Role of Pediococcus Acidilactici J9 in Decreasing the Occurrence of Gastritis Caused by H. pylori Infection and Two-week Repeated-dose Oral Toxicity Study in Mice

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Research

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Abstract

BACKGROUND Helicobacter pylori (H. pylori) is an important pathogen that causes chronic gastritis and peptic ulcer, and is related to the development of gastric carcinoma. Several chemicals, including antibiotics, have been used to eradicate H. pylori. However, more studies are yet required to accomplish a sufficient therapy. Pediococcus acidilactici J9 were studied for inhibition of binding of H. pylori binding to human gastric cell lines. This study was performed in order to investigate the repeated-dose toxicity of Pediococcus acidilactici J9 in male and female mice.

RESULTS C57BL/6 male and female Mus musculus were divided into four groups (n = 10 in each group). Pediococcus acidilactici J9 was administered daily by oral injection of vehicle control at dosage levels to a low-dose group (500 mg/kg/day), middle-dose group (1000 mg/kg/day), and high-dose group (2000 mg/kg/day) for two weeks. After 14 days of exposure, the blood biochemistry and hematology were investigated, along with a histopathology exam. There were no bacterial-related deaths or abnormal clinical signs in either gender of mouse. The data was observed during the period in terms of body weight, food, intake, and water consumption. Also, no alterations in organ weights upon administration of Pediococcus acidilactici J9 alone were observed.

CONCLUSIONS These results suggest that the oral application of Pediococcus acidilactici J9, up to a dosage level of 2,000 mg/kg/day, causes no adverse effects in both male and female mice. Pediococcus acidilactici J9 inhibits the adhesion of H. pylori to AGS gastric cancer cells. When used as probiotics, Pediococcus acidilactici J9 may help decrease the occurrence of gastritis and reduce the risk of H. pylori infection with promising safety issues.

Background

Lactobacillus is a gram-positive microorganism that utilizes carbohydrates as the energy source and produces organic acids like lactic acid and acetic acid as final products. It is used industrially in various fermented products, like fermented vegetables and dairy products, which are broadly involved in the everyday life [1, 2]. Lactobacillus determines the flavor of fermented foods and the characteristics of fermented products, and it plays a critical role in the food preservation. This occurs by extending its shelf life via production of active antibiotic materials like organic acid and bacteriocin. Moreover, various function in the human body is also reported, such as the suppression of intestinal noxious bacteria, the decrease of blood cholesterol levels, anticancer effect, reinforcement of immune function, etc. Because of the Lactobacillus, as a probiotics, intakes living strain, it attracts attention as an antibacterial preparation that solves the residual tolerance problems, in addition to being recently utilized as a healthy functional food [3, 4].

Helicobacter pylori is a macroaerophilic gram-negative bacteria that causes chronic gastritis, peptic ulcer, and presumable gastric cancer. Accumulated evidence demonstrates that the eradication of these bacteria resolves H. pylori-associated disease [5]. Multicenter studies have shown that triple therapy via a
proton pump inhibitor (PPI), clarithromycin, and either amoxicillin or metronidazole (all taken twice daily). This therapy is among the most effective approaches to *H. pylori* eradication [6]. However, 5–10% of *H. pylori* strains are reportedly resistant to clarithromycin [7]. In addition, there was a study noted a clarithromycin-resistant mutation in 63% of *H. pylori* strains from patients in whom treatment with a regimen including clarithromycin was unsuccessful [8]. The treatment of *H. pylori* infection with antibiotics does not eradicate the organism and is also often accompanied by deleterious side effects [9]. Thus, although many experts believe that “untreatable” *H. pylori* is just ill-treated *H. pylori*, no clinical trial. To the best of our knowledge, *H. pylori* has not yielded a treatment that provides 100% eradication [10]. Recently, probiotic lactic acid bacteria (LAB) have been reported to control *H. pylori*. Also, several studies have examined the efficacy of various probiotic preparations for *H. pylori* eradication with and without co-interventions [11]. Moreover, a number of clinical trials have been undertaken to test the hypothesis that probiotic bacteria inhibits *H. pylori* infection [12]. Probiotics inhibit enteric pathogens such as *Salmonella*, *Shigella*, and *Citrobacter rodentium* in both *in vitro* and *in vivo* [13, 14], and potential clinical benefits in preventing or resolving gastrointestinal diseases have been demonstrated [15, 16].

These microorganisms provide gut protection through several mechanisms, including decreasing luminal pH by producing lactic acid [17, 18] and competing with gut pathogens for host surface receptors [19]. Nonetheless, it has been shown that probiotics may inhibit *H. pylori* growth, independent of pH and lactic acid levels [20]. *In vitro* assays were carried out to determine whether the combination of *Pediococcus acidilactici* J9, and its adhesion to gastric cells thus impacting gastric acidity, inhibit the growth of *H. pylori*. The current therapeutic regimen for *H. pylori* aims to eliminate bacterial growth with antibiotics and this reduces the total acidity of gastric acid.

In this study, repeated toxicity tests are performed as the stability test. using mice of C57BL/6 type under the “standard of toxicity test for medicine and medical supplies (Korea food and drug administration notification No. 1999-61)”. We also demonstrated in in vitro models that *Pediococcus acidilactici* J9 in combination have beneficial effects similar to those of antibiotic therapy on *H. pylori*-infected gastric epithelium.

**Results**

**Death rate and normal symptoms**

*Pediococcus acidilactici* J9 was administrated by oral injection for 2 weeks and the Table 1, 2 show the death rate and the normal symptoms of males and females observed for 2 weeks. During the experiment, experimental mice were observed at regular times, and no death was observed in the male and female administration group (Table 1). Also, during all of the experiment, in every administration group – low dose (500 mg/kg/day), medium dose (1,000 mg/kg/day), and high dose (2,000 mg/kg /day) - including the control group, no specific adverse symptoms are observed (Table 2). In this study, a dose of 2,000 mg/kg, which is a maximum dose of oral administration toxicity test, did not generate abnormal
symptoms. It thus seems that the minimal lethal dose of this experimental materials exceeds 2,000 mg/kg/day in both male and female.

