

Microbiota changes with fermented kimchi contributed to either the amelioration or rejuvenation of *Helicobacter pylori*-associated chronic atrophic gastritis

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Korean fermented kimchi is probiotic food preventing *Helicobacter pylori* (*H. pylori*)-associated atrophic gastritis in both animal and human trial. In order to reveal the effect of fermented kimchi against *H. pylori* infection, we performed clinical trial to document the changes of fecal microbiota in 32 volunteers (*H. pylori* (-) chronic superficial gastritis (CSG), *H. pylori* (+) CSG, and *H. pylori* (+) chronic atrophic gastritis (CAG) with 10 weeks kimchi. Each amplicon is sequenced on MiSeq of Illumina and the sequence reads were clustered into operational taxonomic units using VSEARCH and the Chao, Simpson, and Shannon Indices. Though significant difference in α - or β -diversity was not seen in three groups, kimchi intake led to significant diversity of fecal microbiome. As results, *Klebsiella*, *Enterococcus*, *Ruminococcaceae*, *Streptococcus*, *Roseburia*, and *Clostridium* were significantly increased in *H. pylori* (+) CAG, while *Akkermansia*, *Citrobacter*, and *Lactobacillus* were significantly decreased in *H. pylori* (+) CAG. With 10 weeks of kimchi administration, *Bifidobacterium*, *Lactobacillus*, and *Ruminococcus* were significantly increased in *H. pylori* (+) CAG, whereas *Bacteroides*, *Subdoligranulum*, and *Eubacterium coprostanolines* were significantly decreased in *H. pylori* (-) CAG. 10 weeks of kimchi intake significantly improved pepsinogen I/II ratio ($p < 0.01$) with significant decreases in interleukin-1 β . Conclusively, fermented kimchi significantly changed fecal microbiota to mitigate *H. pylori*-associated atrophic gastritis.

Key Words: *H. pylori*, chronic atrophic gastritis, fermented kimchi, fecal microbiota, pepsinogen I/II ratio

Chronic inflammation with *Helicobacter pylori* (*H. pylori*) is a major risk factor for gastric cancer, but only less than 3% of *H. pylori*-infected people develop gastric cancer.⁽¹⁾ As plausible explanations for these discrepancies, host changes in gut microbial composition are associated with intestinal metaplasia (IM), chronic atrophic gastritis (CAG), and gastric adenoma as precancerous lesions and gastric cancer, that is, the role of bacteria other than *H. pylori* is yet to be established.⁽²⁻⁴⁾ A significant percentage of cancers arise from chronic microbial infections and consequent mutagenic inflammation, *H. pylori* infection and gastric cancer via gut microbiota changes is regarded as hallmarks of cancer-inducing microbe.⁽⁵⁾ However, interventions using probiotics are still obscure implication, though the eradication is actively progressing in Japan in order to prevent *H. pylori*-associated CAG and cancer.⁽⁶⁻⁹⁾

Recent publications aimed to explore responsible gut or gastric microbiota in patients with different degree of gastritis including *H. pylori* and non-*H. pylori* bacteria were available,⁽¹⁰⁻¹²⁾ profound alterations in human fecal microbiota of *H. pylori* infected individuals, the increased microbiota diversity associated with *H. pylori* infection, relative abundance of specific genera, all reflected the complex networking between *H. pylori* and its human host and gastric tumor microenvironment. For instances, *H. pylori*-infected individuals was shown to be increased of abundance of *Candida glabrata* and other unclassified fungi in addition to *Succinivibrio*, *Coriobacteriaceae*, *Enterococcaceae*, and *Rikenellaceae*,⁽¹³⁾ linking possible role for *H. pylori*-associated changes in the gut microbiota in producing precancerous lesion as well as early stage gastric cancer, the knowledge and information may eventually lead to the development of novel biomarkers or therapeutic intervention.⁽¹⁴⁾

Put together of above backgrounds, we set hypothesis that fermented diet intervention might be ideal because food contains profuse and diverse kinds of probiotics, safety with long-term intake of food, and associated phytochemicals warranting rejuvenating actions of precancerous atrophic changes in food, ascertained antioxidative and anti-inflammatory actions of phytochemicals included in food,⁽¹⁵⁻²¹⁾ considering the fact that accepted benefits of probiotic supplementation are relieving more gastrointestinal symptoms, some improvement in host immune responses, increasing compliance of eradication regimen, and increases of eradication rate.^(17,22,23) Although consumption of over-the-counter probiotics to promote health has increased worldwide, there are conflicting clinical results that many probiotic strains and formulations can aggravate underlying diseases reported in high impact journal, so called possibility of “probiotics paradox”.⁽²⁴⁻²⁶⁾

Supported with facts that *H. pylori* as well as gut microbiota can be determining environmental factors influencing gastric pathologies including precancerous CAG as well as gastric cancer, in order to maintain the gut homeostasis and defensive condition against *H. pylori* infection, the modulation of gut microbiota with probiotics foods, fermented kimchi in the current study, can be basis for either ameliorating gastritis or rejuvenating atrophy, we have performed clinical trials to explore

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fecal microbiota changes relevant to probiotic foods in patients with gastritis and found significant contribution of probiotic kimchi in gastric homeostasis as well as the rejuvenation of CAG. In this investigation, deep understanding of the association between gut microbiota and *H. pylori*-related gastric lesion is very important for either evaluation of overall benefits of fermented kimchi or the optimization of *H. pylori*-associated gastric cancer prevention strategies.

Material and Methods

Bacteria DNA extraction from human stool samples. The human stool sample is filtered through a 40 µm-pore sized cell strainer after being diluted and incubated in 10 ml of PBS for 24 h. To separate the bacteria from human stool, bacteria in stool samples is isolated using centrifugation at 10,000 × *g* for 10 min at 4°C. After centrifugation, the pellet is comprised of bacteria. To extract the DNA out of the bacteria, bacteria is boiled for 40 min. DNA is extracted by using a DNeasy Power Soil Kit (QIAGEN, Hilden, Germany), for which standard protocol is followed as the kit guides and the DNA from bacteria in each sample is quantified by using QIAxpert system (QIAGEN).

Bacterial metagenomic analysis using DNA from human stool samples. Bacterial genomic DNA was amplified with 16S_V3_F (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3') and 16S_V4_R (5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3') primers, which are specific for V3–V4 hypervariable regions of 16S rDNA gene. The libraries were prepared using PCR products according to MiSeq System guide (Illumina, Coyoacán, Mexico City, Mexico) and quantified using a QIAxpert. Each amplicon is then quantified, set equimolar ratio, pooled, and sequenced on MiSeq (Illumina) according to the manufacturer's recommendations.

Analysis of bacterial composition in the microbiota. Paired-end reads that matched the adapter sequences were trimmed by cutadapt ver. 1.1.6.⁽²⁷⁾ The resulting FASTQ files containing paired-end reads were merged with CASPER and then quality filtered with Phred (Q) score based criteria described by Bokulich.^(28,29) Any reads were shorter than 350 bp and longer than 550 bp after merging, were also discarded. To identify the chimeric sequences, a reference-based chimera detection step was conducted with VSEARCH against the SILVA gold database.^(30,31) Next, the sequence reads were clustered into operational taxonomic units (OTUs) using VSEARCH with *de novo* clustering algorithm under a threshold of 97% sequence similarity. The representative sequences of the OTUs were finally classified using SILVA 132 database with UCLUST (*parallel_assign_taxonomy_uclust.py* script on QIIME ver. 1.9.1) under default parameters.⁽³²⁾ The Chao Indices, an estimator of richness of taxa per individual, were estimated to measure the diversity of each sample.

