Title: KIMCHI LACTIC ACID BACTERIA GROUP HINDERING GROWTH OF HELICOBACTER PYLORI AND HIGH FUNCTIONAL FOOD PROTECTING GASTROENTERIC DISORDER THEREWITH

Abstract: The present invention relates to Kimchi lactic acid bacteria that inhibit growth of Helicobacter pylori, and foods comprising the same, and particularly to a gastrointestinal-tract-protecting high functional food comprising Kimchi lactic acid bacteria that inhibits attachment of Helicobacter pylori to the gastrointestinal tract, and inhibits growth thereof. The Kimchi lactic acid bacteria of the present invention inhibits attachment of Helicobacter pylori that inhibits the gastric mucous membrane tissue of the gastrointestinal tract, and inhibits growth thereof, and thus are very effective for Helicobacter pylori extermination and prevention of reinfection therefrom. In addition, a gastrointestinal-tract-protecting high functional food comprising the Kimchi lactic acid bacteria is very effective for preventing and treating gastritis, peptic ulcers, and duodenal ulcers caused by Helicobacter pylori.
KIMCHI LACTIC ACID BACTERIA GROUP HINDERING GROWTH OF HELICOBACTER PYLORI AND HIGH FUNCTIONAL FOOD PROTECTING GASTROENTERIC DISORDER THEREWITH

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The present invention relates to Kimchi lactic acid bacteria inhibiting the growth of *Helicobacter pylori* and a gastrointestinal-tract-protecting high functional food comprising the same, and particularly to a high functional food that comprises Kimchi lactic acid bacteria which inhibits the attachment of *Helicobacter pylori* to gastric mucous membrane tissues of the gastrointestinal tract, and inhibits growth thereof, and thus is effective for preventing and treating gastritis, peptic ulcers, and duodenal ulcers.

(b) Description of the Related Art

Gastrointestinal diseases are of the worlds most widely occurring diseases, and medical supplies used for treatment thereof form the largest single market item (anti-ulcer drugs: fifteen billion eight hundred million dollars (1999)).

Since 1983, when microbiologists Marchall and Warren first discovered
ichthyosis bacteria in gastric mucous membrane biopsy tissue of B-type gastritis patients, and cultured the bacteria to find it to be a novel species related to the *campylobacter* genus, studies of correlation between *Helicobacter pylori* and upper gastrointestinal diseases such as gastritis and peptic ulcers have been actively undertaken. *Helicobacter pylori*, a gram negative bacillus, has flagella surrounded by thin membranes, is known to secrete a great deal of urease and to be spread through the fecal-oral route, and it attracts world-wide attention because it was confirmed as a certain carcinogen by the WHO in 1994.

The attack mechanism of *Helicobacter pylori* is known to occur through the bacteria entering the gastric inner cavity with food, where it penetrates the gastric mucosa using highly motile flagella and secretes a great deal of urease to raise the pH of the mucosa to an alkaline state. *Helicobacter pylori* inhabits the area between the upper part of the gastric epithelia cell layer and the cells of the gastric mucosa, causing an abnormal secretion of gastrin which in turn induces production of gastric acid, causing inflammation and ulcers. Additionally, according to a recent report proving that *Helicobacter pylori* is an important factor for gastric adenocarcinoma, the WHO designated *Helicobacter pylori* as a group 1 carcinogen.

*Helicobacter pylori* infection has been reported to be particularly prevalent in developing countries, including Korea, and according to report by Paek, et al, approximately 80% or more of the Korean population is assumed to be infected with *Helicobacter pylori* (Paek Seungchul, et al, Korean Microbial Society Paper, 25:45-52, 1990). Therefore, there is an urgent need for
development of a treatment and prevention method for *Helicobacter pylori* infection.

Chemical therapy using antibiotics is the currently most common treatment for diseases caused by microorganism infection. Although this method may be effective in the short term, there is a concern for the appearance of antibiotic-resistant strains of microorganisms.

In addition, as an antibacterial therapy for *Helicobacter pylori*, Rauws et al administrated three kinds of antibacterial bismuth preparations, amoxicillin, and metronidazole with positive results (Rauws EAJ, Langenberg W, Houthoff JH, Zanen HC and Tytgat GHJ, Gastroenterol, 94:33–40, 1988). Borody, et al administrated a bismuth preparation, tetracycline, and metronidazole to peptic ulcer patients and reported positive treatment results of around 80% (Borody T, Lenne J, and Moore-Jones D, Gastroenterol, 98:A24, 1990), as a result of which administration of these three kinds of antibiotics has become the most commonly used treatment of *Helicobacter pylori* infection. However, it is reported that various side effects such as diarrhea, reflux oesophagitis, inhibition of normal intestinal bacteria, and appearance of resistant strains occur due to continuous antibiotic therapy.

