Anti-*Helicobacter pylori* Activity of Antimicrobial Substances Produced by Lactic Acid Bacteria Isolated from Baikkimchi

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**Abstract** The purpose of the present study was to determine the antagonistic activity of the 13 lactic acid bacteria strains isolated from Baikkimchi made with (*Brassica rapa*, subspecies *pekinesis* and *chinensis*) against *Helicobacter pylori* ATCC 43504 in vitro. Relatively good growth properties were found for *Lactobacillus brevis* BK11, *Lactobacillus acidophilus* BK13, and *Leuconostoc mesenteroides* BK26 strains with residual numbers of >10^6 CFU/mL after incubation for 2 h in artificial gastric juice. In co-culturing experiments, *Lactobacillus plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *Pediococcus pentosaceus* BK34, *Lactobacillus paracasei* BK57, *Enterococcus faecalis* BK61, and *Lactococcus lactis* BK65 showed significant antimicrobial ability against *H. pylori*. The cell-free culture supernatants (CFCSs) obtained from *L. brevis* BK11, *L. acidophilus* BK13, *P. pentosaceus* BK34, *L. paracasei* BK57, and *L. lactis* BK65 strains producing very high levels of lactic acid dramatically decreased the viability of *H. pylori*. In addition, the bactericidal activity of *L. plantarum* BK10, *L. brevis* BK11, *L. acidophilus* BK13, *L. paracasei* BK57, and *E. faecalis* BK61 strains was significantly correlated with the bacteriocin production. The CFCS and bacteriocin solutions produced from the strains except for *E. faecalis* BK61 were effective in inhibiting the adhesion of *H. pylori* to human stomach adenocarcinoma cells and their urease activity.

**Keywords** adhesion · antimicrobial substance · *Helicobacter pylori* · lactic acid bacteria · urease

**Introduction**

*Helicobacter pylori*, the pathogen allowing colonization of the harsh stomach environment, has recently been shown to be an important etiologic agent of chronic gastritis as well as peptic ulcer and gastric cancer (Hamilton-Miller, 2003). At present, the standard triple therapy consisting of two kinds of antibiotics (clarithromycin and amoxicillin) and a proton pump inhibitor is regarded as a good treatment to eradicate *H. pylori* infection (Malfertheiner et al., 2002). Although antibiotic-based therapies are efficient, the use of a large dose of antibiotics has caused a rapid emergence of antibiotic-resistant strains and several side effects such as diarrhea, vomiting, nausea, and metallic taste (Matsumoto et al., 1997). As a consequence, the need for novel therapeutic approaches, alternative or complementary to antibiotic therapy, has claimed the attention of many researchers (Canducci et al., 2002).

According to the previous study, one of the alternative anti-*H. pylori* treatments involves an application of probiotic strains, defined as living microorganisms that may confer a health benefit on the host (Patel et al., 2013). Probiotic organisms could be exploited as potential therapeutic agents to eradicate intestinal infections and as adjuncts to current therapy strategies, because lactic acid bacteria (LAB) may improve antibiotic therapy tolerability of the traditional eradication methods by reducing its side effects (De Bortoli et al., 2007).

Because LAB are acid-tolerant and able to persist in the stomach longer than other bacteria, some LAB preparations have been extensively studied for their ability to protect against pathogens such as *H. pylori* (Ryan et al., 2008; Tsai et al., 2004). *In vitro* and animal data indicate that probiotic LAB, *Lactobacillus* sp., and *Bifidobacterium* sp. can inhibit the growth of the pathogens and decrease urease activity necessary for *H. pylori* to remain in the acidic environment of the stomach (Aiba et al., 1998).

