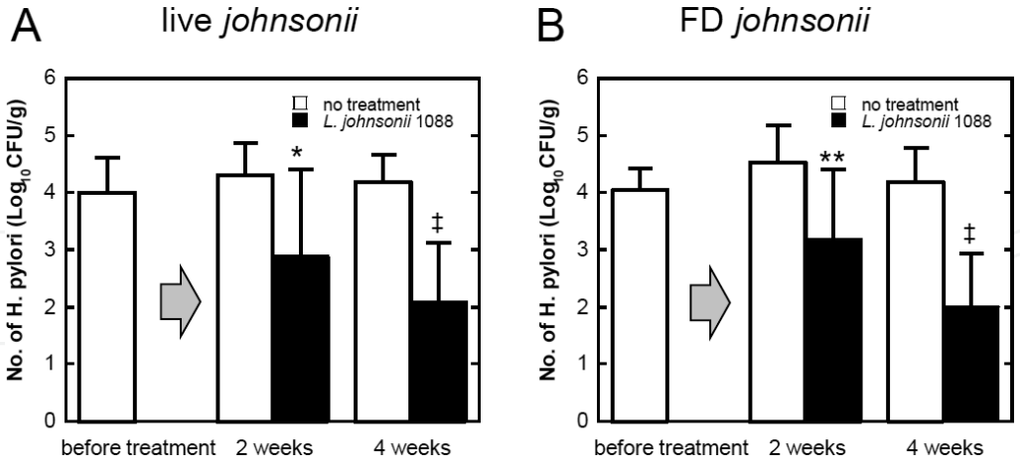

Figure 2. *Anti-H. pylori* effect of *L. johnsonii* No. 1088 (LJ88) in human intestinal microbiota-bearing mice. Mice with human intestinal microbiota were prepared by using germ-free mice and were then infected with *H. pylori* No. 130 (10^9 cfu/mice). *H. pylori*-bearing mice were orally and daily administered live LJ88 (A) or a comparable number of lyophilized cells (B) for two or four weeks. In mice treated with either live or the lyophilized (freeze-dried) form of LJ88, the number of *H. pylori* in the stomach was significantly decreased. Statistical significance was determined by use of Student's *t*-test (*$p < 0.05$, **$p < 0.01$, ‡$p < 0.0001$ vs. no treatment for comparable time periods).

To evaluate the probiotic property of LJ88, we examined the sensitivity of LJ88 to different types of antibiotics. Mueller–Hinton agar plates containing 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.031, 0.016, 0.008, 0.004, 0.002 or 0.001 μg/mL of different antibiotics (ampicillin, oxacillin, cefoxitin, gentamicin, clarithromycin, vancomycin, ciprofloxacin, and chloramphenicol) were prepared; and 5000 cfu of LJ88 (5 μL), after having been cultured in Mueller–Hinton broth for 24 h at 37 °C, was inoculated onto each plate. The minimum inhibitory concentrations (MICs) were determined after cultivation for 48 h at 37°C. The results are depicted in **Table 1**. As shown in **Table 1**, no resistance to any of the antibiotics used was observed, suggesting that LJ88 should be of no concern with respect to the transfer of drug-resistance genes to virulent bacteria.

Antibiotics	**MIC (μg/mL)**
ampicilin	0.004
oxacillin	0.125
cefoxitin	0.004
gentamicin	0.25
clarithromycin	0.5
vancomycin	0.016
ciprofloxacin	0.5
chloramphenicol	0.5

Table 1. MIC of various antibiotics against LJ88.

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To know whether LJ88 is also beneficial to intestinal microbiota, we examined the effect of live LJ88 on the number of bifidobacteria and clostridia in the feces of human intestinal microbiota-bearing mice. These mice were established as described earlier [7]. In brief, 0.5 mL of human feces diluted 100-fold with water were administered to male germ-free Balb/c mice (4 weeks old). Then 10^9 cfu of LJ88 was orally administered once a day for 2 weeks. The amount of lactobacilli, bifidobacteria, and clostridia in the feces of mice were determined before and after LJ88 administration. The results are shown in **Figure 3**. Although lactobacilli were not detected before administration of LJ88, about 10^8 cfu/g of lactobacilli appeared after its administration (Figure 3A), which might reflect the administered LJ88. In association with the administration of LJ88, the number of bifidobacteria and clostridia increased and decreased, respectively (Figure 3B and C). Since bifidobacteria are reportedly beneficial to human health due to their ability to regulate intestinal microbial homeostasis [17], the bifidobacteria-increasing effect of LJ88 is thought to be one of its beneficial effects on the intestines. Although not all of the species belonging to clostridia are virulent, some of them are known to be harmful to human health, e.g. *Clostridium difficile* [18], *Clostridium perfringens* [19], etc. So the effect LJ88 of reducing the number of clostridia in the intestines is another beneficial property of LJ88. These data taken together suggest that LJ88 is a probiotic strain of lactobacilli beneficial to both stomach and intestines.