Recently, the function of lactic acid bacteria fermenting materials has attracted increasing attention for prevention and treatment of gastrointestinal diseases, and particularly, many studies on probiotic *Lactobacillus* which is capable of inhibiting growth of human pathogenic bacteria are in progress. These lactic acid bacteria are fermentation products and they produce antibiotic material related to bacteriocin, as well as lactic acid, acetic acid, and
hydrogen peroxide. Marie-Helen Coconier, et al cultured a human *Lactobacillus acidophilus* strain LB that produces antibiotics for *Helicobacter pylori* *ex vivo*, and confirmed that the supernatant of the culture liquid inhibits attachment of *Helicobacter pylori* to human gastrointestinal cells *in vitro*, and prevents urease activity and inhibits *H. felis* infection in mice (Marie-Helen Coconier et al., *Environ. Microbiol*, Nov. 4573–4580, 1998).

In addition, Yuji Aida M.T. et al have confirmed that *Helicobacter pylori* infection is inhibited in aseptic murine through oral administration of *Lactobacillus* salivarius, and U.S.P. No. 5,578,392 has described a function of *L. johnsonii* for inhibiting attachment of *Helicobacter pylori* to gastrointestinal cells.

In addition, Canducci, et al have prescribed an inert preparation of human *Lactobacillus acidophilus* culture liquid together with an antibiotic therapy using rabeprazol, clarithromycin, and amoxicillin to obtain results of increasing the extermination rate of *Helicobacter pylori* (Canducci F. et al, *Aliment Pharmacol. Ther*, 14:1625–1629, 2000).

Also, Korean Patent Application No. 1999-0040387 has described an antibody for *Helicobacter pylori*, as well as foods containing *Lactococcus* sp. HY 49, *Lactobacillus Casei* HY 2782, *bifidobacterium longum* HY 8001, etc. showing an inhibiting capacity for *Helicobacter pylori*.

Accordingly, there is a need for a material and method capable of preventing attachment of *Helicobacter pylori* to the gastrointestinal tract, and inhibiting its growth, and for studies of foods and a medicine comprising such material.
SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide gastrointestinal-tract-protecting high functional Kimchi lactic acid bacteria that inhibits growth of *Helicobacter pylori* and inhibits its attachment to the gastrointestinal tract, and which are separated from Kimchi, the main lactic acid bacteria supply source of Koreans, to impart beneficial functions to the traditional food.

It is another object of the present invention to provide a gastrointestinal-tract-protecting high functional food comprising the Kimchi lactic acid bacteria that is effective for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers caused by *Helicobacter pylori*.

It is another object of the present invention to provide a gastrointestinal-tract-protecting high functional Kimchi comprising the Kimchi lactic acid bacteria that is effective for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers that are caused by *Helicobacter pylori*.

It is another object of the present invention to provide a medicine for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers caused by *Helicobacter pylori*, comprising the Kimchi lactic acid bacteria.

In order to achieve these objects, the present invention provides Kimchi lactic acid bacteria that inhibit growth of *Helicobacter pylori* and inhibit attachment thereof to the gastrointestinal tract.
The present invention also provides a gastrointestinal-tract-protecting high functional food comprising Kimchi lactic acid bacteria, that inhibits growth of *Helicobacter pylori*, and that is effective for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers.

The present invention also provides a gastrointestinal-tract-protecting high functional Kimchi comprising Kimchi lactic acid bacteria, that inhibits growth of *Helicobacter pylori*, and that is effective for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers.

The present invention also provides a medicine for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers, comprising a Kimchi lactic acid bacteria that inhibits growth of *Helicobacter pylori*.

**DETAILED DESCRIPTION AND THE PREFERRED EMBODIMENTS**

The present invention will now be explained in detail.

Kimchi is known to have various useful functions, including anti-mutation and anti-cancer effects, etc. because of the Kimchi ingredients such as cabbage and radishes and many lactic acid bacteria involved in Kimchi. Microorganisms involved in Kimchi fermentation include *L. plantarum*, *L. brevis*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Pediococcus*, etc., and the kinds and compositional ratio of bacteria forming the total microflora change according to the fermentation stage.