Volunteers recruitment and clinical trials. After Institutional Review Board, we have recruited 32 volunteers to accept the current clinical trial that 10 weeks of fermented kimchi intake can benefits against *H. pylori* infection, changes of pepsinogen I/II as marker for gastric atrophy, the urea breath test (UBT) as assessing *H. pylori*, measurement of serum IL-1β and malondialdehyde (MDA) as marker for inflammation as well as fecal microbiota changes. Volunteer subjects with *H. pylori* (–) chronic superficial gastritis (CSG), *H. pylori* (+) CSG, and *H. pylori* (+) chronic atrophic gastritis (CAG) were recruited at clinical trial center located at Digestive Disease Center, CHA University Bundang Hospital (Seongnam, Korea). The informed consents were obtained after explaining the aim and object of the current study. As shown in Fig. 1C, they were administered with fermented kimchi for 10 weeks after informed consent and stools

were donated for microbiota analysis (MD Healthcare, Inc., Seoul, Korea) and blood samplings were saved for biochemical analysis including CBC, biochemistry, and levels of IL-1β, MDA, and pepsinogen I/II for lipid peroxidation as well as UBT for detecting the presence of *H. pylori*. Compliance for taking kimchi was checked above intake more than 95% and kimchi was prepared every 5 days to keep optimal fermentation state. All of these studies were approved with IRB (IRB #18-0202) of CHA University Bundang Hospital. There was no significant difference in mean ages, gender difference, and history of smoking and alcohol among groups on demographic analysis. As shown in Fig. 1A and B, representative gastroscopic pictures showing CSG, mild to moderate, in 10 patients, Male:Female = 5:5, mean ages, 44 ± 5, who was negative in *H. pylori* infection, CLO (–) and UBT (–), and Giemsa staining (–), the other group *H. pylori* (+) CSG, mild to moderate, in 11 patients, Male:Female = 6:5, mean ages, 48 ± 6, who was positive in *H. pylori* infection, CLO (+) and UBT (+), and Giemsa staining (+), and chronic atrophic gastritis (CAG), moderate to severe by Updated Sydney System, in 9 patients, Male:Female = 5:4, mean ages, 51 ± 3, who was positive in *H. pylori* infection, CLO (+) and UBT (+), Giemsa staining (+), and scored according to Updated Sydney System score. All the participating volunteers were sampled with blood to check the ratio of PG I and PG II, IL-1β, and MDA levels by ELISA. All volunteers were administered with fermented kimchi (CJ Food, Blossom Park, Suwon, Korea), 100 g/day for 10 weeks. Kimchi was prepared every 5 days to keep fermentation and was delivered to house of volunteers on exact day of delivery and keep at refrigerator. 100 g of kimchi was packed and supplied.

Biochemical tests, GastroPanel®, IL-1β. Serum levels of basal gastrin 17, pepsinogen (PG) I, PG II, and *H. pylori* antibody were measured by a chemiluminescent enzyme immunoassay using commercial kits (Biohit plc, Helsinki, Finland) and serum levels of IL-1β were measured with R/G human IL-1β ELISA kit (Mineapolis, MN). A 10 h fasting blood sample was obtained from all patients. Patients were not receiving anti-secretory treatment (including PPIs) 2 weeks before the extraction. EDTA tubes were centrifuged at 2,000 *g* for 15 min; 50 µl of G17 stabilizer was then added to plasma. Blood was stored at –20°C until the assay was performed.

Urea breath test (UBT). The ¹³C urea breath test was performed two times, before clinical trial and after 10 weeks of kimchi intake using 75 mg urea (Urea ¹³C, Otsuka, Tokushima, Japan) according to the manufacturer's instructions, briefly, as follows: a basal breath sample was obtained by asking the patient to take a deep breath then holding it for 10 s before blowing the exhale into a specific bag at zero time. After this, the patient was asked to drink the reagent that contains urea attached to a ¹³C carbon atom in 90 ml of water. Then, 30 min later, the patient was similarly asked to give a breath sample again, which was collected into a new specified bag. Samples were analyzed by infrared spectrophotometer.

Statistical analysis. Comparison of relative abundances (RA) of OTUs and α-diversity between groups was performed with the Mann-Whitney test. Statistical significance was considered if the *p* value was <0.05. The α-diversity of microbial composition was measured using the Observed, Chao1, Shannon, Simpson index and rarefied to compare species richness. Statistical analyses were performed with R software (ver. 3.6.0).

Results

α- and β-Diversity analysis according to gastric pathology and *H. pylori* status. α-Diversity is the analysis of species diversity in samples, for which Chao1, Shannon, and Simpson indexes were explored in order to describe the diversity features of gut tract community according to gastric pathologic condition,

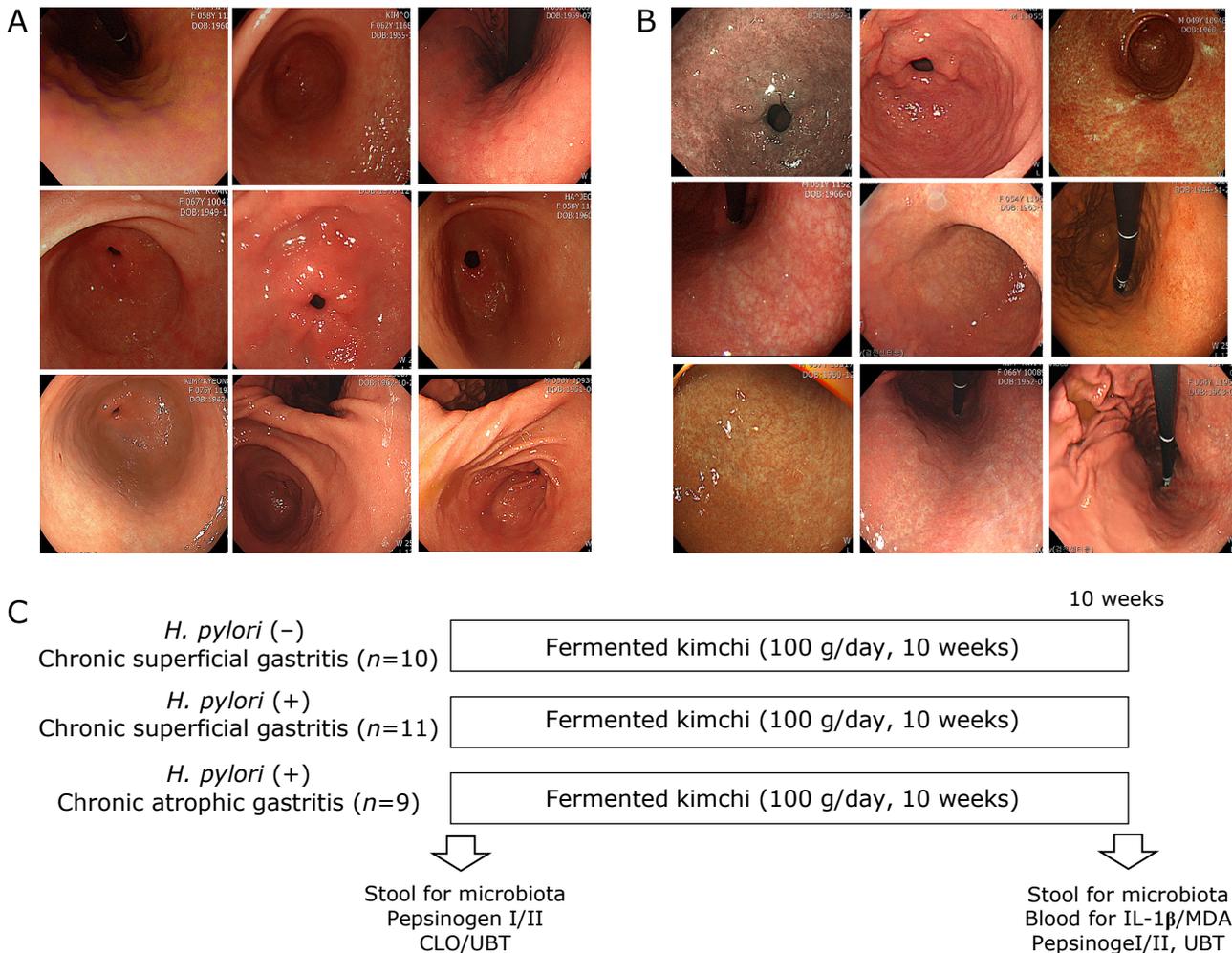


Fig. 1. Clinical trial with fermented kimchi in patients with gastritis. (A) Representative gastroscopic pictures showing *H. pylori*(-) chronic superficial gastritis (CSG), 10 patients, Male:Female = 5:5, mean ages, 44 \pm 5, who was negative in *H. pylori* infection assessed with CLO (-), UBT (-), and Giemsa staining (-), *H. pylori* (+) CSG, mild to moderate, in 11 patients, Male:Female = 6:5, mean ages, 48 \pm 6, who was positive in *H. pylori* infection assessed with CLO (+), UBT (+), and Giemsa staining (+), and *H. pylori* (+) chronic atrophic gastritis (CAG), moderate to severe by Updated Sydney System, in 9 patients, Male:Female = 5:4, mean ages, 51 \pm 3, who was positive in *H. pylori* infection assessed with CLO (+), UBT (+), documentation of presence of *H. pylori* through Giemsa staining scored according to Updated Sydney System score. (B) Representative gastroscopic pictures showing *H. pylori* (+) CAG in 9 patients, Male:Female = 4:5, mean ages, 54 \pm 4. (C) Schematic protocol for fecal microbiota measurement. All volunteers were administered with fermented kimchi (CJ Food, Suwon, Korea), 100 g/day for 10 weeks. All the volunteers were included after informed consent and their compliance for kimchi intake were more than 95%. Before starting, all were performed CLO/UBT/ Giemsa staining and stool/blood collection for microbiota and serology analysis. See color figure in the on-line version.