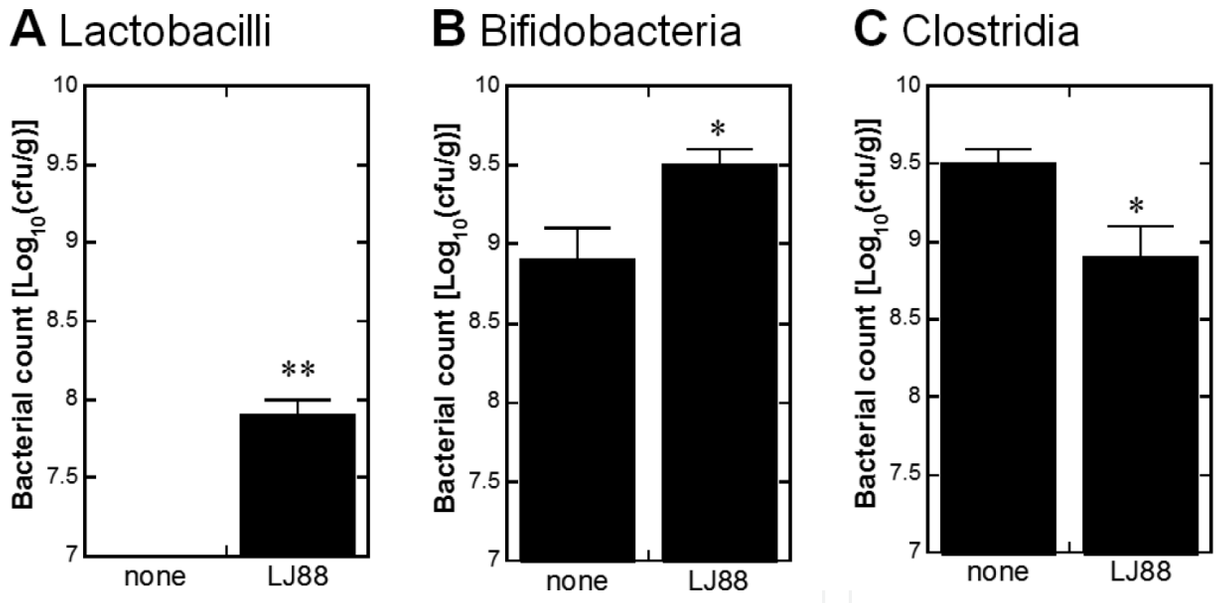

Figure 3. Effect of live *Lactocacillus johnsonii* No.1088 (LJ88) on the number of lactobacilli (A), bifidobacteria (B), and chlostoridia (C) in feces of human intestinal microbiota-bearing mice. LJ88 in the measure of 10^9 cfu was orally administered once a day for two weeks, and the number of bacteria in feces was determined. Each bar represents mean with standard deviation ($n = 5$). *$p < 0.05$, and **$p < 0.001$ vs. control by Student's *t*-test.

2.1.4. Limitation of probiotics against H. pylori

Although many reports including in vitro, in vivo, and clinical studies have suggested the effectiveness of probiotics against *H. pylori* infection, complete eradication cannot be at-

tained by probiotics alone. The standard and more effective way to eradicate *H. pylori* infections is the so-called "triple therapy" consisting of two antibiotics and one proton pump inhibitor (PPI) [20]. But the cost for such a therapy is expensive, and so a lower cost way to control the extent of *H. pylori* at a level under the asymptomatic one is needed. Moreover, since the eradication rate of this triple therapy is not 100%, probiotics effective in increasing the eradication rate of triple therapy might be meaningful. In fact, some strains have been reported to have such a property [21].

2.1.5. Possible mechanism underlying anti-H. pylori activity of probiotics

Although the exact mechanisms underlying the anti-*H. pylori* activity of probiotics have not yet been fully elucidated, some putative ones have been proposed, as shown in **Table 2**. We describe them in brief here.