The present inventors have confirmed, during studies on Kimchi lactic acid bacteria that inhibit growth of *Helicobacter pylori*, that among microorganisms involved in the Kimchi fermentation process, Kimchi lactic acid
bacteria that inhibit growth of *Helicobacter pylori* and simultaneously inhibit attachment of Helicobactor pylori to the gastrointestinal tract exist, and completed the present invention.

The present invention provides Kimchi lactic acid bacteria that inhibit growth of *Helicobacter pylori* and inhibit attachment thereof to the gastrointestinal tract.

The Kimchi lactic acid bacteria are preferably separated and identified while fermenting Kimchi prepared by a common method at 1 to 40 °C up to 90 days or until its pH becomes about 3.0. More preferably, they are separated while the Kimchi is being aged at 1 to 25 °C for 90 days, or while being aged at 30 to 40 °C for 30 days.

The lactic acid bacteria are separated by culturing samples taken from Kimchi prepared by a common method on a MRS agar medium to select numbers of Kimchi lactic acid bacteria that inhibit growth of *Helicobacter pylori*, and sequentially selecting Kimchi lactic acid bacteria that inhibit attachment of *Helicobacter pylori* to the gastrointestinal tract therefrom.

Therefore, the Kimchi lactic acid bacteria are preferably, among the lactic acid bacteria found in the Kimchi fermentation process, *Leuconostoc* and *Lactobacillus* species, and more preferably, they are one or more kinds selected from a group consisting of *Leuconostoc* species EUG4427 (KCCM-10274), *Lactobacillus* species EUG6017 (KCCM-10273), *Lactobacillus* species EUG4066 (KCCM-10270), *Lactobacillus* species EUF4048 (KCCM-10271), and *Lactobacillus* species EUG6404 (KCCM-10272).
The *Leuconostoc* species or *Lactobacillus* species isolated from Kimchi inhibit growth of *Helicobacter pylori* that inhabit the gastric mucous membrane tissue and inhibit attachment thereof to the gastrointestinal tract, and are very effective for *Helicobacter pylori* extermination and prevention of reinfection therewith.

Also, the lactic acid bacteria are not derived in human intestines or from fermentation products of milk, but the functional lactic acid bacteria are selected from Kimchi, the Korean main lactic acid bacteria supply source. The functions can be supplied by adding the bacteria to traditional Kimchi and to other foods, hence the functional lactic acid bacteria supply source can be diversified. Therefore, the present invention also provides a gastrointestinal-tract-protecting high functional food comprising *Leuconostoc* species or *Lactobacillus* species from Kimchi that is effective for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers.

A functional food comprising the Kimchi lactic acid bacteria that inhibits growth of *Helicobacter pylori* and inhibits attachment thereof to the gastrointestinal tract can be a starter, such as a culture liquid, or it can be a lyophilized lactic acid bacteria powder to be added to foods, such as beverages, fermented milk, bean curd, fermented soybean paste, bean-paste soup prepared with ground fermented soybeans, jelly, etc., and there is no limitation in their preparations so long as functions of the lactic acid bacteria are not inhibited, and other commonly used food additives can also be added according to nutritional requirements, consumer’s preference, etc.

The present invention also provides a gastrointestinal-tract-protecting
high functional Kimchi comprising the *Leuconostoc* species or *Lactobacillus* species from Kimchi, that is effective for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers that are caused by *Helicobacter pylori*.

The Kimchi comprising Kimchi lactic acid bacteria that inhibits growth of *Helicobacter pylori* and inhibits attachment thereof to the gastrointestinal tract can be prepared by adding Kimchi lactic acid bacteria culture liquid or lyophilized lactic acid bacteria powder as a starter to Kimchi seasonings, and preferably lyophilized lactic acid bacteria powder is added in order to maintain quality.

Preparation conditions such as temperature, pH, preparation methods, etc. for gastrointestinal-tract-protecting high functional Kimchi comprising Kimchi lactic acid bacteria are not specifically limited if they do not inhibit the functions of the lactic acid bacteria, and commonly used food additives can be added according to nutritional requirements, consumer’s preference, etc.

The present invention also provides a medicine for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers caused by *Helicobacter pylori*, comprising *Leuconostoc* species or *Lactobacillus* species from Kimchi. The medicine can be prepared as a gel, a suspension, a spray, a tablet, or a liquid preparation.