H. pylori (-), mild to moderate degree of CSG, *H. pylori* (+), mild to moderate CSG, and *H. pylori* (+) CAG (Fig. 2A). In detail, Shannon, diversity indexes reflecting the diversity features of gut community, Chao indexes reflecting the species richness in the sample, and Simpson indexes reflecting community diversity including species richness and species evenness^(33,34) were shown in Fig. 2 according to patient group. Generally, the larger the Shannon index and the smaller the Simpson index, the higher the species diversity in the sample were noted, regarding α -diversity according to gastric condition as shown in Fig. 2A, there was no significant difference in *p* value of these indexes between *H. pylori* (-) CSG and *H. pylori* (+) CSG (Chao1 index = 0.46, Shannon index = 0.82, and Simpson index = 0.71) and in *p* value of these indexes between *H. pylori* (+) CSG and *H. pylori* (+) CAG (Chao1 index = 0.13, Shannon index = 0.31, and Simpson index = 0.72). In order to further display differences in species diversity among samples, principal coordinate analysis was used to display differences among samples. In case that the

two samples are close together, the species composition of the two samples is interpreted as similar. As result, no significant separation in bacterial community composition between *H. pylori* (-) CSG and *H. pylori* (-) CSG with 10 weeks of kimchi intake (Supplemental Fig. 1A*), between *H. pylori* (+) CSG and *H. pylori* (+) CSG with 10 weeks of kimchi intake (Supplemental Fig. 1B*), and between *H. pylori* (+) CAG and *H. pylori* (+) CAG with 10 weeks of kimchi intake (Supplemental Fig. 1C*). Relative abundance of gastritis associated microbiota including microbial composition of *H. pylori* (-) CSG, *H. pylori* (+) CSG, and *H. pylori* (+) CAG at the phylum level was shown in left and at genus level at right of Supplemental Fig. 1*.

Phyla and genus result according to gastric pathology and *H. pylori* status. The most abundant phylum in total samples was *Firmicutes*, followed by followed by *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* (Fig. 2B and C). Conclusively, relative abundance of the phylum level in feces by pyrosequencing showed that there was no significant

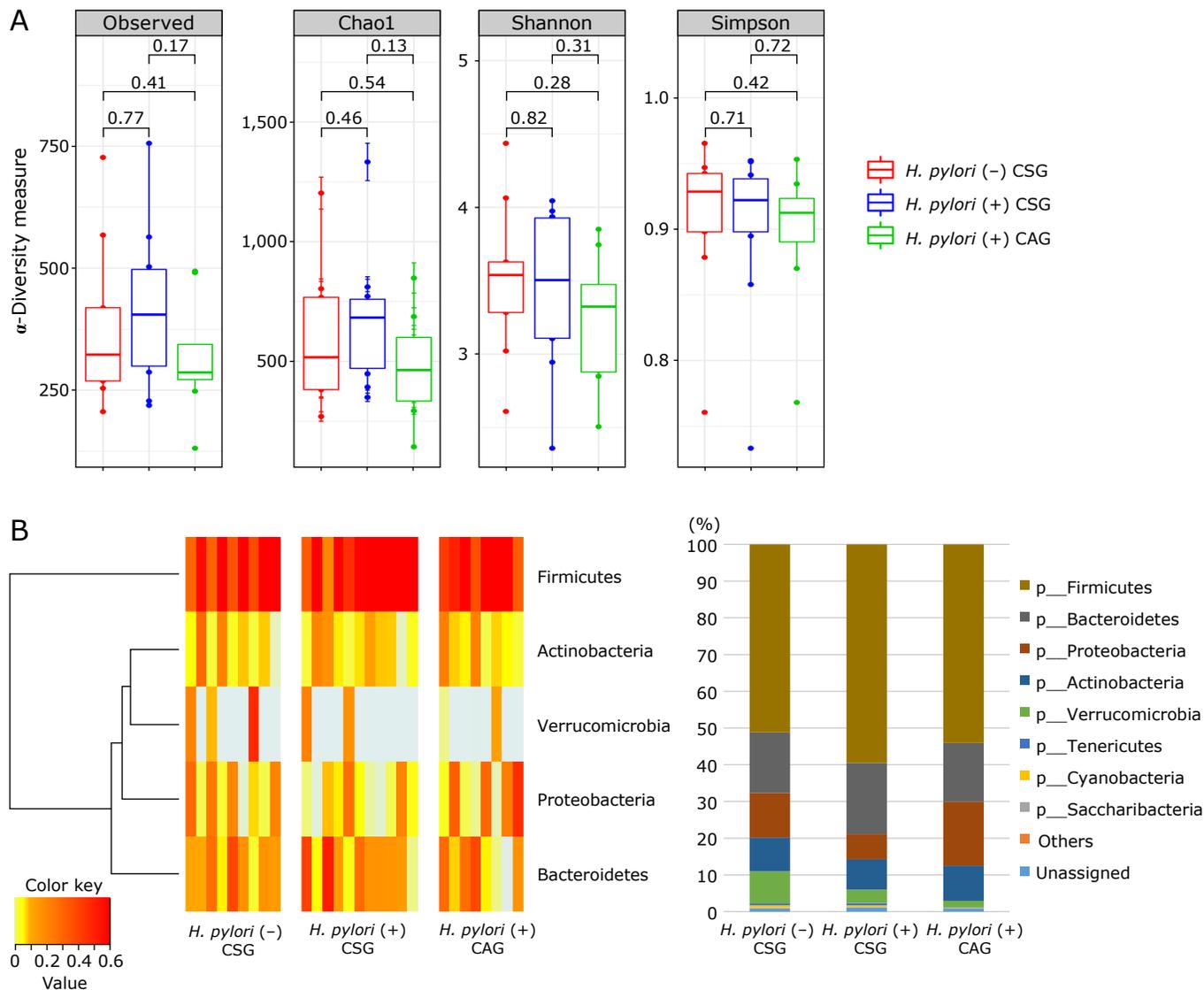


Fig. 2. Relative abundance of the phylum level (% similarity) in collected feces samples by pyrosequencing. (A) α -Diversity in *H. pylori* (-) CSG, *H. pylori* (+) CSG, mild to moderate, and *H. pylori* (+) CAG, moderate to severe degree. (B) Microbiota at phylum levels, Heatmap and bar display. (C) Microbiota at phylum levels. (D) Relative abundance of the genus level (% similarity) in collected feces samples by pyrosequencing. Significant changes in microbiota among *H. pylori* (-) CSG, *H. pylori* (+) CSG, and *H. pylori* (+) CAG, all before kimchi intake. See color figure in the on-line version.

difference in RA of most abundant phyla among the three groups, although looks different according to gastric pathology and *H. pylori* status, but no statistical significance ($p > 0.05$). RAs of gastritis-associated microbiota including microbial composition of *H. pylori* (-) CSG, *H. pylori* (+) CSG, and *H. pylori* (+) CAG at the genus level was shown in Fig. 2D and 3. RAs of microbiota among the three groups, *H. pylori* (-) CSG, *H. pylori* (+) CSG, and *H. pylori* (+) CAG in the genus level was shown in Fig. 2C depicting *Klebsiella*, *Enterococcus*, *Ruminococcaceae*, *Streptococcus*, *Roseburia*, and *Clostridium sensu* significantly increased in *H. pylori*-associated CAG, whereas *Akkermansia*, *Citrobacter*, and *Lactobacillus* significantly decreased in *H. pylori*-associated CAG ($p < 0.05$). Individual microbiome was compared according to group, which was presented in Fig. 3 that *Enterococcus* and *Intestinalis* was significantly decreased in *H. pylori*-associated CSG compared to *H. pylori* negative CSG ($p < 0.05$), whereas *Eubacterium rectalis* was significantly increased in *H. pylori* (+) CSG compared to

H. pylori (-) CSG ($p < 0.05$). In cases of *H. pylori* (+) CAG, *Eubacterium rectalis* was significantly decreased in *H. pylori* (+) CAG compared to *H. pylori* (+) CSG and *Intestinalbacter* was further decreased in *H. pylori* (+) CAG ($p < 0.001$, Fig. 3).