Proposed Mechanisms	Described in
Lactic acid production	2.1.5.1
Production of antimicrobial products	2.1.5.2
Competition for adherent sites	2.1.5.3
Immunological mechanisms	2.1.5.4
Co-aggregation with *Helicobacter pylori*	2.1.5.5

Table 2. Putative mechanisms by which probiotics inhibit *H. pylori*.

2.1.5.1. Lactic acid

H. pylori can survive in the highly acidic gastric mucosa by producing urease, which degrades urea to ammonia and carbon dioxide, and the resulting ammonia neutralizes the gastric acid to elevate pH of surrounding environment. Lactic acid produced by probiotic bacteria competes with the pH elevation by urease mentioned above, which makes the environment unsuitable for *H. pylori* to survive [22–24]. In addition to acidification, lactic acid inhibits urease activity itself [22], which might be another molecular mechanism for lactic acid to inhibit survival of *H. pylori* in the stomach. But since not all lactic acid bacteria producing the same level of lactic acid can inhibit *H. pylori* to the same extent [3], the production of lactic acid may only be part of the anti-*H. pylori* effect of lactic acid bacteria.

2.1.5.2. Antimicrobial products

Some probiotic strains have reported to secrete antimicrobial substances other than lactic acid. The culture supernatants of *L. johnsonii* La1 [3] and *L. acidophilus* Strain LB [12] can inhibit the growth of *H. pylori* in vitro and in vivo in a pH-independent manner, but the molecular structures of these active substances have not yet been determined. Moreover, some strains of *L. delbrueckii supsp. bulgaricus* reportedly inhibit the growth of *H. pylori* in an agar-well diffusion

http://dx.doi.org/10.5772/62828

assay under both acidic and neutral pH conditions, suggesting secretion of anti-*H. pylori* substances [15]. Bacteriocins are being widely investigated as proteinaceous antimicrobial substances produced by bacteria [25]. Kim et al. examined the anti-*H. pylori* activity of selected known bacteriocins and found that lacticins A164 and BH5 produced by *Lactococcus lactis subsp. lactis* A164 and BH5, respectively, strongly inhibit the growth of *H. pylori* [26]. Other than lactic acid bacteria, another probiotic strain of bacillus, *B. subtilis* 3, has been reported to produce aminocoumacin A, another anti-*H. pylori* substance [13]. As described here, the molecular nature of almost all of the anti-*H. pylori* substances produced by probiotic bacteria is unknown and remains to be elucidated.

2.1.5.3. Competition

For *H. pylori* to grow in gastric mucosa, it is necessary first for the bacteria to adhere to the inner surface of the stomach. So if probiotics and/or its products can compete with the sites where *H. pylori* adhere, the growth of *H. pylori* might be inhibited. Kabir et al. reported that an anti-*H. pylori* strain of *Lactobacillus salivarius* inhibit the attachment of *H. pylori* to human gastric cell lines (MKN45 and KATO-III) and murine gastric epithelial cells, whereas other lactic acid bacteria not inhibiting *H. pylori* (*Enterococcus faecalis*, and also *Streptococcus aureus*) do not [27]. Furthermore, *L. reuteri* has been reported to compete with the specific binding sites of *H. pylori*, i.e., asialo-GMI and sulfatide [28]. Such competition which is either specific or nonspecific, might be one of the potential mechanisms underlying the anti-*H. pylori* activity of probiotics.

2.1.5.4. Immunological mechanisms

H. pylori infection of the stomach stimulates the production of inflammatory cytokines, such as IL-8, resulting in the activation of monocytes and dendritic cells, which then produce Tumor necrosis factors (TNF)-α, Interleukin (IL)-1, and IL-6, which in turn stimulate Th1 helper T cells [29]. Such reactions promote inflammation in the stomach to combat *H. pylori*, but these inflammatory reactions are unsuccessful to eradicate the bacteria. However, some probiotic strains have reported to reduce the extent of inflammation and to decrease the level of specific Immunoglobulin (IgG) against *H. pylori* in animal models [22, 24, 27].