| Sex   | Dose (mg) | No. of animal | Days after treatment | Final mortality |
|-------|-----------|---------------|----------------------|----------------|
|       |           |               | 0 | 7 | 14 | End |                   |
| Male  | 0         | 10            | 0/10 | 0/10 | 0/10 | T.S | 0/10 |
|       | 500       | 10            | 0/10 | 0/10 | 0/10 | T.S | 0/10 |
|       | 1,000     | 10            | 0/10 | 0/10 | 0/10 | T.S | 0/10 |
|       | 2,000     | 10            | 0/10 | 0/10 | 0/10 | T.S | 0/10 |
| Female| 0         | 10            | 0/10 | 0/10 | 0/10 | T.S | 0/10 |
|       | 500       | 10            | 0/10 | 0/10 | 0/10 | T.S | 0/10 |
|       | 1,000     | 10            | 0/10 | 0/10 | 0/10 | T.S | 0/10 |
|       | 2,000     | 10            | 0/10 | 0/10 | 0/10 | T.S | 0/10 |

Values are expressed as the numbers of dead animals/total numbers of animals

T.S: terminal sacrifice.

| Sex   | Dose (mg) | Clinical signs | Days after treatment |
|-------|-----------|----------------|----------------------|
|       |           |                | 0 | 7 | 14 |
| Male  | 0         | NAD            | 0/10 | 0/10 | 0/10 |
|       | 500       | NAD            | 0/10 | 0/10 | 0/10 |
|       | 1,000     | NAD            | 0/10 | 0/10 | 0/10 |
|       | 2,000     | NAD            | 0/10 | 0/10 | 0/10 |
| Female| 0         | NAD            | 0/10 | 0/10 | 0/10 |
|       | 500       | NAD            | 0/10 | 0/10 | 0/10 |
|       | 1,000     | NAD            | 0/10 | 0/10 | 0/10 |
|       | 2,000     | NAD            | 0/10 | 0/10 | 0/10 |

NAD: no abnormalities detected.

Values are expressed as number of animals with the sign/number of animals examined.
Changes In Body Weight

*Pediococcus acidilactici* J9 was orally administered for 2 weeks with varying concentrations and the changes in body weight are shown in Table 3. Changes in body weight during the whole period of experiment were negligible for the control group, low dose group (500 mg/kg/day), medium dose group (1,000 mg/kg/day), and high dose group (2,000 mg/kg/day). Additionally, from the date of administration of experimental materials to the end of the experiment, there was a normal weekly increase in body weight in the control group and the administration group (Fig. 1A).

| Sex   | Dose (mg) | Days after treatment |
|-------|-----------|----------------------|
|       |           | 0        | 7         | 14         |
| Male  | 0         | 19.07 ± 0.80<sup>NS</sup> | 19.64 ± 0.95<sup>NS</sup> | 20.62 ± 1.13<sup>NS</sup> |
|       | 500       | 18.79 ± 1.32 | 19.40 ± 1.53 | 20.22 ± 1.39 |
|       | 1,000     | 19.21 ± 0.70 | 19.90 ± 1.04 | 20.59 ± 1.11 |
|       | 2,000     | 19.08 ± 0.92 | 19.89 ± 1.50 | 20.83 ± 1.79 |
| Female| 0         | 16.60 ± 0.96<sup>NS</sup> | 16.67 ± 0.77<sup>NS</sup> | 17.79 ± 0.77<sup>NS</sup> |
|       | 500       | 16.64 ± 0.64 | 16.94 ± 0.40 | 17.49 ± 0.47 |
|       | 1,000     | 16.41 ± 0.64 | 16.78 ± 0.71 | 17.37 ± 0.79 |
|       | 2,000     | 16.30 ± 0.79 | 16.16 ± 0.96 | 16.92 ± 0.85 |

Values are expressed as mean ± SE (n = 10).

NS: not significantly different among groups.

Intake Of Nutrition And Water

There was no significant change in the control group and the experimental material administration group in the amount of intake of feed and water during the experiment period (Tables 4 and 5). Therefore, it seems that the administration of experimental materials does not affect significantly the amount of intake of feed and water (Fig. 1B and C).
Table 4
Daily food consumption of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

| Sex    | Dose (mg) | Food consumption (g/day/mouse) | Days after treatment |
|--------|-----------|--------------------------------|---------------------|
|        |           |                                | 7                   | 14                  |
| Male   | 0         | 74 NS                          | 87 NS               |
|        | 500       | 92                             | 91                  |
|        | 1,000     | 71                             | 95                  |
|        | 2,000     | 113                            | 93                  |
| Female | 0         | 103 NS                         | 83 NS               |
|        | 500       | 85                             | 81                  |
|        | 1,000     | 108                            | 79                  |
|        | 2,000     | 65                             | 70                  |

Values are expressed as mean (n = 2).

NS: not significantly different among groups.
Table 5
Daily water consumption of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

| Sex     | Dose (mg) | Days after treatment | Water consumption (mL/day/mouse) |
|---------|-----------|----------------------|----------------------------------|
|         |           | 7                    | 14                               |
| Male    | 0         |                      | 139 ± NS                         |
|         |           |                      | 120 ± NS                         |
|         | 500       |                      | 141±                             |
|         | 1,000     |                      | 137±                             |
|         | 2,000     |                      | 154±                             |
| Female  | 0         |                      | 127 ± NS                         |
|         |           |                      | 124 ± NS                         |
|         | 500       |                      | 148±                             |
|         | 1,000     |                      | 142±                             |
|         | 2,000     |                      | 132±                             |

Values are expressed as mean (n = 2).

NS: not significantly different among groups.