Evaluation of α - and β -diversity in the 3 groups after 10 weeks of kimchi intake. The Chao1 Richness index of the fecal microbiota was significant different between *H. pylori* (-) CSG and *H. pylori* (-) with 10 weeks of kimchi intake ($p = 0.01$, Fig. 4A), between *H. pylori* (+) CSG and *H. pylori* (+) CSG with 10 weeks of kimchi intake ($p = 0.0019$, Fig. 4B), between *H. pylori* (+) CAG and *H. pylori* (+) CAG with 10 weeks of kimchi intake ($p = 0.05$, Fig. 4C), while other indexes were only significant in Simpson index between *H. pylori* (+) CSG and *H. pylori* (+) CSG with 10 weeks of kimchi intake ($p = 0.047$, Fig. 4B). α -Diversity among *H. pylori* (-) CSG, *H. pylori* (+) CSG, and *H. pylori* (+) CAG was displayed in Fig. 5A. In order to further display differences in species diversity among samples including *H. pylori* (-) CSG, *H. pylori* (+) CSG, and *H. pylori*

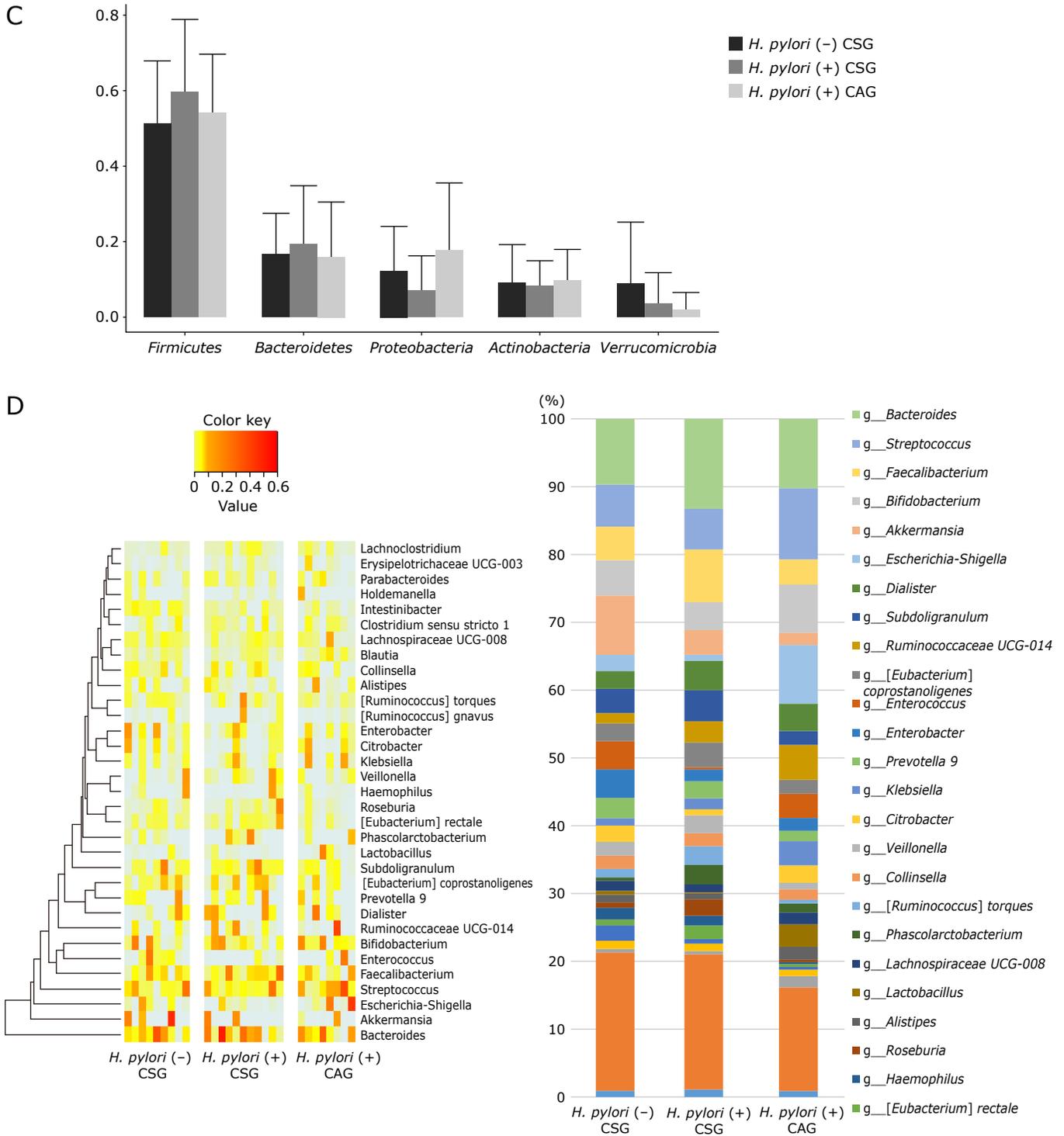


Fig. 2. (continue)

(+) CAG before and after 10 weeks of kimchi intake, principal coordinate analysis (PCoA) based on the unweighted UniFrac distance metrics is used to display differences among samples was presented in Supplemental Fig. 2A*: *H. pylori* (-) CSG, Supplemental Fig. 2B*: *H. pylori* (+) CSG, Supplemental Fig. 2C*: *H. pylori* (+) CAG, and all included group (Supplemental Fig. 2D*).

Relative abundance of the three groups after 10 weeks of kimchi intake (the phylum level). The most abundant phylum in fecal samples was *Firmicutes*, followed by *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* (Fig. 5B), not significantly changes compared to before kimchi intake as shown in Fig. 2B. Conclusively, RA of the phylum level in feces by pyrosequencing showed that there was no significant difference in RA of most abundant

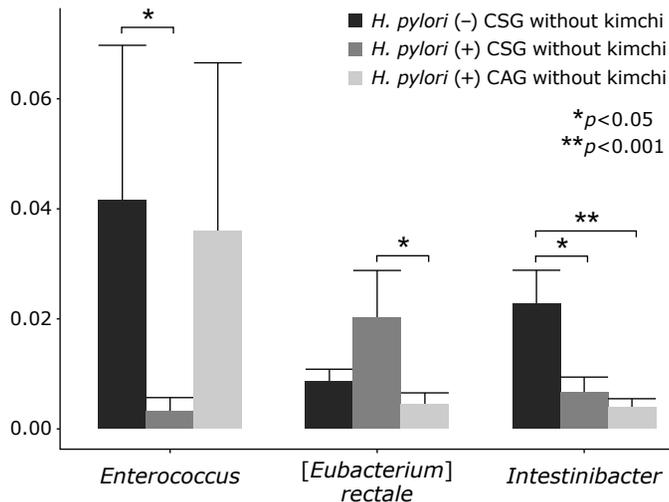


Fig. 3. Individual microbiota changes according to gastric pathology and *H. pylori* status.

phyla among the three groups, although looks different according to gastric pathology and *H. pylori* status, but no statistical significance ($p > 0.05$). Individual separate Heatmap results compared between group before kimchi and group after kimchi at phylum levels were shown on Supplemental Fig. 1*.