2.1.5.5. Coaggregation

Coaggregation with pathogenic bacteria has been proposed as a mechanism by which probiotic bacteria can inhibit the growth of pathogenic bacterial. Recently, Holtz et al. reported that nonviable *L. reuteri* DSM17648 coaggregates with *H. pylori* and exerts anti-*H. pylori* activity [30]. So this mechanism can also be thought as one of the possible mechanisms for probiotic bacteria to inhibit *H. pylori*.

2.2. Gastric acid-reducing activity of probiotics

2.2.1. Gastroesophageal reflux disease (GERD)

Gastroesophageal reflux disease (GERD) is a chronic disease caused by backflow of gastric acid to the esophagus and is subjectively recognized mainly as heartburn. Although proton pump inhibitors (PPIs) have been strongly recommended, and their effectiveness against GERD is widely recognized, hypergastrinemia is a concern as a side-effect of long-term usage of PPIs [31]. In relation to *H. pylori*, it had been debated whether *H. pylori* infection is possibly beneficial to the host by moderating the extent of acidity of gastric juice to weaken GERD [32]. However, infection by *H. pylori* itself has not been reported to bring about any difference in subjective or objective measures of GERD [33]. *H. pylori* has another implication in GERD that is related to the adverse effects of drugs used for treat *H. pylori* infection, e.g., PPIs. Mentioned earlier, the recent standard therapy for *H. pylori* is the so-called "triple therapy" including two antibiotics and one PPI. But even after successful eradication of *H. pylori* by triple therapy, cessation of PPI may possibly bring about GERD as a side effect, which might arise because of the hypergastrinemia induced by PPIs via increased gastrin production by gastrin-producing cells (G-cells) and/or an increase in the number of G-cells in the gastric epithelia. So it would be beneficial to have the way to suppress hypergastrinemia possibly caused by PPI administration. Also, in GERD without *H. pylori* infection, a way to avoid a kind of PPI-addiction to control heartburn is desirable.

2.2.2. Probiotics effective in reducing the production of gastric acid

LJ88, as mentioned above, can reduce the number of *H. pylori* in the stomach. Moreover, LJ88 has another interesting property, i.e., that of reducing the production of gastric acid. The mechanism underlying this effect has been investigated, and it was found that LJ88 reduces the number of G-cells. Because gastrin is the hormone secreted by G-cells when stimulated by a variety of stimuli [34, 35]; e.g., distension of gastric antrum, vagal stimulation, presence of partially digested proteins (amino acids, etc.), and hypercalcemia, if the number of G-cells decreases, the maximal level of production of gastrin might be reduced without cessation of the stimuli-induced increase in the production of gastrin itself. Although the standard way to treat GERD and hyperacidity might be drugs directly inhibiting production of gastric acid, e.g., PPI, H2-blocker, and Potassium-Competitive Acid Blocker (P-CAB), probiotics reducing the number of G-cells are thought to be a mild way to treat GERD and hyperacidity. In addition to LJ88, another probiotic bacteria, *L. gasseri* OLL2716 has been reported to reduce the number of gastrin-positive cells in the stomach [36]. The exact mechanism by which these bacteria reduce the number of G-cells has not been elucidated to date, although stimulation of Toll-like receptor 2 by cell-wall components has been proposed as one candidate [7].

2.3. Implications of proton-pump inhibitors for viability of gastric microbiota

The stomach is considered to be a barrier to prevent virulent bacteria from entering the gastrointestinal tract due to its high acidity. However, irrespective of such a harmful condition for bacteria, a significant number of live bacteria exist in the stomach environment.

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Namely, in healthy persons, the number of live bacteria in gastric fluid is reportedly about 10^2–10^4 cfu/mL [9, 37]. But in subjects administered PPI, this number is reported to be increased 1000-fold or more over that of the subjects without PPI treatment, i.e., about 10^7 cfu/mL [9]. Since the pH value of gastric fluid in subjects treated or not with PPI is about 3.2 or 1.6, respectively [9], such an increase in live bacteria in the stomach is thought to be caused by the increase in pH due to the PPI administration. Interestingly, the number of bacteria quantified by real-time polymerase chain reaction (PCR) with universal primers to bacterial 16S rRNA is about 10^8 cfu/mL in gastric fluid, irrespective of treatment with PPI [9]. Because the quantitative PCR method counts not only living bacteria but also dead ones, almost all of the bacterial bodies are thought to exist in stomach as their dead form in normal subjects (>99.99% = $(1–10^4/10^8) \times 100$). In PPI-administered subjects, about 10% (= $10^7/10^8 \times 100$) exist alive in the stomach, suggesting that in such a condition, probiotics ingested might affect the stomach partly as their living form. In addition to the total number (both living and dead) of bacteria in gastric fluid, the composition of bacteria at the genus level is not different between PPI-treated and not-treated groups [9], so that a part of the effects of probiotic bacteria will be retained in the stomach even after bacterial death due to high acidity (as biogenics; see below).