**Autopsy Results**

As the result of the observation of main organs with naked eyes after the autopsy of experimental mice, there was no significant change in organs and specific autopsy opinion dependent on the dose of administration (Table 6). However in both control group and administration group, blackish red discoloration at the spleen terminal, shrinkage of the right testicle, and thinning of the right atrium were observed. (CTR-F-001: discoloration of spleen; CTR-F-005: discoloration of spleen; 500 mg-F-001: discoloration of spleen; CTR-M-004: discoloration of spleen; CTR-M-005: thinning of right atrium; 500 mg-M-004: discoloration of spleen; 1000 mg-M-004: shrinkage of right testicle)
Table 6
Gross findings of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

| Sex   | Male          | Female         |
|-------|---------------|----------------|
| Dose (mg) | 0 | 500 | 1,000 | 2,000 | 0 | 500 | 1,000 | 2,000 |
| Brain  | NGF 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Lung   | NGF 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Liver  | NGF 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Heart  | NGF 9(90) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Kidney (L) | NGF 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Kidney (R) | NGF 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Testis (L) | NGF 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Testis (R) | NGF 10(100) | 10(100) | 10(100) | 9(90) | 10(100) |
| Ovary (L) | NGF | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Ovary (R) | NGF | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |
| Spleen | NGF 9(90) | 9(90) | 10(100) | 10(100) | 7(70) | 9(90) | 10(100) | 10(100) |
| Thymus | NGF 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) | 10(100) |

NGF: no gross finding

Values are expressed as animal numbers (the percentage of animal numbers)

**The Weight Of Organs**

The weight of organs were measured after repeated administration of *Pediococcus acidilactici* J9 which varied to low dose (500 mg/kg/day), medium dose (1,000 mg/kg/day), and high dose (2,000 mg/kg/day) for 2 weeks (Table 7). No changes were observed in the weight of brain, lung, testis, ovary, kidney, heart, spleen, and liver with respect to the administration of experimental materials and no abnormal changes were dependent on dose of administrations. Generally, when the toxic materials were ingested, liver takes...
the largest effect since the detoxication starts at the liver. However, there were no significant changes in each group on the observed weight of the liver. From the results above, the administration of *Pediococcus acidilactici* J9 does not affect the weight of organs.

Table 7
Organ weights of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

| Sex   | Organs  | Dose (mg) | 0          | 500        | 1,000      | 2,000      |
|-------|---------|-----------|------------|------------|------------|------------|
|       |         |           | 500        | 1,000      | 2,000      |            |
| Male  | Brain   | 0.445 ± 0.014 | 0.440 ± 0.014 | 0.444 ± 0.015 | 0.437 ± 0.016 |            |
|       | Lung    | 0.124 ± 0.013 | 0.126 ± 0.006 | 0.125 ± 0.010 | 0.134 ± 0.023 |            |
|       | Liver   | 0.984 ± 0.208 | 0.920 ± 0.065 | 0.929 ± 0.183 | 0.904 ± 0.176 |            |
|       | Heart   | 0.104 ± 0.011 | 0.101 ± 0.007 | 0.100 ± 0.008 | 0.105 ± 0.010 |            |
|       | Kidney(L)| 0.129 ± 0.025 | 0.128 ± 0.025 | 0.127 ± 0.130 | 0.126 ± 0.188 |            |
|       | Kidney(R)| 0.133 ± 0.025 | 0.136 ± 0.011 | 0.134 ± 0.017 | 0.140 ± 0.029 |            |
|       | Testis(L)| 0.071 ± 0.020 | 0.067 ± 0.000 | 0.067 ± 0.008 | 0.071 ± 0.006 |            |
|       | Testis(R)| 0.074 ± 0.024 | 0.070 ± 0.000 | 0.063 ± 0.018 | 0.074 ± 0.007 |            |
|       | Spleen  | 0.057 ± 0.009 | 0.052 ± 0.007 | 0.050 ± 0.006 | 0.054 ± 0.014 |            |
|       | Thymus  | 0.042 ± 0.010 | 0.046 ± 0.007 | 0.044 ± 0.007 | 0.045 ± 0.008 |            |
| Female| Brain   | 0.443 ± 0.014 | 0.434 ± 0.019 | 0.437 ± 0.013 | 0.431 ± 0.018 |            |
|       | Lung    | 0.120 ± 0.006 | 0.120 ± 0.008 | 0.134 ± 0.014 | 0.121 ± 0.017 |            |
|       | Liver   | 0.775 ± 0.065 | 0.788 ± 0.199 | 0.755 ± 0.092 | 0.727 ± 0.103 |            |
|       | Heart   | 0.091 ± 0.007 | 0.089 ± 0.005 | 0.105 ± 0.007 | 0.084 ± 0.007 |            |
|       | Kidney(L)| 0.114 ± 0.025 | 0.102 ± 0.011 | 0.126 ± 0.007 | 0.102 ± 0.010 |            |
|       | Kidney(R)| 0.110 ± 0.011 | 0.110 ± 0.013 | 0.140 ± 0.012 | 0.105 ± 0.012 |            |
|       | Ovary (L)| 0.001 ± 0.000 | 0.002 ± 0.000 | 0.071 ± 0.000 | 0.001 ± 0.000 |            |
|       | Ovary(R)| 0.002 ± 0.000 | 0.002 ± 0.000 | 0.074 ± 0.000 | 0.002 ± 0.000 |            |
|       | Spleen  | 0.058 ± 0.007 | 0.052 ± 0.010 | 0.053 ± 0.010 | 0.051 ± 0.007 |            |
|       | Thymus  | 0.077 ± 0.007 | 0.071 ± 0.010 | 0.045 ± 0.011 | 0.076 ± 0.014 |            |

Values are expressed as mean ± SE (n = 10).

NS: not significantly different among groups.
Hematological Tests

As the result of the measurement of percentages of Red blood cells, RBC, hematocrit, HCT, hemoglobin, Hb, mean corpuscular volume, MCV, mean corpuscular hemoglobin, MCH, mean corpuscular hemoglobin concentration, MCHC, white blood cells, WBC, Hemoglobin, HGB, Cellular Hb Concentration Mean, CHCM, Red Cell Distribution Width, RDW, Hb Distribution Width, HDW, Cellular Hb content, CH, Cellular Hb Distribution Width, CHDW, Platelet, PLT, Platelet Distribution Width, PDW, Plateletcrit, PCT, Neutrophil, NEUT, Neutrophil, NEUT%, Lymphocyte, LYMPH, Lymphocyte %, LYMPH%, Monocyte, MONO, Monocyte %, MONO%, Eosinophil, EOS, Eosinophil %, EOS%, Basophil, BASO, Basophil %, BASO%, Large Unstained Cells, LUC, Large Unstained Cells, LUC%, Reticulocyte Count, Retic#, Reticulocyte %, Retic%, Mean Corpuscular Volume of Retics, MCVr, Mean Corpuscular Volume of Retics %, MCVr%, Red Cell Distribution Width of Retics, RDWr*, Hb Distribution Width of Retics, HDWr*, Cellular Hb of Retics, CHr, and Cellular Hb Distribution Width of Retics, CHDWr* after the autopsy using blood auto-analyzer (System SE-9000, TOA Medical Electronics Co., Ltd., Kobe, Japan), no significant changes were observed in control and administration groups ($p \leq 0.05$) (Table 8). As a result of hematological examination, both the control group and the administration group were included in normal range and no dependence on dose was observed. This result is similar to the range previously reported in hematological fundamental database of which there is a repeated toxicity test for 2 weeks using mice.
Table 8
Hematology of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