The present invention will be explained in more detail with reference to the following examples. However, these are to illustrate the present invention and the present invention is not limited to them.

[Examples]

**Example 1 : Separation of Kimchi lactic acid bacteria inhibiting growth**
of *Helicobacter pylori*

In order to select lactic acid bacteria that inhibit growth of *Helicobacter pylori* from Kimchi lactic acid bacteria in vitro, Kimchi lactic acid bacteria were separated and lactic acid bacteria inhibiting *Helicobacter pylori* were separated therefrom.

(Separation of Kimchi lactic acid bacteria)

Kimchi was prepared by a common method, and a sample was taken every 24 hours or every 48 hours for 90 days or until the pH became 3.0 while storing at 1 °C, 4 °C, 8 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C (1~25 °C: aging for 90 days; 30~40 °C: aging for 30 days). The samples taken from Kimchi were cultured on MRS agar media at 37°C for 72 hours.

(Separation of *Helicobacter pylori* growth inhibiting lactic acid bacteria)

On a brucella agar containing 10% horse serum, a *Helicobacter pylori* suspension was spread and dried for 5 minutes, and then approximately 48,000 lactic acid bacteria colonies cultured on the MRS agar medium were moved by a tooth-picking method and cultured at 37°C for 72 hours under 10% CO₂ partial pressure, and then 588 lactic acid bacteria colonies forming transparent rings were selected.

Example 2: Search for lactic acid bacteria inhibiting attachment of *Helicobacter pylori* to cell line

(Primary selection)

For the 588 kinds of Kimchi lactic acid bacteria inhibiting growth of *Helicobacter pylori* in vitro selected in Example 1, activities for inhibiting *Helicobacter pylori* attachment to Kato III gastric carcinoma cells were
investigated, and lactic acid bacteria having such capacities were selected.

The *Helicobacter pylori* #26695 were separated from the stomach of a
Korean having gastritis and peptic ulcers, and obtained from Kyungsang
University Medical School, and human Kato III gastric carcinoma cells were
obtained from the Korean Cell Line Bank.

Primarily, the number of *Helicobacter pylori* attached to cells was
measured by absorbence at 625 nm of color-forming urease reaction solution,
an indicator for measuring the number of *Helicobacter pylori*, and lactic acid
bacteria that decreased the number of attached *Helicobacter pylori* to \(10^6\) or
less were selected, and as a result, a total of 70 kinds were selected as shown
in Table 1 (if the A625 value is 1.7 or less, number of *Helicobacter pylori* is
calculated to be \(10^6\) or less.).