3. Prebiotics for the stomach

Prebiotics were defined by Gibson and Roberfroid as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health" [38]. So if indigenous bacteria exist in stomach beneficial to host health, e.g., those corresponding to bifidobacteria in the colon, then the concept "prebiotics for the stomach" will become meaningful. However, since we do not have any evidence showing the existence of such resident bacteria in the stomach, "prebiotics for stomach" remains as a mere hypothesis for now. Of course, some beneficial indigenous bacteria may possibly be found in the stomach in the future. In such a case, "prebiotics for the stomach" will come to have a factual basis for further research and development.

4. Biogenics for the stomach

Biogenics were originally defined by Mitsuoka as "food ingredients that beneficially affect the host by direct immunostimulation, suppression of mutagenesis, tumorigenesis, peroxidation, hyper-cholesterolemia or intestinal putrefaction" [8]. He proposed the following agents as candidates of biogenics: i.e., biological response modifier (BRM), carotenoids, flavonoids, eicosapentaenoic acid, docosahexaenoic acid, lacto-tripeptide, immunopotentiators, etc. [8] Although Mitsuoka's original concept of biogenics seems not to have included beneficial effect to the stomach, we think that agents directly affecting the stomach could be thought as a kind of biogenic as well.

4.1. Heat-killed bacteria as biogenics for the stomach

4.1.1. Gastric acid-reducing activity of heat-killed bacteria

One of the characteristic effects of our LJ88 is the reduced production of gastrin, as mentioned above. We found that such an effect is the property of not only living bacteria but also heat-killed ones [7, 36], allowing LJ88 to be thought as a kind of biogenics for the stomach. We already mentioned about a possible side effect of PPI, i.e., an increase in the number of G-cells, which might cause gastric hyperacidity after cessation of PPI. Especially, such a side effect might be of concern after triple therapy to eradicate a *H. pylori* infection.

