Probiotic Mechanism of *Lactobacillus gasseri* OLL2716 Strain against *Helicobacter pylori*

*Helicobacter pylori* infection is a major risk factor for several gastroduodenal diseases, including gastric ulcer, duodenal ulcer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and distal gastric cancer (4, 6, 9). Although it is unknown when and how the human population became infected with *H. pylori* in developed countries, Weyermann et al. reported that *H. pylori* is almost always acquired in early childhood (11). Probiotics intended for the control of *H. pylori* as well as for antibacterial chemotherapy against *H. pylori* have attracted attention (10). Current systematic reviews describe probiotics as an adjunct to first-line triple therapy and not as antibacterial chemotherapy. Whether adjunctive therapy enhances eradication rates remains

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**FIG 1** Coccoid conversion of *H. pylori* strains caused by *L. gasseri* OLL2716 or DL-lactic acid. (A) *H. pylori* ATCC 43504. (B) *L. gasseri* OLL2716. *L. gasseri* OLL2716 was added to *H. pylori* on agar plates and cocultured for 24 h (C) and 48 h (D). Coccoid conversion of *H. pylori* was observed after coculture for 24 h (C), and after 48 h, the number of coccoids decreased (D). Either 0.1% (0.9 g/liter) (E) or 1% (9 g/liter) (F) DL-lactic acid was added to *H. pylori* cultured on agar plates, and coccoid conversion of *H. pylori* was observed (E). However, reduction of the size and number of *H. pylori* organisms was observed with 9% lactic acid (F). Magnification, ×5,000. Bar, 2 μm.
FIG 2 Quantities of \( \text{D}-\text{lactic acid} \) produced by \( \text{Lactobacillus gasseri OLL2716} \) and other \( \text{Lactobacillus} \) species.

controversial, but there is more evidence supporting the role of probiotics in reducing the frequency of side effects caused by the triple-therapy regimen (7). \( \text{Lactobacillus gasseri OLL2716} \) proved effective in both suppressing \( \text{H. pylori} \) colonization of the stomach and reducing gastric mucosal inflammation (8). We reported that an \( \text{L. gasseri OLL2716} \) strain contained in a yogurt drink can colonize the gastric mucus layer in infected patients (3). However, the mechanism by which that \( \text{L. gasseri OLL2716} \) strain controlled \text{H. pylori} \) strains was unknown.

Probiotic effects of \( \text{L. gasseri OLL2716} \) against the \( \text{H. pylori} \) ATCC 43504 strain were investigated. A total of 10 \( \mu \text{g/ml} \) of \( \text{L. gasseri OLL2716} \) solution (10\(^{5}\) CFU/ml) was added to \( \text{H. pylori} \) cultured for 24 h on 5% sheep blood agar plates, and the strains were cocultured at 37°C under microaerobic conditions for 24 h and 48 h. The two cocultured strains were adhered to cover glasses by treating them with Cell-Tak (BD Biosciences, Tokyo). The seven \( \text{L. gasseri OLL2716} \) strains cocultivated with \( \text{L. gasseri OLL2716} \) for 24 h converted from rods to coccoids (Fig. 1). We cultured these \( \text{H. pylori} \) coccoids again. However, they did not grow on sheep blood agar plates.

The quantity of \( \text{D}-\text{lactic acid} \) produced by \( \text{Lactobacillus} \) organisms, including \( \text{L. gasseri OLL2716} \), was measured. Both \( \text{H. pylori} \) strains (10\(^{5}\) CFU/ml) and each \( \text{Lactobacillus} \) strain (10\(^{5}\) CFU/ml) were cocultured at 37°C under microaerobic conditions for 48 h in Brucella broth (Difco, MD) with 5% defibrinated sheep blood. The quantity of \( \text{D}-\text{lactic acid} \) produced by each \( \text{Lactobacillus} \) strain was determined by spectrophotometry using an F kit (Roche Diagnostic, Japan, Tokyo). The seven \( \text{Lactobacillus} \) strains investigated were \( \text{Lactobacillus gasseri OLL2716} \) and clinical isolates of \( \text{Lactobacillus gasseri} \)-1, \( \text{Lactobacillus plantarum} \), \( \text{Lactobacillus salivarius} \), \( \text{Lactobacillus fermentum} \)-1, \( \text{L. fermentum} \)-2, and \( \text{L. fermentum} \)-3. The quantity of \( \text{D}-\text{lactic acid} \) produced by \( \text{L. gasseri OLL2716} \) was about 1.4 g/liter (Fig. 2). That of other \( \text{Lactobacillus} \) organisms except \( \text{L. fermentum} \)-1, -2, and -3 was almost the same. To confirm the morphological conversion of \( \text{H. pylori} \), 10 \( \mu \text{l} \) of either 0.1% (0.9 g/liter) or 1% (9 g/liter) \( \text{D}-\text{lactic acid} \) (Wako, Osaka) was added to the \( \text{H. pylori} \) strain cultured on agar plates. After 24 h, a similar coccoid conversion of \( \text{H. pylori} \) was observed in cells treated with 0.1% \( \text{D}-\text{lactic acid} \) (Fig. 1E). Furthermore, the number of \( \text{H. pylori} \) cells decreased when exposed to 9 g/liter of \( \text{D}-\text{lactic acid} \). These coccoid-shaped \( \text{H. pylori} \) cells did not grow on sheep blood agar plates. These results showed that the \( \text{L. gasseri OLL2716} \) strain suppressed the growth of \( \text{H. pylori} \). Generally, bacteria of a coccoid form are in a dormant state and do not multiply, although they are live bacteria. However, whether coccoid forms of \( \text{Helicobacter} \) spp, are dormant or simply dead is the subject of ongoing controversy (1).

The effect of probiotics to completely eradicate \( \text{H. pylori} \) by \( \text{Lactobacillus} \) supplementation is not yet shown. However, they can suppress \( \text{H. pylori} \) colonization (2). In this study, we confirmed coccoid conversion of \( \text{H. pylori} \) by \( \text{D}-\text{lactic acid} \) produced by the \( \text{L. gasseri OLL2716} \) strain and the suppression of \( \text{H. pylori} \) multiplication. Because the quantities of \( \text{D}-\text{lactic acid} \) produced by various \( \text{Lactobacillus} \) strains, such as \( \text{L. plantarum} \) and \( \text{L. salivarius} \), were similar, those strains may be effective against \( \text{H. pylori} \) infection. However, after screening over 200 \( \text{Lactobacillus} \) strains, Kimura found that the \( \text{L. gasseri OLL2716} \) strain has strong anti-acid properties (5). Furthermore, this strain can compete with \( \text{H. pylori} \) in the human gastric mucus layer and suppresses \( \text{H. pylori} \) infection (3).

In conclusion, though further studies including factors other than lactate production are necessary, we showed that the mechanism of the probiotic \( \text{L. gasseri OLL2716} \) strain against \( \text{H. pylori} \) was coccoid conversion of \( \text{H. pylori} \) due to \( \text{D}-\text{lactic acid} \). This is the first report that showed that the \( \text{L. gasseri OLL2716} \) strain induces the coccoid conversion of \( \text{H. pylori} \) by using an electron microscope. The incidence of antibiotic-resistant \( \text{H. pylori} \) has increased in recent years. The combination of probiotics using \( \text{L. gasseri OLL2716} \) and conventional eradication therapy may be effective for the control of these resistant strains. When they are ingested through yogurt, \( \text{L. gasseri OLL2716} \) strains which are acid proof to gastric juice will be effective as probiotics against \( \text{H. pylori} \) infection.

REFERENCES

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