| Sex   | Parameters       | Dose (mg) |
|-------|------------------|-----------|
|       |                  | 0         | 500     | 1,000 | 2,000 |
| Male  | CBC              |           |         |       |       |
|       | WBC (x10^3/µL)   | 2.672 ± 0.65 | 2.066 ± 0.63 | 2.016 ± 1.01 | 2.474 ± 0.96 |
|       | RBC (x10^6/µL)   | 9.88 ± 0.33  | 9.518 ± 0.26 | 10.00 ± 0.14 | 9.876 ± 0.32 |
|       | HGB (g/dL)       | 15.14 ± 0.64 | 14.72 ± 0.36 | 15.36 ± 0.28 | 15.26 ± 0.53 |
|       | HCT (%)          | 51.62 ± 2.24 | 49.42 ± 1.43 | 51.38 ± 1.17 | 50.90 ± 2.09 |
|       | MCV (fL)         | 52.26 ± 0.69 | 51.9 ± 0.42 | 51.36 ± 1.09 | 51.54 ± 0.72 |
|       | MCH (pg)         | 15.32 ± 0.22 | 15.46 ± 0.21 | 15.34 ± 0.22 | 15.46 ± 0.23 |
|       | MCHC (g/dL)      | 29.30 ± 0.23 | 29.78 ± 0.29 | 29.88 ± 0.43 | 29.96 ± 0.40 |
|       | CHCM (g/dL)      | 28.16 ± 0.35 | 28.16 ± 0.11 | 28.64 ± 0.44 | 28.68 ± 0.45 |
|       | RDW (%)          | 13.36 ± 0.38 | 13.88 ± 0.40 | 13.30 ± 0.50 | 13.26 ± 0.94 |
|       | HDW (g/dL)       | 1.45 ± 0.02  | 1.45 ± 0.06 | 1.45 ± 0.04 | 1.45 ± 0.03 |
|       | CH (pg)          | 14.68 ± 0.19 | 14.58 ± 0.08 | 14.66 ± 0.17 | 14.74 ± 0.09 |
|       | CHDW (%)         | 2.00 ± 0.05  | 2.07 ± 0.08 | 2.00 ± 0.05 | 2.01 ± 0.11 |
|       | PLT (x10^3/µL)   | 1263.20 ± 73.52 | 1244.80 ± 57.87 | 1238.60 ± 61.75 | 1202.00 ± 60.76 |
|       | MPV (fL)         | 7.44 ± 0.17  | 7.88 ± 0.05 | 7.52 ± 0.08 | 7.48 ± 0.26 |
|       | PDW (%)          | 60.38 ± 1.85 | 57.30 ± 1.62 | 55.80 ± 1.62 | 55.96 ± 2.34 |
|       | PCT (%)          | 0.94 ± 0.06  | 0.98 ± 0.05 | 0.93 ± 0.05 | 0.90 ± 0.06 |

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.
| DIFF  | NEUT (x10³/µL)         | 0.34 ± 0.13 | 0.18 ± 0.05 | 0.19 ± 0.09 | 0.21 ± 0.05 |
|-------|-------------------------|-------------|-------------|-------------|-------------|
| NEUT (%) | 12.5 ± 3.19 | 9.26 ± 2.15 | 9.56 ± 1.06 | 8.94 ± 1.50 |
| LYMHP (x10³/µL) | 2.25 ± 0.52 | 1.83 ± 0.56 | 1.78 ± 0.89 | 2.22 ± 0.89 |
| LYMHP (%) | 84.46 ± 3.46 | 88.48 ± 2.27 | 88.52 ± 1.21 | 89.46 ± 1.17 |
| MONO (x10³/µL) | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| MONO (%) | 0.54 ± 0.34 | 0.32 ± 0.28 | 0.28 ± 0.18 | 0.32 ± 0.16 |
| EOS (x10³/µL) | 0.05 ± 0.02 | 0.03 ± 0.04 | 0.02 ± 0.01 | 0.01 ± 0.01 |
| EOS (%) | 1.64 ± 0.50 | 1.30 ± 1.31 | 0.76 ± 0.30 | 0.48 ± 0.21 |
| BASO (x10³/µL) | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| BASO (%) | 0.28 ± 0.13 | 0.40 ± 0.20 | 0.34 ± 0.24 | 0.26 ± 0.19 |
| LUC (x10³/µL) | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 |
| LUC (%) | 0.62 ± 0.16 | 0.24 ± 0.27 | 0.48 ± 0.33 | 0.56 ± 0.23 |
| RETI  | Reticulocyte (x10⁹/µL) | 301.60 ± 47.96 | 258.32 ± 30.72 | 302.89 ± 10.26 | 298.02 ± 63.69 |
| Reticulocyte (%) | 3.15 ± 0.51 | 2.71 ± 0.25 | 3.03 ± 0.12 | 3.00 ± 0.56 |
| MCVr (fL) | 58.00 ± 0.51 | 57.46 ± 0.48 | 57.60 ± 0.65 | 57.86 ± 1.13 |
| CHCMr (g/dL) | 26.24 ± 0.17 | 25.92 ± 0.13 | 26.30 ± 0.19 | 26.18 ± 0.31 |
| RDWr (%) | 12.06 ± 0.65 | 12.36 ± 0.66 | 11.96 ± 0.64 | 12.20 ± 0.85 |
| HDWr (%) | 2.34 ± 0.12 | 2.40 ± 0.10 | 2.43 ± 0.07 | 2.52 ± 0.10 |
| CHr (pg) | 15.20 ± 0.25 | 14.86 ± 0.22 | 15.12 ± 0.11 | 15.12 ± 0.16 |