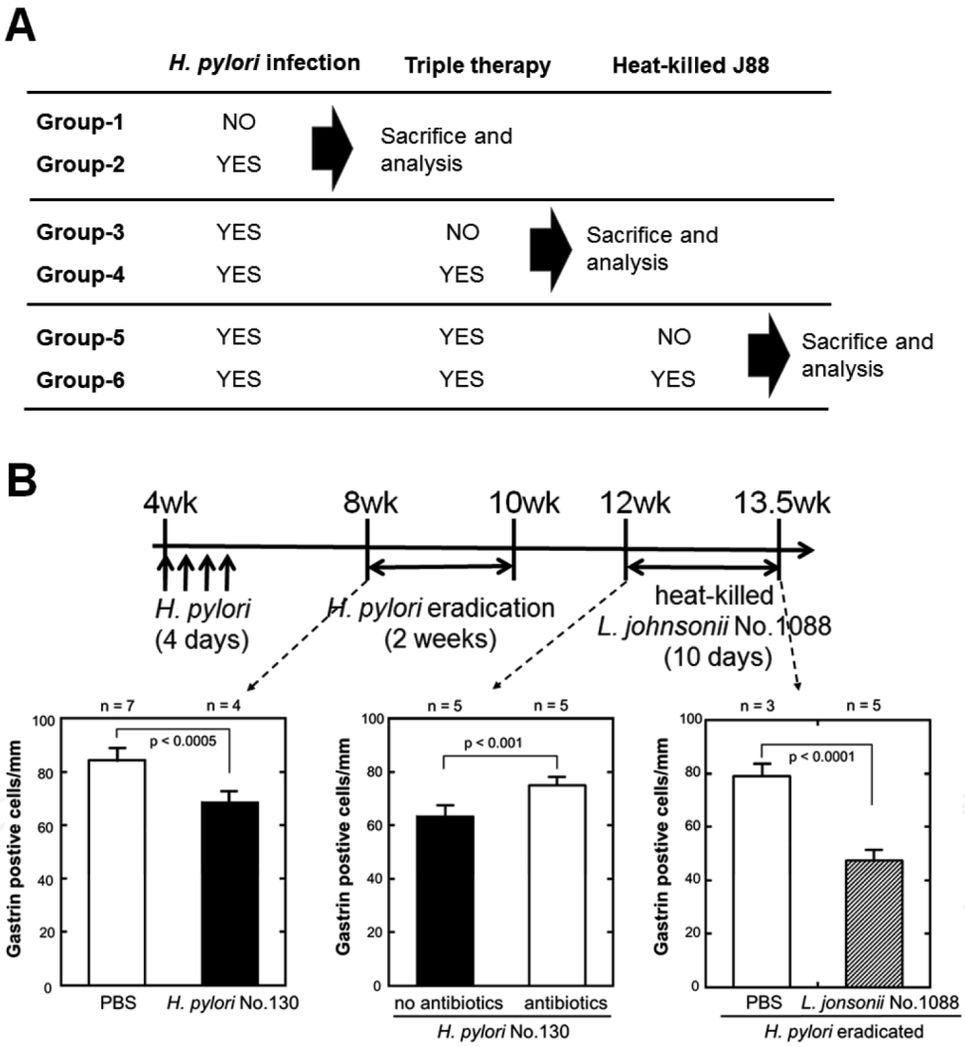

Figure 4. Increase in the number of gastrin-positive cells by *H. pylori* eradication with triple therapy including PPI, and its decrease by treatment with heat-killed *L. johnsonii* No. 1088 (LJ88). (A) Summary of different treatments of six experimental groups. (B) Results of the experiment. *H. pylori* infection of germ-free mice decreased the number of gastrin-positive cells (left-side bar graph), whereas treatment with triple therapy including PPI reverted the number of gastrin-positive cells to a higher level (middle bar graph). However, treatment with heat-killed LJ88 significantly decreased the number of gastrin-positive cells (right-side bar graph). Statistical significance was determined by use of Student's *t*-test.

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To determine if LJ88 would ameliorate such a side effect of PPI in the context of triple therapy, we did an animal experiment with germ-free Balb/c mice infected with *H. pylori*. Six groups of germ-free Balb/c mice (four weeks old), each consisting of three to seven mice, were used for this experiment. The different treatments of these six experimental groups (Groups-1 to -6) are summarized in (**Figure 4A**). The mice of five groups (Groups-2 to -6) were orally administered 10^9 cfu of *H. pylori* once a day for four consecutive days, and the remaining group (Group-1) was administered PBS by the same route as a control. Four weeks after the administration of *H. pylori* or PBS, two groups (PBS and *H. pylori* groups; Groups-1 and −2) were sacrificed and examined for the difference in the number of gastrin-positive cells in their stomach as described previously [7, 36]. Three of the remaining four groups (Groups-4 to -6) with *H. pylori* administration were started to be treated with triple therapy [20, 39] (omeprazole, 150 μg/day; amoxicillin 3.75 mg/day; and clarithromycin, 2 mg/day), which was continued for two weeks. The last group (Group-3) was not administered any drugs for the same two weeks. After the triple therapy, two groups (with and without triple therapy; Groups-3 and -4) were sacrificed and analyzed for the number of gastrin-positive cells as above. Finally, the remaining two groups were orally administered (Group-6) or not (Group-5) 10^9 heat-killed LJ88 cells for 10 days; and 24 h after the last administration, these two groups were examined for their number of gastrin-positive cells.