Relative abundance of the three groups after 10 weeks of kimchi intake (genus level). Relative abundance of microbiota among the three groups, *H. pylori* (-) CSG, *H. pylori* (+) CSG, and *H. pylori* (+) CAG in the genus level was shown in Fig. 5C depicting *Bifidobacterium*, *Lactobacillus*, and *Ruminococcus* were significantly increased in *H. pylori* (+) CAG with 10 weeks intake of kimchi intake, whereas *Bacteroides*, *Subdoligranulum*, and *Eubacterium coprostanolines* were significantly decreased in *H. pylori* (+) CAG with 10 weeks of kimchi intake (Fig. 5C). After assessing all the results from Heatmap and genus analysis (Fig. 6), we reached to findings that *Megamonas* was significantly increased in *H. pylori* (+) CSG compared to *H. pylori* (-) CSG ($p < 0.05$), but its level was significantly increased with 10 weeks of kimchi intake compared to before ($p < 0.001$), whereas *Enterococcus* was significantly decreased in *H. pylori* (+) CSG compared to *H. pylori* (-) CSG, its levels was slightly increased with kimchi intake ($p < 0.05$), *Peptoclostridium* was significantly decreased with *H. pylori* infection, but increased with kimchi intake ($p < 0.05$) (Fig. 6A). When analysis was extended to patients with *H. pylori* (+) CAG, as seen in Fig. 6B, *Enterococcus* was significantly decreased in patients with *H. pylori* (+) CAG with kimchi intake ($p < 0.05$), whereas *Intestinibacter* was significantly decreased after *H. pylori* infection, but significantly restored with 10 weeks of fermented kimchi intake ($p < 0.05$). Similar and significant findings were noted in *Bacteroidetes*, *Peptoclostridioium*, *Anaerotruncus*, and *Megamonas* (Fig. 6B).

Changes of IL-1 β and MDA after kimchi intake. Kimchi intake significantly decreased sera levels of IL-1 β between *H. pylori* (-) CSG and *H. pylori* (+) CSG with 10 weeks of kimchi intake, though no significant changes were noted in MDA levels, reflecting antioxidative levels, in spite of kimchi intake and increases in PG I/II ratio. Interestingly, 10 weeks of kimchi intake led to unanticipated successful eradication with 10 weeks of fermented kimchi alone in whole 7 patients among 21 patients, by which we have high curiosity to analyze taxon between successful eradicated group and non-eradicated group (Supplemental Fig. 3C*).

Discussion

Though this study, we could document the real worlds evidence that fermented kimchi can be anticipatorily applied to either mitigate *H. pylori*-associated CSG or rejuvenate precancerous CAG. Though not assessed with follow up histology evaluation after kimchi intervention, GastroPanel® (Biohyt, Finland) dealing with the change of PG I/II ratio as marker for interpreting gastric atrophy and IL-1 β levels as marker for gastric inflammation were significantly decreased with kimchi intake in addition to significant changes in fecal microbiota. In 7 out of 21 cases, kimchi intake alone led to eradication of bug. Furthermore, we could discover that *Klebsiella*, *Enterococcus*, *Ruminococcaceae*, *Streptococcus*, *Roseburia*, and *Clostridium sensu* were significantly increased in *H. pylori* (+) CAG, while *Akkermansia*, *Citrobacter*, and *Lactobacillus* were significantly decreased in *H. pylori* (+) CAG ($p < 0.05$). With 10 weeks of kimchi administration, *Bifidobacterium*, *Lactobacillus*, and *Ruminococcus* were significantly increased in *H. pylori* (+) CAG, whereas *Bacteroides*, *Subdoligranulum*, and *Eubacterium coprostanolines* were significantly decreased in *H. pylori* (+) CAG (Fig. 7). Our study showed the role of the gut microbiome in *H. pylori* pathogenesis⁽³⁵⁾ and the possibility of prevention of *H. pylori*-associated gastric lesions through intake of probiotic foods.⁽³⁶⁾

The human gastric lumen is one of the most hostile environments of the human body, featured with very acidic pH condition, to be sterile, and highly peristaltic condition until the discovery of *H. pylori* and the general advancement of next generation sequencing and bioinformatic analysis.^(37,38) Now, functional genomics as well as the colonization of the gastric habitat can be documented by the 16S rRNA gene amplicon based bacterial microbiome using gastric biopsy or feces. For instance, the genera *Actinomyces*, *Granulicatella*, *Veillonella*, *Fusobacterium*, *Neisseria*, *Helicobacter*, *Streptococcus*, and *Prevotella* are significantly different between the *H. pylori* (+) and *H. pylori* (-) sample groups including gastric cancer.⁽³⁹⁻⁴¹⁾ In detail, according to Coker *et al.*⁽⁴²⁾ data, OTUs whose species identification corresponds to *Parvimonas micra*, *Dialister pneumosintes*, *Slackia exigua*, *Peptostreptococcus stomatis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Prevotella oris*, and *Catonella morbi* were found to be significantly enriched in microbiome of gastric cancer compared with precancerous stages. Pathological gastric microbes including *H. pylori* and more bacteria especially in changed atrophic condition directly modulate its pathogenicity and carcinogenic potential.⁽⁴³⁾ Minor changes in diversity indices in gastritis, reduced microbial diversity, by decreased abundance of *H. pylori*, and by the enrichment of other bacterial genera was featured in the gastric carcinoma microbiota analysis,⁽⁴⁴⁾ concluding that dysbiosis can discriminate between gastritis and gastric cancer and the possible intervention of probiotics or probiotic foods as for preventive way.^(45,46)

In the gut beyond stomach, the human gut microbiota is critical for maintenance of human health and plays an integral role in energy metabolism, absorption of nutrients, and defense against invading pathogens, but microbiota should exist within a delicate balance, homeostasis and hormesis, because in altered condition, dysbiosis contributed to aberrant pro-inflammatory, abnormal immune responses, susceptibility to invading pathogens, and initiation of disease processes including cancer.⁽⁴⁷⁾ In detail, *H. pylori* was present at relatively low abundance in patients with advanced premalignant lesion, but the microbiota of patients with gastric cancer were dominated by species of *Lactobacillus*, *Streptococcus*, *Veillonella*, and *Prevotella*⁽²⁾ and a steady decrease in bacterial diversity of the gastric microbiota, with an increasing abundance of *Lactobacillus* and *Lachnospiraceae* in patients progressing to