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.
|                | CHDWr (%)     | CBC WBC (x10³/µL) | RBC (x10⁶/µL) | HGB (g/dL)     | HCT (%)       | MCV (fL)     | MCH (pg)     | MCHC (g/dL)  | CHCM (g/dL)  | RDW (%)      | HDW (g/dL)   | CH (pg)       | CHDW (%)     | PLT (x10³/µL) | MPV (fL)     | PDW (%)       | PCT (%)      | NEUT (x10³/µL) | NEUT (%)     |
|----------------|---------------|--------------------|---------------|---------------|---------------|--------------|--------------|--------------|--------------|---------------|--------------|----------------|--------------|----------------|--------------|--------------|---------------|--------------|----------------|--------------|
| Female         | 1.98 ± 0.08   | 2.95 ± 0.60        | 9.51 ± 0.13   | 14.72 ± 0.21  | 49.12 ± 0.73  | 51.64 ± 0.43 | 15.50 ± 0.23 | 29.98 ± 0.36 | 28.74 ± 0.34 | 13.68 ± 0.46  | 1.50 ± 0.05  | 14.76 ± 0.13  | 2.07 ± 0.06  | 955.60 ± 64.27 | 7.56 ± 0.35  | 57.78 ± 3.04  | 0.73 ± 0.07   | 0.28 ± 0.07  | 9.48 ± 0.42   |
|                | 1.97 ± 0.07   | 2.53 ± 1.08        | 10.19 ± 0.15  | 15.80 ± 0.47  | 52.20 ± 1.70  | 51.16 ± 1.04 | 15.48 ± 0.33 | 30.28 ± 0.35 | 28.94 ± 0.40 | 13.90 ± 0.46  | 1.54 ± 0.03  | 14.74 ± 0.21  | 2.11 ± 0.05  | 1102.40 ± 63.27 | 7.70 ± 0.16  | 57.10 ± 2.82  | 0.85 ± 0.06   | 0.29 ± 0.06  | 12.38 ± 2.50  |
|                | 1.92 ± 0.05   | 2.75 ± 0.70        | 10.07 ± 0.22  | 15.60 ± 0.26  | 51.48 ± 1.52  | 51.12 ± 0.79 | 15.50 ± 0.33 | 30.32 ± 0.61 | 28.96 ± 0.43 | 13.00 ± 0.52  | 1.52 ± 0.04  | 14.76 ± 0.15  | 2.00 ± 0.08  | 1129.80 ± 94.82 | 7.82 ± 0.11  | 54.94 ± 1.31  | 0.89 ± 0.08   | 0.25 ± 0.08  | 9.70 ± 3.01   |
|                | 1.97 ± 0.06   | 3.32 ± 1.34        | 9.97 ± 0.31   | 15.74 ± 0.31  | 50.44 ± 1.77  | 50.12 ± 0.62 | 15.78 ± 0.42 | 31.20 ± 0.91 | 29.5 ± 0.35  | 13.02 ± 0.46  | 1.59 ± 0.02  | 14.86 ± 0.06  | 2.03 ± 0.06  | 1035.00 ± 107.62 | 7.38 ± 0.47  | 57.68 ± 3.19  | 0.77 ± 0.11   | 0.23 ± 0.12  | 6.94 ± 1.34   |

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.
| Parameter                | Group 1 (x10^3/µL) | Group 2 (x10^3/µL) | Group 3 (x10^3/µL) | Group 4 (x10^3/µL) |
|-------------------------|---------------------|---------------------|---------------------|---------------------|
| LYMPH                   | 2.61 ± 0.53         | 2.20 ± 1.01         | 2.45 ± 0.72         | 3.03 ± 1.19         |
| LYMPH (%)               | 88.50 ± 0.39        | 86.24 ± 2.40        | 88.74 ± 3.30        | 91.42 ± 1.61        |
| MONO (x10^3/µL)         | 0.01 ± 0.00         | 0.00 ± 0.01         | 0.01 ± 0.01         | 0.01 ± 0.00         |
| MONO (%)                | 0.46 ± 0.15         | 0.20 ± 0.12         | 0.22 ± 0.13         | 0.22 ± 0.08         |
| EOS (x10^3/µL)          | 0.02 ± 0.01         | 0.01 ± 0.01         | 0.01 ± 0.01         | 0.01 ± 0.01         |
| EOS (%)                 | 0.80 ± 0.25         | 0.34 ± 0.38         | 0.34 ± 0.26         | 0.42 ± 0.29         |
| BASO (x10^3/µL)         | 0.01 ± 0.01         | 0.01 ± 0.01         | 0.01 ± 0.00         | 0.01 ± 0.01         |
| BASO (%)                | 0.32 ± 0.22         | 0.30 ± 0.12         | 0.38 ± 0.19         | 0.30 ± 0.19         |
| LUC (x10^3/µL)          | 0.01 ± 0.01         | 0.02 ± 0.01         | 0.02 ± 0.01         | 0.03 ± 0.02         |
| LUC (%)                 | 0.48 ± 0.13         | 0.54 ± 0.18         | 0.64 ± 0.51         | 0.66 ± 0.38         |
| RETI Reticulocyte (x10^9/µL) | 309.34 ± 52.93     | 304.60 ± 43.32     | 277.50 ± 38.28     | 351.10 ± 49.29     |
| RETI Reticulocyte (%)   | 3.25 ± 0.53         | 2.99 ± 0.42         | 2.76 ± 0.41         | 3.53 ± 0.60         |
| MCVr (fL)               | 57.90 ± 0.50        | 57.86 ± 1.25        | 57.36 ± 0.70        | 58.12 ± 0.75        |
| CHCMr (g/dL)            | 26.46 ± 0.18        | 26.42 ± 0.33        | 26.44 ± 0.11        | 26.76 ± 0.23        |
| RDWr (%)                | 13.08 ± 0.93        | 12.14 ± 1.01        | 13.40 ± 0.70        | 13.22 ± 1.00        |
| HDWr (%)                | 2.58 ± 0.11         | 2.63 ± 0.11         | 2.75 ± 0.12         | 2.81 ± 0.12         |
| CHr (pg)                | 15.30 ± 0.23        | 15.24 ± 0.42        | 15.12 ± 0.22        | 15.52 ± 0.13        |
| CHDWr (%)               | 2.03 ± 0.04         | 2.01 ± 0.08         | 2.07 ± 0.07         | 2.13 ± 0.10         |

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.