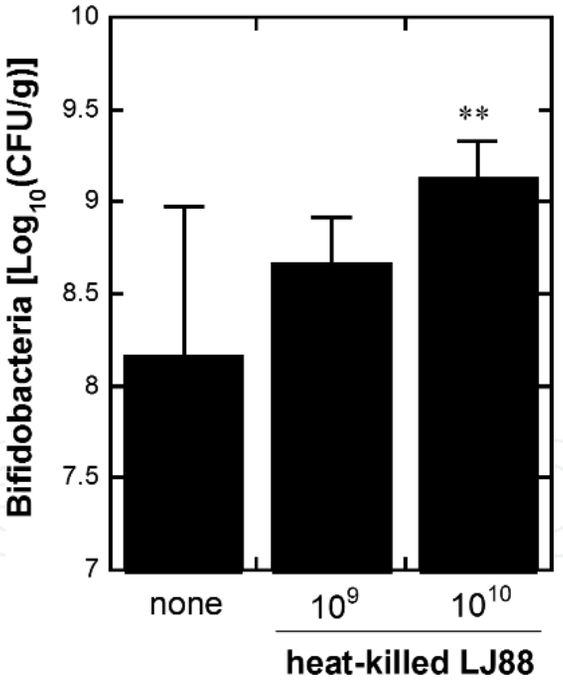

Figure 5. Effect of heat-killed *L. johnsonii* No. 1088 (LJ88) on the number of bifidobacteria in feces of human intestinal microbiota-bearing mice. Heat-killed LJ88 (10^9 and 10^{10} cells) were orally administered once a day for two weeks, and the number of bifidobacteria in the feces was determined. Each bar represents the mean with standard deviation ($n = 5$). $^{**}p < 0.01$ vs. control by Dunnett's *t*-test.

The results are shown in Figure 4B. *H. pylori* infection decreased the number of gastrin-positive cells (left-side bar graph), whereas treatment with antibiotics including PPI reverted the

number of gastrin-positive cells to a higher level (middle bar graph). However, treatment with heat-killed LJ88 significantly decreased the number of gastrin-positive cells (right-side bar graph). These results suggest that LJ88, even in its heat-killed form, can prevent the increase in gastric acid after triple therapy by decreasing the number of gastrin-positive cells, the effect of which might be beneficial for prophylaxis of GERD. Since this result was obtained by using a mouse model, it should be examined whether or not the same mechanism works also in humans.

Since live LJ88 were beneficial not only to the stomach but also to intestinal microbiota, as shown in **Figure 3**, we examined the effect of heat-killed LJ88 on intestinal bacteria by determining the number of bifidobacteria in the feces of human intestinal microbiota-bearing mice. As shown in **Figure 5**, heat-killed LJ88 increased the number of bifidobacteria in the feces by the administration of 10^{10} cells for two weeks, suggesting that heat-killed LJ88 might also be beneficial to not only the stomach but also to the intestines as well.

4.1.2 Anti-H. pylori activities of heat-killed bacteria

We already described that some probiotic strains have anti-*H. pylori* activity, and possible mechanisms underlying such an activity were discussed (Section 3 and listed in **Table 2**). Among them, some mechanisms can be expected to belong not only to live bacteria (probiotics) but also to heat-killed ones (biogenics).

One possible mechanism might be competition between *H. pylori* and probiotic bacteria for adherence sites on gastric epithelial cells. So some probiotic strains proposed to compete for adherence sites on gastric surface might have anti-*H. pylori* activity even in their heat-killed forms. However, no such examples have been reported to date.

Another potential mechanism might be coaggregation with *H. pylori*. Examining the anti-*H. pylori* effect of heat-killed *Lactobacius reuteri* DSM17648, Holz et al. found that it coaggregates well with *H. pylori* both in vitro and in vivo, and that it exerts anti-*H. pylori* activity also in the clinical situation [30]. This pioneering result suggests that other probiotic strains having anti-*H. pylori* activity are worth being examined for their ability to coaggregate with *H. pylori*.

4.2 Soybean-related products as biogenics for the stomach

Historically, it has been suggested that soy products prevent the incidence of various cancers including gastric cancer, and several meta-analysis studies concluded that nonfermented and fermented soy foods reduce and increase, respectively, the risk of gastric cancer [40, 41]. However, it has also been suggested that "nonfermented" and "fermented" soy foods are possibly associated with "fruit/vegetable" and "salt intake," respectively [40, 41]. So preventive and stimulatory effects of nonfermented and fermented soy foods should be considered taking these factors in mind. Since isoflavones are one of the proposed molecular candidates for preventing gastric cancer, a large-scale, population-based, prospective, cohort study was conducted to investigate the relationship between isoflavone-intake and risk of gastric cancer in Japan [42]. The results suggested that higher intake of isoflavones does not prevent gastric

cancer [42]. So even if nonfermented soy foods can reduce the risk of gastric cancer, the responsible molecules might not be isoflavones in soy foods. However, since genistein, which is one of the soybean isoflavones, reportedly has a protective effect against stress-induced gastric mucosal lesions in rats [43], soy foods might be beneficial to the stomach even if their cancer-preventing effects are not so large.