*See online. <https://doi.org/10.3164/jcfn.20-123>

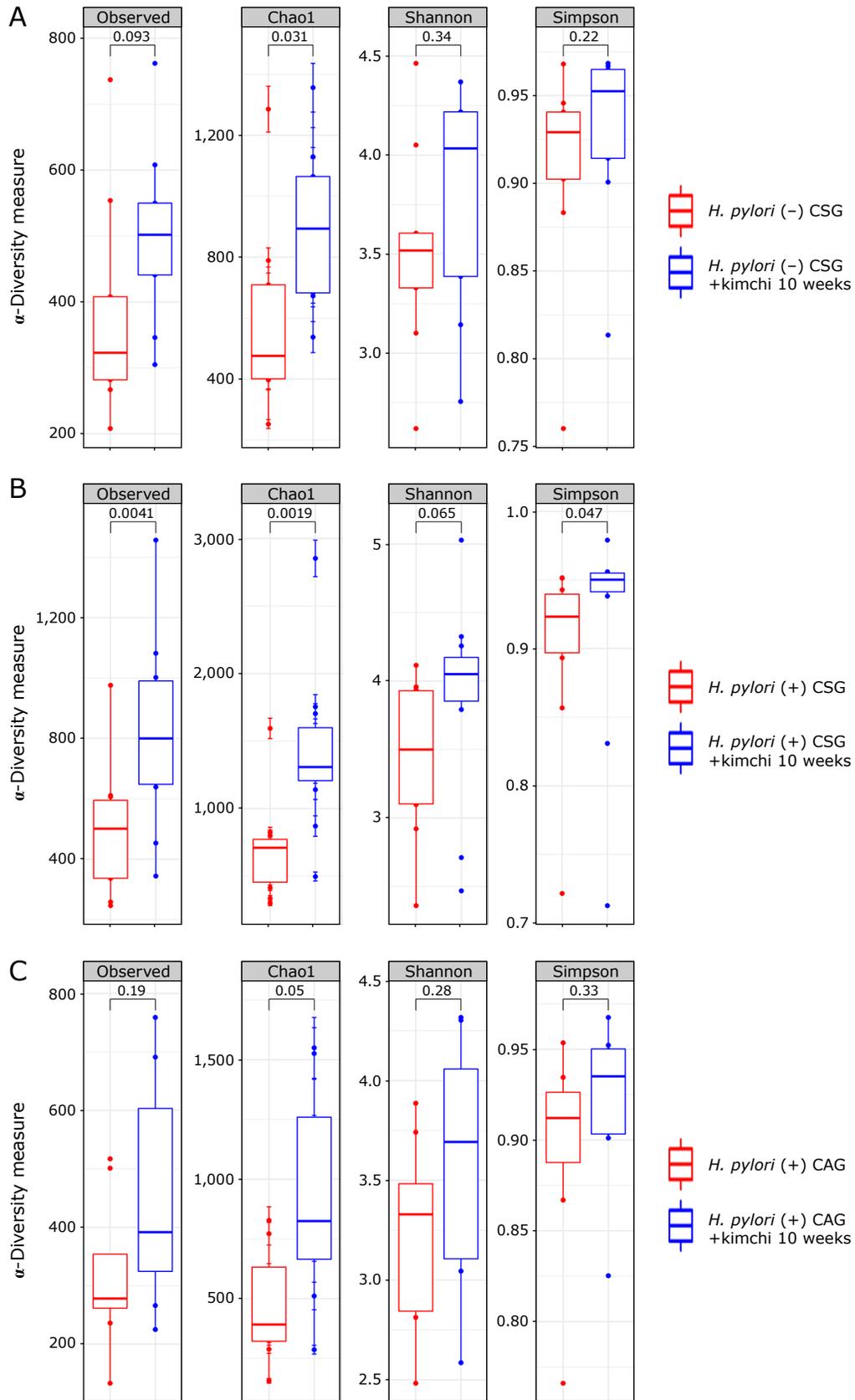


Fig. 4. α -Diversity, phylum and genus level analysis after 10 weeks kimchi intake. (A) α -Diversity in *H. pylori* (-) CSG and *H. pylori* (-) CSG with 10 weeks of kimchi intake, (B) α -diversity in *H. pylori* (+) CSG and *H. pylori* (+) CSG with 10 weeks of kimchi intake, (C) α -diversity in *H. pylori* (+) CAG and *H. pylori* (+) CAG with 10 weeks of kimchi intake.

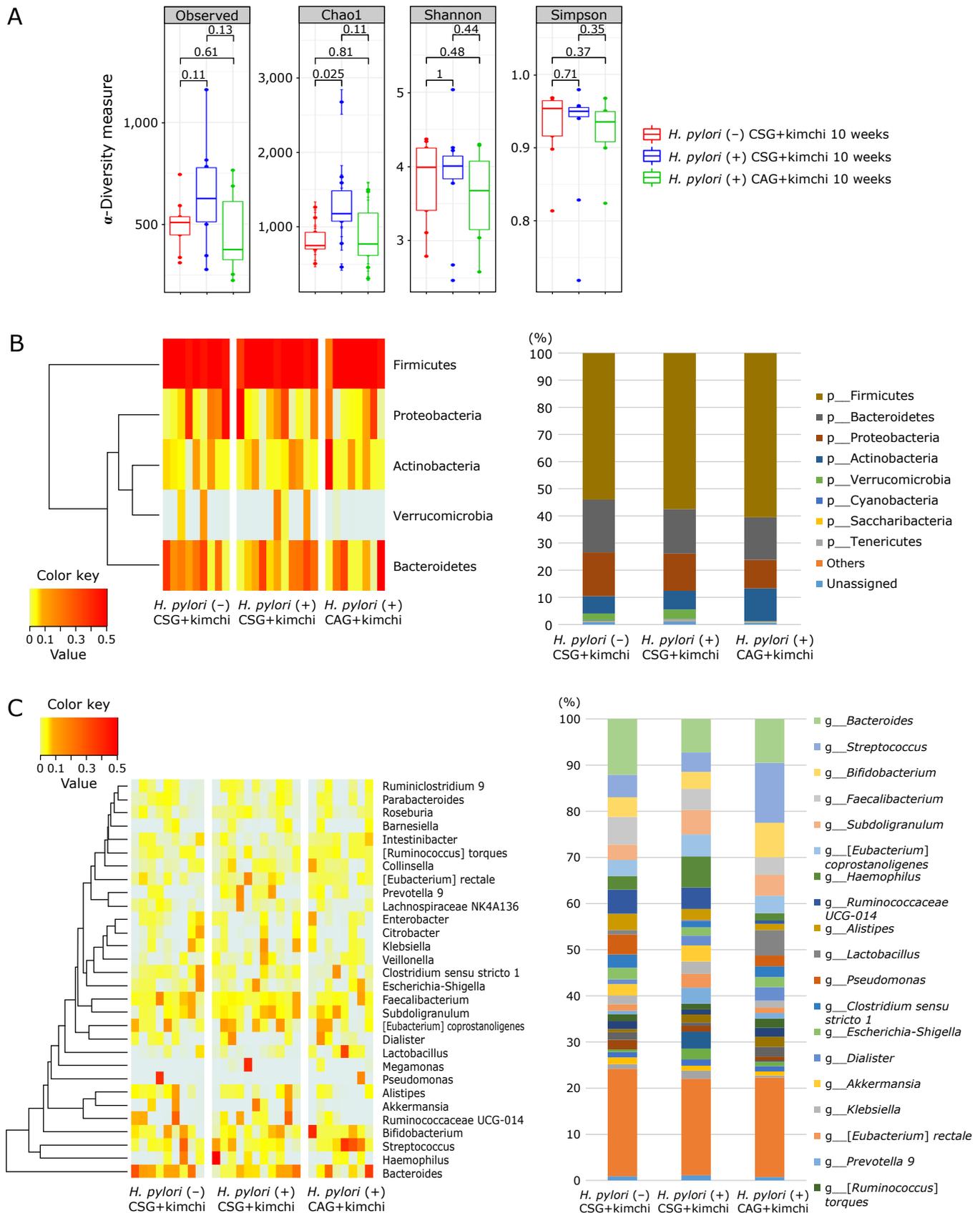


Fig. 5. (A) α -Diversity in *H. pylori* (-) CSG, *H. pylori* (+) CSG, and *H. pylori* (+) CAG with 10 weeks of kimchi intake, respectively. (B, C) Significant changes in microbiota among *H. pylori* (-) CSG, *H. pylori* (+), and *H. pylori* (+) CAG with 10 weeks kimchi intake. (B) Phylum level analysis; Phylum levels bar analysis (right). (C) Genus levels analysis; Heatmap analysis (left) and genus levels bar analysis (right). See color figure in the on-line version.