Blood biochemical analysis.
As the result of the measurement of Glucose; GLU, Blood Urea Nitrogen; BUN, Creatinine; CREA, Total cholesterol; T-CHOL, Albumin; ALB, Total Bilirubin; T-BIL, Alkaline Phosphatase; ALP, Aspartate Aminotransferase; AST(GOT), Alanine Aminotransferase; ALT(GPT), Triglyceride; TG, Total protein; TP using auto-analyzer (Hitachi-747, Hitachi Medical Co., Tokyo, Japan) from blood serum, which is the indicator of blood biochemistry, no significant changes dependent on the administration of experimental materials were observed in the whole administration groups with respect to the control group. Both the control group and *Pediococcus acidilactici* J9 administration group showed normal parameters ($p \leq 0.05$) (Table 9).
Table 9
Levels of serum biochemical indices of male and female (mice) treated orally with *Pediococcus acidilactici* J9 for 14 days

| Sex     | Parameters | Dose (mg) | 0        | 500      | 1,000     | 2,000     |
|---------|------------|-----------|----------|----------|-----------|-----------|
| Male    | Glu        |           | 256.40 ± 11.91 | 243.00 ± 42.57 | 234.20 ± 25.82 | 230.80 ± 31.32 |
|         | BUN        |           | 18.68 ± 3.87  | 21.54 ± 5.78  | 20.50 ± 6.22  | 18.24 ± 2.60  |
|         | Crea       |           | 0.28 ± 0.04   | 0.29 ± 0.02   | 0.27 ± 0.02   | 0.27 ± 0.03   |
|         | T-Chol     |           | 79.00 ± 4.36  | 85.20 ± 4.92  | 82.40 ± 6.80  | 81.80 ± 4.66  |
|         | TP         |           | 4.74 ± 0.05   | 4.82 ± 0.24   | 4.76 ± 0.11   | 4.68 ± 0.16   |
|         | ALB        |           | 1.70 ± 0.07   | 1.72 ± 0.08   | 1.68 ± 0.04   | 1.68 ± 0.13   |
|         | T-BIL      |           | 0.06 ± 0.05   | 0.04 ± 0.05   | 0.08 ± 0.04   | 0.08 ± 0.04   |
|         | ALP        |           | 133.00 ± 19.51| 137.80 ± 9.65| 126.20 ± 19.82| 137.40 ± 15.96|
|         | AST(GOT)   |           | 56.20 ± 15.07 | 65.20 ± 13.18| 48.00 ± 7.35 | 57.40 ± 13.28 |
|         | ALT(GPT)   |           | 28.80 ± 4.15  | 28.00 ± 4.18  | 22.20 ± 1.30  | 25.20 ± 4.49  |
|         | TG         |           | 66.40 ± 26.88 | 64.40 ± 31.45 | 49.80 ± 23.85 | 39.40 ± 17.94 |
|         | A/G        |           | 0.56 ± 0.05   | 0.56 ± 0.05   | 0.52 ± 0.04   | 0.56 ± 0.05   |
|         | B/C        |           | 66.61 ± 12.59 | 74.13 ± 18.80 | 76.83 ± 22.46 | 69.05 ± 11.10 |
| Female  | Glu        |           | 214.40 ± 37.40| 229.60 ± 47.45| 228.40 ± 40.32| 231.00 ± 66.87 |
|         | BUN        |           | 25.16 ± 5.42  | 24.70 ± 5.07  | 23.06 ± 3.26  | 22.58 ± 4.50  |
|         | Crea       |           | 0.27 ± 0.05   | 0.25 ± 0.05   | 0.28 ± 0.03   | 0.26 ± 0.03   |
|         | T-Chol     |           | 76.80 ± 8.11  | 70.20 ± 5.97  | 78.80 ± 13.44 | 80.00 ± 9.85  |
|         | TP         |           | 4.82 ± 0.08   | 4.72 ± 0.16   | 4.78 ± 0.20   | 4.70 ± 0.23   |
|         | ALB        |           | 1.74 ± 0.05   | 1.74 ± 0.05   | 1.74 ± 0.05   | 1.72 ± 0.04   |
|         | T-BIL      |           | 0.00 ± 0.00   | 0.02 ± 0.04   | 0.02 ± 0.04   | 0.02 ± 0.04   |
|         | ALP        |           | 168.20 ± 8.93 | 173.40 ± 10.31| 154.20 ± 13.88| 153.60 ± 26.37|

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.
|                |                  |                |                |                |
|----------------|------------------|----------------|----------------|----------------|
| AST(GOT)       | 68.20 ± 17.02    | 67.60 ± 14.57  | 66.80 ± 8.20   | 78.40 ± 18.01  |
| ALT(GPT)       | 21.00 ± 12.88    | 22.80 ± 3.11   | 24.60 ± 3.51   | 23.00 ± 3.39   |
| TG             | 0.56 ± 0.05      | 43.00 ± 9.70   | 27.00 ± 3.00   | 34.20 ± 15.45  |
| A/G            | 0.56 ± 0.05      | 0.58 ± 0.04    | 0.56 ± 0.05    | 0.58 ± 0.04    |
| B/C            | 94.97 ± 12.02    | 103.82 ± 32.56 | 82.32 ± 13.29  | 87.08 ± 24.74  |

Values are expressed as mean ± SE (n = 5).

NS: not significantly different among groups.

**Histopathology Observations**

For the histopathology test of *Pediococcus acidilactici* J9-administrated mice, liver and kidney were stained by hematoxylin and eosin. As the result of the histopathology test, no lesions were observed in the liver, like infection, necrosis, iron pigmentation, and bilirubin pigmentation. The structure of liver cells were also normal (Fig. 2A). There was no lesions in the kidney, like infection and necrosis, and no changes were observed in kidney cells (Fig. 2B). Therefore, there is no significant changes in liver and kidney, and no extraordinary pathologic abnormality dependent on dose of experimental materials were observed in both the control group and administration group as the result of the histopathology test. This opinion seems to correspond with the long-term change of weight as well as the blood biochemical change.

**Inhibition of adhesion and growth of H.pylori in gastric epithelial cells in the presence of Pediococcus acidilactici J9**

The adhesion and growth of *H. pylori* were inhibited by a 24 h treatment of *H. pylori* and *Pediococcus acidilactici* J9 at a 200 ug / ml concentration on AGS cells, which are gastric cancer cells. Compared to the control group (AGS cell and H.pylori), the number of H. pylori analyzed by FACS significantly (p < 0.01) decreased after incubation of AGS cell with Pediococcus acidilactici J9 for 24 hours. Control biological triplicate groups are also analyzed for statistical options. (Fig. 3 and Fig. S1 in Additional file 1).