Several authors have previously indicated that probiotic...
lactobacilli are able to inhibit potential pathogens growth owing to the production of antimicrobial substances (Midolo et al., 1995; Hamilton-Miller, 2003). Furthermore, distinct probiotic strains may facilitate the stabilization of gut mucosal barrier and the coaggregation with pathogens (Patel et al., 2013). Other mechanisms include the strengthening of gastric barrier function due to mucin production and the competition with *H. pylori* for adhesion sites on gut wall and nutrients (Kim et al., 2008). Animal studies suggested that probiotic bacteria could modify the humoral immune response of the host by interacting with epithelial cells and controlling the balance of proinflammatory and anti-inflammatory cytokines, which may result in reduction of gastric trouble symptoms (Muroskai et al., 2000). Probiotic LAB alter the composition of gastrointestinal flora and inhibit the growth of pathogens by producing the antimicrobial substances, such as short-chain fatty acids, diacetyl, hydrogen peroxide, as well as various bacteriocins (Midolo et al., 1995; Hamilton-Miller, 2003). Michetti et al. (1990) reported that a strain of *Lactobacillus acidophilus* produces an antimicrobial substance that reduced the viability of *H. pylori*. Tsai et al. (2004) indicated that the spent culture supernatant of *Enterococcus faecium* TM39 significantly inhibited the viability and the urease activity of *H. pylori*.

The purpose of the present study was to determine the antagonistic activity of the LAB strains isolated from Baikkimchi (Kimchi made without red pepper powder; *Brassica rapa*, subspecies *pekinesis* and *chinensis*) against *H. pylori* ATCC 43504 *in vitro*. Furthermore, the inhibitory effects of the adhesion of *H. pylori* cells to the epithelial cell lines and the urease activity of the adhered strains by the antimicrobial substances obtained from the isolated strains were evaluated.

**Materials and Methods**

**LAB isolation, growth conditions, and identification.** A total of 13 LAB strains were collected directly from 10 samples of Korean fermented vegetable food, Baikkimchi. The samples were serially diluted in sterile phosphate buffer solution (PBS, pH 7.0), spread directly onto the surface of Lactobacilli MRS agar (Difco, USA) supplemented with 1% CaCO₃, and incubated at 37°C for 48 h. Distinct colonies from each plate were randomly picked, purified by replating on MRS agar plates, and maintained on MRS agar broth containing 5% (v/v) fetal bovine serum (FBS, Gibco BRL, USA), 0.2% (w/v) 2,6-di-β-methyl-β-cyclodextrin (CD), and antibiotics (cefuroxim, vancomycin, trimethoprim, and amphotericin B) kept in microaerobic conditions (10% CO₂, Anaeromat system, MART Co., Netherlands). Stock culture was kept at −80°C in broth containing 20% (v/v) glycerol until further study.

**H. pylori cell growth.** *H. pylori* ATCC 43504 strain used in the present study was obtained from American Type Culture Collection (ATCC). *H. pylori* strain was cultured at 37°C for 48 h in Brucella broth (Difco) supplemented with 5% (v/v) fetal bovine serum (FBS, Gibco BRL, USA), 0.2% (v/v) 2,6-di-β-methyl-β-cyclodextrin (CD), and antibiotics (cefuroxim, vancomycin, trimethoprim, and amphotericin B) kept in microaerobic conditions (10% CO₂, Anaeromat system, MART Co., Netherlands). Stock culture was kept at −80°C in broth containing 20% (v/v) glycerol until further study.

**Co-culture of H. pylori with the LAB strain.** For co-culture experiment, the LAB strains cultured for 24 h were harvested and washed twice (7,000×g, 10 min, 4°C) with sterile PBS (pH 7.2), and finally resuspended in Brain Heart Infusion (BHI) broth (Difco) at 1.0×10⁸ CFU/mL. *H. pylori* cells were grown in Brucella broth containing 5% (v/v) FBS, CD, and antibiotics at 37°C under microaerobic conditions. Cell pellets of two strains collected by centrifugation (7,000×g, 10 min, 4°C) were washed twice with sterile PBS (pH 7.0). Fresh *H. pylori* cells (1.0×10⁹ CFU/mL) suspended in antibiotic-free Brucella broth (0.1 mL) containing the LAB cells were incubated under microaerobic condition for 12 to 48 h at 37°C. Viability of *H. pylori* was evaluated based on the number of viable cells of *H. pylori* cultured under optimal condition.

**Measurements of viable cell counts, pH, and titratable acidity.** Overnight cultures of the LAB strains were inoculated in MRS broth and proliferated at 37°C for 24 h. After incubation, viable