4.3. Brassicaceae vegetable-related products as biogenics for the stomach

Vegetables of Brassicaceae classification, including cabbage and broccoli, reportedly contain S-methylmethionine, also known as vitamin U. S-methylmethionin is a useful ingredient originally found as anti-ulcerogenic factors in raw cabbage juice [44, 45], and has been used as an ingredient of gastrointestinal drugs in Japan for over 50 years, e.g., Cabagin U. [46]. So Brassicaceae vegetables might be thought as good biogenics for the stomach for treatment and/or prevention of gastric ulcer.

Furthermore, broccoli sprouts especially contain sulforaphane, an isothiocyanate compound reported to have anti-*H. pylori* activity both in vitro [47] and in vivo [48]. Sulforaphone also has been reported to have protective and reparative effects against oxidative stress in gastric mucosa by stimulating nrf2 gene-dependent antioxidant enzyme activities, and also to have anti-inflammatory effects on gastric mucosa during *H. pylori* infections [49]. So among Brassicaceae vegetables, broccoli sprouts are thought to be an especially beneficial biogenic for the stomach.

4.4. Other natural products beneficial to the stomach, including those with anti-H. pylori activity

Because of the wide variety and expected low toxicity of natural products, extracts and essential oils prepared from various plants have been examined their anti-ulcer and anti-*H. pylori* activities. Bonifácio extensively reviewed such products, including 21 different plant extracts and 18 different essential oils [50]. Most of the extracts and essential oils, described in the review mentioned above, were examined only in vitro, although some of them have been evaluated in vivo as well. Bonamin et al. reported that a methanol extract and its enriched alkaloid fraction of a Brazilian plant, *Strychnos pseudoquina* St. Hil. (Loganiaceae), were effective against gastric ulcer induced by acetic acid, and also had anti-*H. pylori* activity in vitro [51]. Extracts of other Brazilian plants, e.g., *Qualea parviflora* Mart. (from bark) [52], *Hancornia speciosa* Gomez (Mangaba; from bark) [53], and *Byrsonima intermedia* A. Juss. (Malpighiaceae; from leaves) [54], have also been reported to have anti-ulcer activity in vivo and anti-*H. pylori* activity in vitro. Ohno et al. reported that 13 different essential oils prepared from a variety of plants inhibited the growth of *H. pylori* in vitro [55]. Among them, essential oils from *Cymbopogon citratus* (lemongrass) and *Lippia citriodora* (lemon verbena) were found to be bactericidal [55]. They also found that essential oil from lemongrass inhibited *H. pylori* in a murine model [55]. Thus, natural sources including herbal and medicinal plants can be thought of as future promising sources of new biogenics for the stomach.

5. Future directions

In this report, we discussed probiotics, prebiotics, and biogenics for the stomach. As shown in **Figure 1**, this research area remains small to date, as only 2% of the total volume of publications concerning "probiotics, prebiotics, or biogenics" as a whole has focused on the stomach. However, the research efforts made related to this interesting research field, as mentioned in this review, are none the less very significant. We think future research in this field will go in the following directions:

Concerning probiotics for the stomach, a search for new probiotic strains beneficial to the stomach is warranted. Although no probiotic bacteria able to reside and grow in the stomach have yet been found, the possible existence of such a kind of so-called "extremophile" [56] type of probiotic bacteria cannot be denied in principle. Indeed, most researchers did not believe in the existence of indigenous bacteria in the stomach until 1984, when *H. pylori* was first described to exist there [57]. Other extremely acid-resistant probiotic strains that can survive in the stomach for a significant time period even if not able to grow there, such as our LJ88, will be a more promising type of bacteria as probiotics for the stomach.

However, since "extremophile" probiotics or indigenous bacteria beneficial to the stomach have not been found to date, prebiotics for such bacteria are also unknown as well. If such bacteria are found in the future, compounds supporting the growth of these bacteria in the stomach may be regarded as "prebiotics for the stomach." Specific substances specifically utilized by supposed stomach bacteria beneficial to the host might be such candidates.

As described in this report, some strains of heat-killed bacteria are thought to be good biogenics for the stomach, as they, like LJ88, might be effective as anti-*H. pylori* agents and also as gastrin-inhibiting ones. Such novel kinds of more effective bacteria may possibly be found in the future. Moreover, the possibility of new biogenics for the stomach, derived from natural sources, e.g., vegetables, fruits, traditional medicinal plants, fungi, products of microorganisms, and marine organisms, should be examined, and promising candidates may well be found in the future.

Practically speaking, appropriate combinations of probiotics, prebiotics (putative), and biogenics might be important for stomach health.

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