**Discussion**

*Pediococcus acidilactici* J9 was obtained from a bean paste soup prepared with ground fermented soybeans and beneficially affects to human body by improving its intestinal microbial balances. *Pediococcus acidilactici* J9 has been emerged as a potential probiotic. *Pediococcus acidilactici* J9 exerts as an antagonism against other enteric pathogens, primarily through the production of lactic acid and secretion of pediocin. Thermo-stable pediocin is an antimicrobial peptide is known to have a strong
activity against food bacteria and pathogenic enteric bacteria [21]. For these reasons, Probiotics including *Pediococcus acidilactici* J9 were used for commercial healthcare products like beverages and foods. Pediocin secreted by *Pediococcus acidilactici* J9 has a potential to inhibit other pathogenic bacteria.

*H.pylori* is known to be an important causative agent of peptic ulcer, gastritis, gastric cancer, or mucosa associated lymphoid tissue lymphoma [22]. Various antibiotics have been used for *H.pylori* eradication [23]. These antimicrobial agents have been pointed out for various problems such as adverse effects, risk of re-infection due to increased pH, appearance of resistant bacteria, and high cost [24]. Recently, *H.pylori* inhibitory activity of natural products has been reported as a new treatment method of *H.pylori*. There is growing interest in probiotic lactic acid bacteria, which can play a role in the treatment of *H.pylori* by directly acting on *H.pylori*, with minimal clinical side effects of antibiotics [25].

This study investigated the toxicity and anti-*H.pylori* effect of *Pediococcus acidilactici* J9. Daily administration of *Pediococcus acidilactici* J9 in mice for two week showed no abnormal clinical signs in body weight, hematology, food intake and water consumption. In all test groups, no general symptoms and deaths from the test substance were observed. During the entire test period, body weight continuously increased but no significant change was observed with the control group. In addition, there were no significant differences in the gross observation, long-term weight change, hematology, blood biochemical and histopathologic examination of the organ in all the test substance administration groups, and all of them were within the normal range. As a result of repeated toxicity test for 2 weeks, *Pediococcus acidilactici* J9 was judged to be a safe and low-toxic substance. *Pediococcus acidilactici* J9, inhibits the adhesion of *H.pylori* to AGS gastric cancer cells. Probiotics refers to living microorganisms that are beneficial to the human body when consumed in moderate quantities [26, 27]. Most probiotics known to date are lactic acid bacteria [28, 29]. Probiotic bacteria such as lactic acid bacteria and beneficial bacteria survive in the stomach acid and bile acid in the body, reach the small intestine, multiply in the intestines and settle [30, 31]. It has a beneficial effect on health in the colon, and these probiotics should be non-toxic and non-pathogenic [32, 33]. Ingestion of probiotics not only helps maintain health, it also helps to improve various diseases such as infants, irritable bowel syndrome, and inflammatory bowel disease [34].

Based on our in vivo and in vitro results, when used as probiotics, *Pediococcus acidilactici* J9 may help decrease the occurrence of gastritis and reduce the risk of *H.pylori* infection with promising safety issues, without side effects.

**Conclusions**

In conclusion, we reported the toxicity and anti-*H.pylori* effect of *Pediococcus acidilactici* J9. Daily administration of *Pediococcus acidilactici* J9 in mice for two week showed no abnormal clinical signs in body weight, hematology, food intake and water consumption. Also, *Pediococcus acidilactici* J9, inhibited the adhesion of *H.pylori* to AGS gastric cancer cells. Based on our in vivo and in vitro results, when used...
as probiotics, *Pediococcus acidilactici* J9 may help decreasing the occurrence of gastritis and reducing the risk of *H. pylori* infection with promising safety issues, without side effects.

**Methods**

**Model organisms and conditions**

C57BL/6 mice of 4 weeks of age without certain pathogens are purchased at an amount of 20 males and females each. Normal and healthy mice without any weight loss are used in experiment by clinical observation during 7 days of education. Feeds are the following; solid feeds for laboratory animal are freely offered, and drinking water. The filtration-purified water is also freely offered to mice.

**Configuration Of Test Group And Set Of Dosage Setting**

Dosage was set by MFDs standards. Maximum dosage is set to 2,000 mg/kg/day for both male and female, with the geometric ratio of 1/2, low dose group, medium dose group, and high dose group are set at 500, 1000, and 2000 mg per body weight(kg) respectively. The number of mice in each group are set to 5 males and females each. Dosage is set to not exceed 0.2 ml per 10 g and calculated according to the body weight measured just before administration. Test materials are well-mixed to sterile distilled water before administration, and they are directly injected into the stomach by sonde for oral administration for once a day during 2 weeks. Sterile distilled water for injection is used as reference material.

**Normal Symptoms And Observation Of Lethality In Mice**

Observation is conducted for 6 hours after oral administration and starting from the next day, to observe change of general condition, as well as expression of addiction. This was held in presence of dead mice and symptoms that can be expressed by the test materials are observed carefully. In the case of abnormality, the type and the extent of symptoms are recorded individually. All mice were checked for death or critical condition.

**Measurement Of Weight, Feed And Water Intake**

For every animal, change of weight is measured just before the administration of test materials once a week at a certain time during 2 weeks. Intake of feeds and water is measured and calculated weekly.

**Autopsy And Naked Eye Examination**

After administration going up to 14 days, the body weight of the surviving mice is measured, before anesthesia with CO2 and autopsy. External findings such as abnormality of subcutaneous, internal
organs and brain were observed with the naked eye. The brain, kidney, liver, lung, reproductive organ, heart, spleen, and thymus are extracted and weighed.

**Blood Biochemical**

The hematologic analysis of the serum is performed the same day of the autopsy, which is collected from a 3,000 rpm, 20 minutes long, centrifugation of the blood and conducted by auto-analyzer (Hitachi-747, Hitachi Medical Co., Tokyo, Japan). Glucose; GLU, Blood Urea Nitrogen; BUN, Creatinine; CREA, Total cholesterol; T-CHOL, Albumin; ALB, *Total Biliubin*; *T-BIL*, Alkaline Phosphatase; ALP, Aspartate Aminotransferase; AST(GOT), Alanine Aminotransferase; ALT(GPT), Triglyceride; TG, and Total protein; TP are measured.