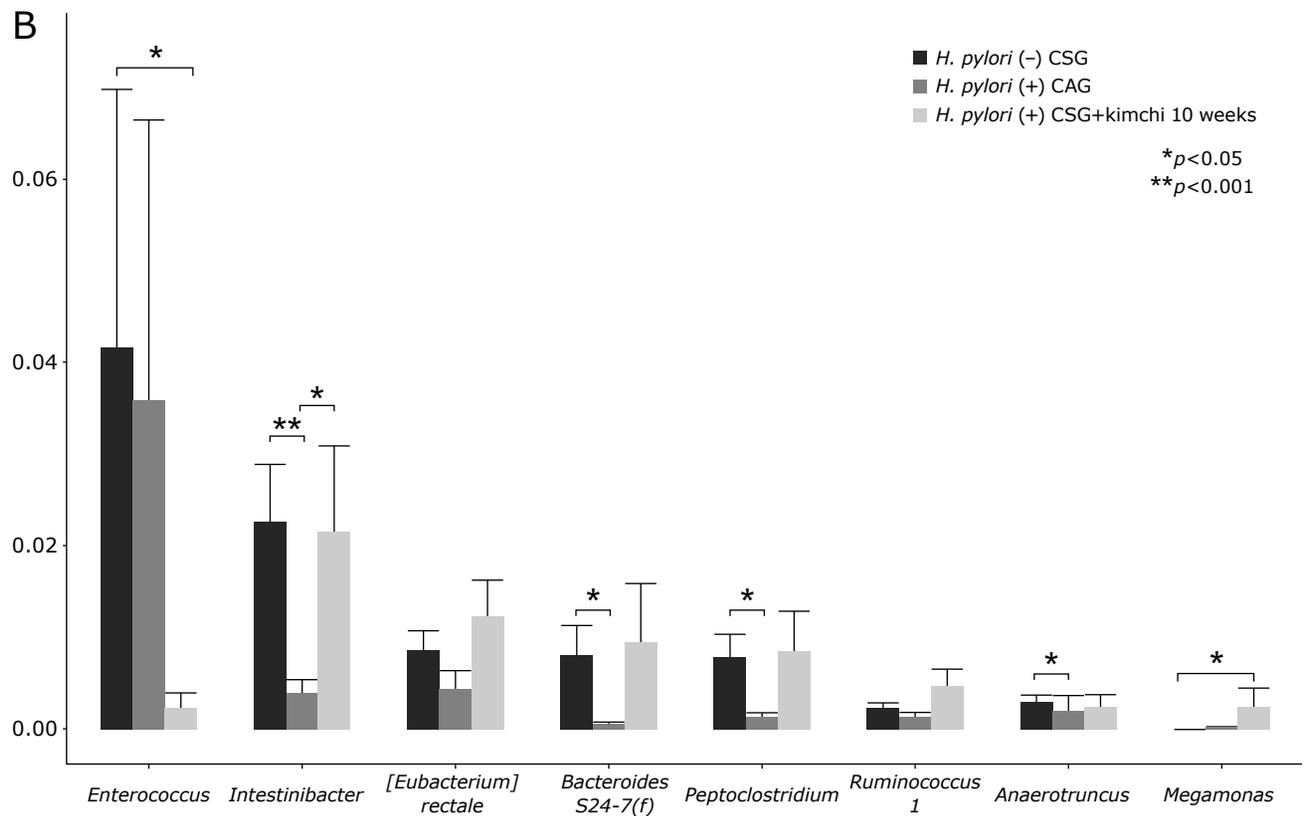
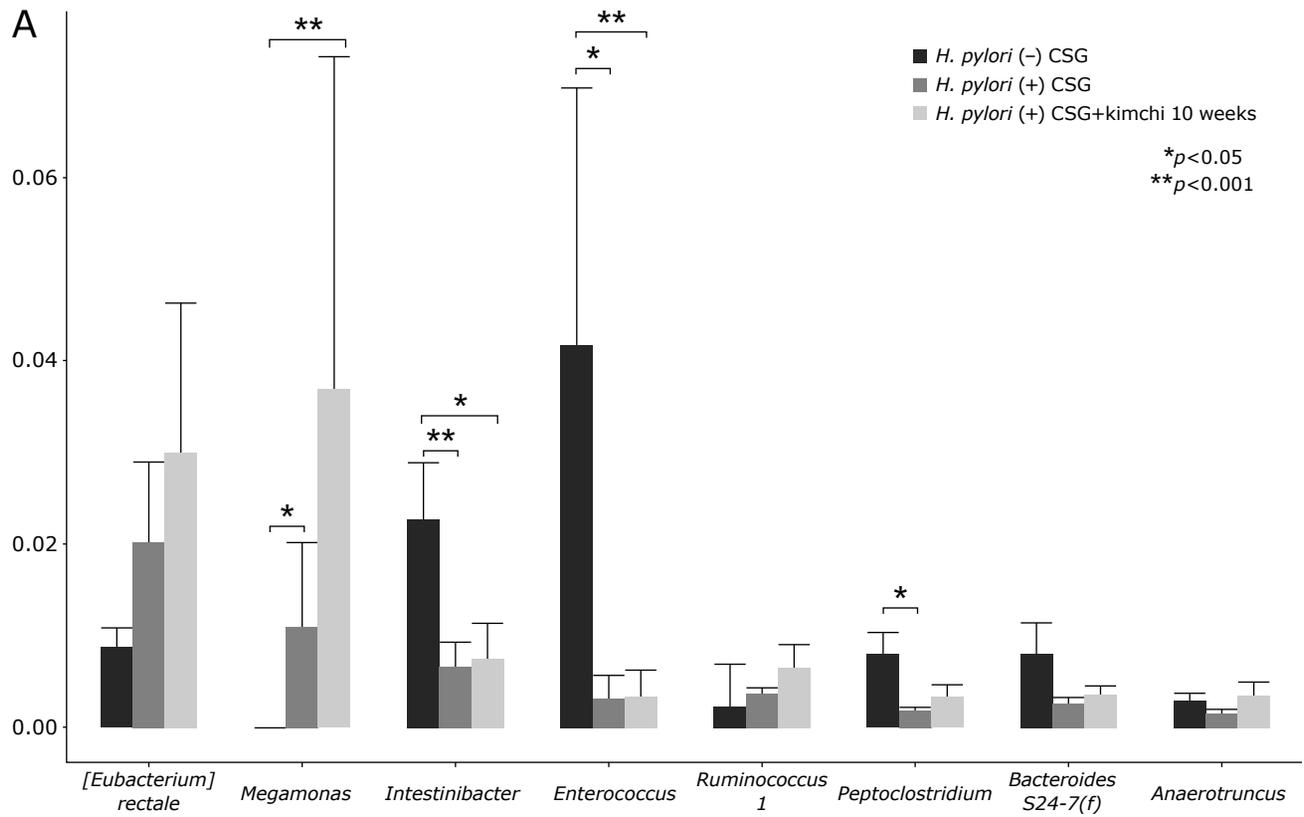
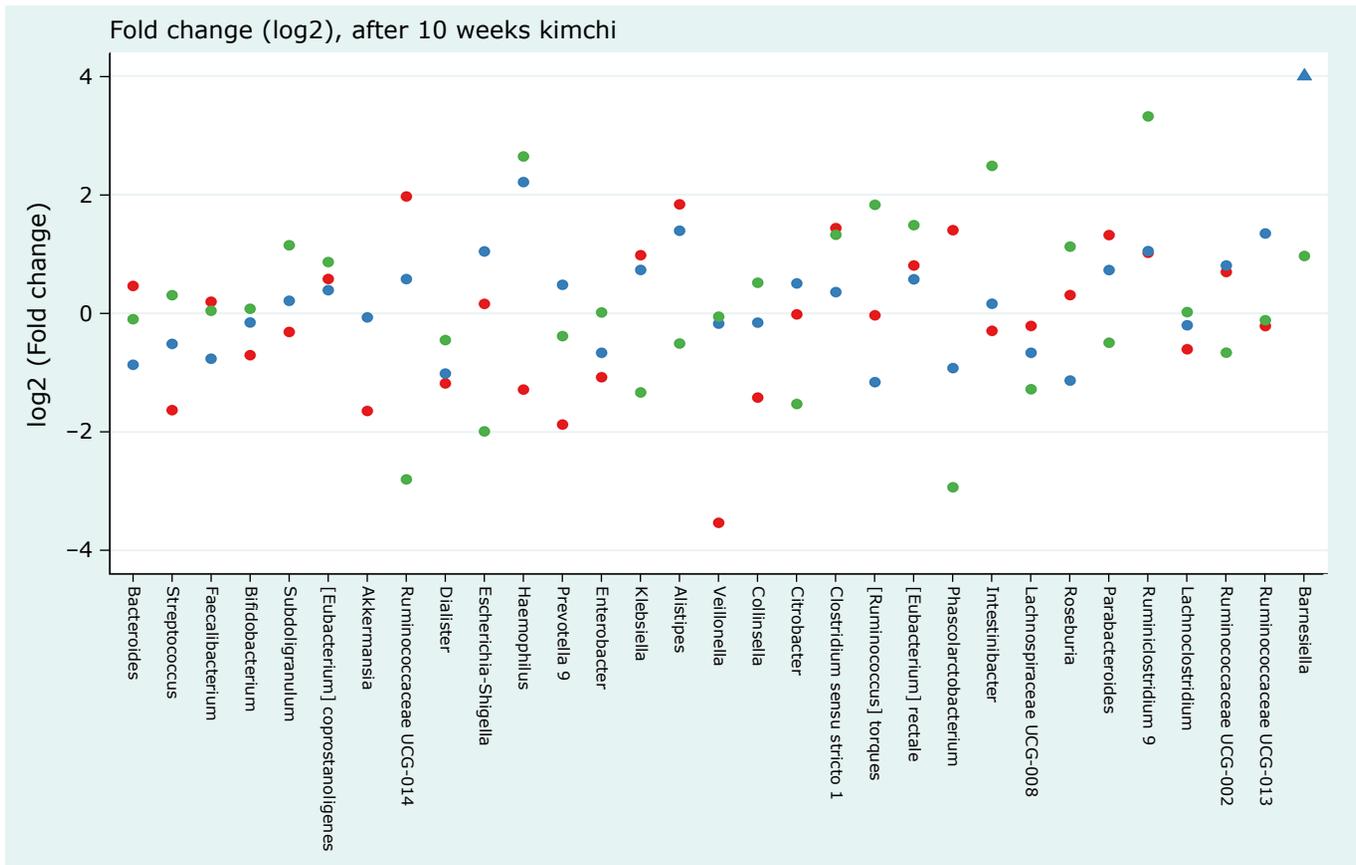