**Hematology**

Mice fasted for 8 hours before the autopsy are slightly anesthetized with CO$_2$. Part of the blood from the exsanguination is EDTA-treated and stored in tubes and then analyzed by blood auto-analyzer (System SE-9000, TOAMedical Electronics Co., Ltd., Kobe, Japan). Red blood cells, RBC, hematocrit, HCT, hemoglobin, Hb, mean corpuscular volume, MCV, mean corpuscular hemoglobin, MCH, mean corpuscular hemoglobin concentration, MCHC, white blood cells, WBC, Hemoglobin, HGB, Cellular Hb Concentration Mean, CHCM, Red Cell Distribution Width, RDW, Hb Distribution Width, HDW, Cellular Hb content, CH, Cellular Hb Distribution Width, CHDW, Platelet, PLT, Platelet Distribution Width, PDW, Plateletcrit, PCT, Neutrophil, NEUT, Neutrophil, NEUT%, Lymphocyte, LYMPH, Lymphocyte %, LYMPH%, Monocyte, MONO, Monocyte %, MONO%, Eosinophil, EOS, Eosinophil %, EOS%, Basophil, BASO, Basophil %, BASO%, Large Unstained Cells, LUC, Large Unstained Cells, LUC%, Reticulocyte Count, Retic#, Reticulocyte %, Retic%, Mean Corpuscular Volume of Retics, MCVr, Mean Corpuscular Volume of Retics %, MCVr%, Red Cell Distribution Width of Retics, RDWr*, Hb Distribution Width of Retics, HDWr*, Cellular Hb of Retics, CHr, and Cellular Hb Distribution Width of Retics, CHDWr* are measured.

**Histopathology**

Liver and kidney were extracted and fixed with a 10% neutral buffered formalin solution the day of final autopsy, after the observation of gross lesions on every animal which were administered with test materials. Then paraffin embedding was conducted and hematoxylin & eosin dye performed with the sections of 3 ~ 4 um sections.

**Helicobacter Pylori Preparation**
Helicobacter pylori (ATCC 43504) used in this study were obtained and inoculated onto chocolate media, incubated for 5 ~ 7 days at 37°C in a 10% CO₂ incubator under aerobic conditions and then used for the examination. When the chocolate media is filled over 90%, Helicobacter pylori is swabbed with sterilized swabs and suspended in 20 ml of RPMI-1640 media to form the Helicobacter pylori suspension.

细胞培养

The human gastric adenocarcinoma cell lines AGS (KCLB 21739; Korea) cells were seeded at a density of $1 \times 10^5$ cells in 2 ml of RPMI-1640 (RPMI-1640; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) and 1% penicillin-streptomycin (Invitrogen, USA) into 6 well culture plates (SPL) and cultured for 2 ~ 3 days at 37°C in a 5% CO₂ incubator.

粘附试验

When the AGS cell reach a density of 80% of the seeding plate, we eliminate the media from the plate and wash with phosphate buffered saline (PBS : Welgene, Daegu, Korea) 3 times. Experimental groups are as follows. For negative control, only AGS is seeded. For positive control, AGS is treated by 1 ml of H. pylori suspension. For the measurement of suppression of attachment, AGS is treated by 1 ml of H. pylori suspension and Pediococcus acidilactici J9 (200ug/ml). The culture plates seeded with AGS treated by H. pylori and Pediococcus acidilactici J9 are incubated for 90 minutes at 37°C in a 5% CO₂ incubator. The culture media is eliminated and the cells are carefully harvested. The cells are suspended in 500 ul of PBS then examined with FACS.

统计分析

All values shown in the figures are presented as mean ± standard error. Western blot results were analyzed by Student’s t-test. A 2-tailed probability value below 0.05 was considered statistically significant. Data were analyzed using SPSS version 17.0 (SPSS Inc., USA).

声明

伦理审批和同意参加

All experimental animal procedures performed were approved by the Institutional Animal Care and Use Committee (IACUC, Approval number: 16-0043-C2A1) of Seoul National University Hospital, which was accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

同意发表

Not applicable.
Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ML performed experiments, analyzed data, wrote paper, JC provided advice in study design, critically discussed results, co-edited paper, KYK provided advice in study design, critically discussed results, co-edited paper, WI provided advice in study design, critically discussed results, co-edited paper, MK provided advice in study design, critically discussed results, co-edited paper.

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Figures
Figure 1

Changes in body weight and intake of C57BL/6 mice which treated Pediococcus acidilactici J9. Dosage is set by the standard of MFDS. Maximum dosage is set to 2,000 mg/kg/day for both male and female, and with the geometric ratio of 1/2, low dose group, medium dose group, and high dose group are set by per body weight (kg) respectively. a For every animal, change of weight is measured just before the administration of test materials once a week at certain time during 2 weeks. b, c Intake of feeds and water is measured and calculated once a week. Feeds; solid feeds for laboratory animal are freely offered, and drinking water; filtration-purified water is also freely offered. N = 10 samples per group.
Figure 2

Histopathological examinations of the liver and Kidney. a, b Female and male C57BL / 6 mice were orally administered with Pediococcus acidilactici J9 for 14 days. The liver and kidneys of the control and Pediococcus acidilactici J9 administration animals were extracted and fixed with 10% neutral buffered formalin solution on the final autopsy day of all animals after gross lesion observation. Pathological lesions and structures such as liver, kidney infection, necrosis, and iron and bilirubin pigmentation were confirmed by H & E staining. Bar = 30μm, N = 10 samples per group.
Figure 3

Pediococcus acidilactici J9 inhibits adhesion and growth of H. pylori in gastric epithelial cells. a After H. pylori supernatant and Pediococcus acidilactici J9 at 200 ug / ml concentration were treated for 24 hours in AGS cells, H.Pylori count was confirmed by flow cytometry. In the control group, the H.pylori number was confirmed by flow measurement after 24 h of treatment with H.pylori in the AGS cell. b H. pylori number was quantified by a flow cytometer. The experiment was repeated three times and the data are shown as the mean (SEM). **p<0.01 versus control group.

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