Fig. 6. Significant microbiota changes according to group, (A) between *H. pylori* (+) CSG and *H. pylori* (+) CSG with 10 weeks of fermented kimchi, (B) between *H. pylori* (+) CAG and *H. pylori* (+) CAG with 10 weeks of fermented kimchi.



● *H. pylori* (-) CSG+kimchi

● *H. pylori* (+) CSG+kimchi

● *H. pylori* (+) CAG+kimchi

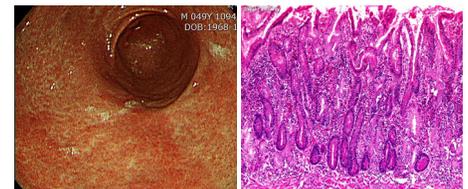
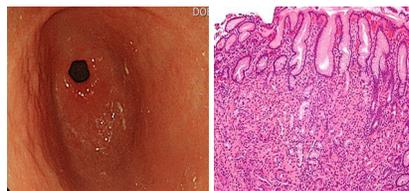


Fig. 7. Schematic drawing explaining the significant contribution of fermented kimchi in either inhibition of *H. pylori* (+) CSG and blocking the advancement from *H. pylori* (+) CSG and *H. pylori* (+) CAG. Red dot; *H. pylori* (-) CSG, Blue dot; *H. pylori* (+) CSG, Green dot; *H. pylori* (+) CAG after 10 weeks of fermented kimchi intake. Fold changes (log₂). See color figure in the on-line version.

gastric carcinogenesis.⁽⁴⁸⁻⁵¹⁾

Also, we could include detailed microbiota changes between successful eradicated subjects and non-eradicated subjects only with kimchi intake. Though we did not include gastric cancer patients, Instead, IM patients were included in our study, as per IM significance, as multiple risk factors associated with gastric carcinogenesis, IM interplays with *H. pylori* infection and bacterial genomics, host genetic factors, environmental factor such as hygiene, salt intake, diet, and gut microbiota.^(52,53) Coker *et al.*⁽⁴²⁾ observed significant mucosa microbial dysbiosis in IM and gastric cancer subjects that enrichment of 21 and depletion of 10 bacterial taxa, *Peptostreptococcus stomatis*, *S. anginosus*, *Parvimonas micra*, *Slackia exigua*, and *Dialister pneumosintes*. Though a few in number, Sohn *et al.*⁽⁵⁴⁾ analyzed gastric biopsy samples from body and antrum according to *H. pylori* and non-*Helicobacter* infection in gastric cancer and found that the number of non-*H. pylori* urea-producing and non-*H. pylori* nitro-reducing bacteria was higher in *H. pylori*-(-) cancer groups than

the others and these differences were more pronounced in the body, higher composition of *Streptococcus pseudopneumoniae*, *S. parasanguinis*, and *S. oralis*, emphasizing the importance of our study that long-term nutritional intervention like fermented food seems to be important in preventing *H. pylori*-associated gastric pathologies.⁽⁵⁵⁾

Investigation correlating the changes of gut microbiota and parameter changes relevant to *H. pylori* infection is increasing, for instances, changes of IL-1 β cytokine and PG I/II ratio in the current study adopting surrogate biomarker reflecting the improvement of atrophy with fermented kimchi via gut microbiota changes. Others published that changes in the gut microbiota such as the *Bacteroidetes:Firmicutes* (B:F) ratio after treatment with antimicrobials changed the plasma ghrelin level.⁽⁵⁶⁾ In a paper dealing with that *H. pylori* eradication has been found to be effective for gastric cancer prevention, but uncertainties remain about the possible adverse consequences such as microbial dysbiosis, the dominant phyla in fecal

samples were *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* with average relative and microbial diversity analysis showed that observed species and Shannon index were increased in subjects with past or current *H. pylori* infection compared with negative subjects,⁽⁵⁷⁾ signifying that the alterations of fecal microbiota, especially the dominant phyla of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, may be involved in the process of *H. pylori*-related gastric lesion progression. After eradication of *H. pylori*, 1 year later, microbial co-occurrence was reduced and *Acinetobacter lwoffii*, *Streptococcus anginosus*, and *Ralstonia* were enriched, while *Roseburia* and *Sphingomonas* were depleted in patients with persistent inflammation.⁽⁵⁸⁾ Unexpectedly, kimchi intake alone for 10 weeks resulted in eradication in 7 patients, in which we analyzed genus level and found similar outcome (Supplemental Fig. 3C*).

Among 6 gene polymorphisms highly reported to be associated with *H. pylori* infection including MUC1, toll-like receptor 4 (TLR4), protein tyrosine phosphatase, non-receptor type 11 (PTPN11), IL-1 β , and pepsinogens 3–5,⁽¹⁴⁾ IL-1 β 511 C/T SNP and levels reflect *H. pylori*-associated gastritis as well as cancer risk.^(59–61) Though follow-up endoscopy for Updated Sydney System to evaluate atrophic gastritis in the current study is prerequisite, the analysis of serum biomarkers for the assessment of CAG using stomach-specific diagnostic performance of GastroPanel[®] was done.^(62–64) As seen in Supplemental Fig. 3*, the detection of corpus CAG and severe CAG was significantly improved in group taken 10 weeks of fermented kimchi relevant to gut microbiota changes. In addition, though spontaneous eradication rate of *H. pylori* is regarded as very low, 1–3%,⁽⁶⁵⁾ 10 weeks of fermented kimchi intake led to 33% eradication.

As limitations, small in size, short-follow up interval, and analysis in phylum and genus level, we strictly excluded volunteers such as previous administration of proton pump inhibitor (PPI), other probiotics or nutraceuticals, case with autoimmune disease since prior PPI use or probiotics can influence the results.^(66,67) Further with our *in vitro* and *in vivo* model study,⁽³⁶⁾ we dare to conclude long-term intake of fermented kimchi can be anticipating strategy either to mitigate *H. pylori*-associated gastric inflammation/carcinogenesis or to rejuvenate *H. pylori*-associated atrophic gastritis. Conclusively,

though different ethnicity, different dietary style, and different cultural habits exist, our study should be extended more to reach to fermented kimchi as pharmanutrient fulfilling the efficacy of gut microbiota modulation against troublesome *H. pylori* infection, especially in Asian countries including Korea, Japan, and China.

Author Contributions

Study concept and design: JMP and KBH; acquisition of data: JMP, WHL, HS, and DYL; analysis and statistical analysis: JMP, WHL, and KBH; interpretation of data: HS, JYO, SJK, and KBH; drafting of manuscript: KBH.

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Abbreviations

CAG	chronic atrophic gastritis
CSG	chronic superficial gastritis
IM	intestinal metaplasia
MDA	malondialdehyde
OTUs	operational taxonomic units
PCoA	principal coordinate analysis
PG I/II	pepsinogen I/II
RA	relative abundance
UBT	urea breath test

Conflict of Interest

No potential conflicts of interest were disclosed.

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