[Table 1]
<table>
<thead>
<tr>
<th>Bacteria name</th>
<th>Number of attached Hp bacteria (Attachment-inhibiting capacity : $&lt; 10^9$)</th>
<th>Bacteria name</th>
<th>Number of attached Hp bacteria (Attachment-inhibiting capacity : $&lt; 10^9$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Eug 12142</td>
<td>O</td>
<td>36  Eug 44251</td>
<td>O</td>
</tr>
<tr>
<td>2  Eug 6013</td>
<td>O</td>
<td>37  Eug 2253</td>
<td>O</td>
</tr>
<tr>
<td>3  Eug 6014</td>
<td>O</td>
<td>38  Eug 2613</td>
<td>O</td>
</tr>
<tr>
<td>4  Eug 6017</td>
<td>O</td>
<td>39  Eug 14414</td>
<td>O</td>
</tr>
<tr>
<td>5  Eug 0113</td>
<td>O</td>
<td>40  Eug 4416</td>
<td>O</td>
</tr>
<tr>
<td>6  Eug 0141</td>
<td>O</td>
<td>41  Eug 4417</td>
<td>O</td>
</tr>
<tr>
<td>7  Eug 0340</td>
<td>O</td>
<td>42  Eug 4418</td>
<td>O</td>
</tr>
<tr>
<td>8  Eug 0352</td>
<td>O</td>
<td>43  Eug 4419</td>
<td>O</td>
</tr>
<tr>
<td>9  Eug 0358</td>
<td>O</td>
<td>44  Eug 44115</td>
<td>O</td>
</tr>
<tr>
<td>10 Eug 0445</td>
<td>O</td>
<td>45  Eug 4118</td>
<td>O</td>
</tr>
<tr>
<td>11 Eug 0442</td>
<td>O</td>
<td>46  Eug 4427</td>
<td>O</td>
</tr>
<tr>
<td>12 Eug 4547</td>
<td>O</td>
<td>47  Eug 4439</td>
<td>O</td>
</tr>
<tr>
<td>13 Eug 8126</td>
<td>O</td>
<td>48  Eug 14449</td>
<td>O</td>
</tr>
<tr>
<td>14 Eug 8130</td>
<td>O</td>
<td>49  Eug 4410</td>
<td>O</td>
</tr>
<tr>
<td>15 Eug 4842</td>
<td>O</td>
<td>50  Eug 4414</td>
<td>O</td>
</tr>
<tr>
<td>16 Eug 4848</td>
<td>O</td>
<td>51  Eug 24013</td>
<td>O</td>
</tr>
<tr>
<td>17 Eug 8540</td>
<td>O</td>
<td>52  Eug 0618</td>
<td>O</td>
</tr>
<tr>
<td>18 Eug 8553</td>
<td>O</td>
<td>53  Eug 40312</td>
<td>O</td>
</tr>
<tr>
<td>19 Eug 9617</td>
<td>O</td>
<td>54  Eug 0317</td>
<td>O</td>
</tr>
<tr>
<td>20 Eug 6322</td>
<td>O</td>
<td>55  Eug 0315</td>
<td>O</td>
</tr>
<tr>
<td>21 Eug 6713</td>
<td>O</td>
<td>56  Eug 4034</td>
<td>O</td>
</tr>
<tr>
<td>22 Eug 6123</td>
<td>O</td>
<td>57  Eug 40410</td>
<td>O</td>
</tr>
<tr>
<td>23 Eug 6416</td>
<td>O</td>
<td>58  Eug 0412</td>
<td>O</td>
</tr>
<tr>
<td>24 Eug 20553</td>
<td>O</td>
<td>59  Eug 6404</td>
<td>O</td>
</tr>
<tr>
<td>25 Eug 14435</td>
<td>O</td>
<td>60  Eug 4047</td>
<td>O</td>
</tr>
<tr>
<td>26 Eug 16811</td>
<td>O</td>
<td>61  Eug 4048</td>
<td>O</td>
</tr>
<tr>
<td>27 Eug 16828</td>
<td>O</td>
<td>62  Eug 4049</td>
<td>O</td>
</tr>
<tr>
<td>28 Eug 8215</td>
<td>O</td>
<td>63  Eug 40511</td>
<td>O</td>
</tr>
<tr>
<td>29 Eug 8220</td>
<td>O</td>
<td>64  Eug 4056</td>
<td>O</td>
</tr>
<tr>
<td>30 Eug 16842</td>
<td>O</td>
<td>65  Eug 40514</td>
<td>O</td>
</tr>
<tr>
<td>31 Eug 8756</td>
<td>O</td>
<td>66  Eug 4065</td>
<td>O</td>
</tr>
<tr>
<td>32 Eug 16871</td>
<td>O</td>
<td>67  Eug 4063</td>
<td>O</td>
</tr>
<tr>
<td>33 Eug 9218</td>
<td>O</td>
<td>68  Eug 4066</td>
<td>O</td>
</tr>
<tr>
<td>34 Eug 44211</td>
<td>O</td>
<td>69  Eug 0615</td>
<td>O</td>
</tr>
<tr>
<td>35 Eug 2242</td>
<td>O</td>
<td>70  Eug 40614</td>
<td>O</td>
</tr>
</tbody>
</table>
(Secondary selection)

For the primarily-selected 70 kinds of strains, reproduction tests were conducted and simultaneously strains having high *Helicobacter pylori* attachment-inhibiting activities were selected. 35 of the lactic acid bacteria that decreased the number of attached *Helicobacter pylori* to approximately $10^5$ or less were selected, and these are shown in Table 2.

[Table 2]
(Tertiary selection)

For the secondarily-selected 35 kinds of lactic acid bacteria, secondary reproduction tests were conducted and 22 strains were selected as shown in Table 3.

[Table 3]
Example 3: Identification of Strain

Results of identifying 22 lactic acid bacteria strains selected by the methods in Examples 1 and 2 according to a BIOLOG kit and Bergey's manual are shown in Table 4.

<table>
<thead>
<tr>
<th>Bacteria name</th>
<th>Gram</th>
<th>Shape of bacteria</th>
<th>Shape of spore</th>
<th>Catalase</th>
<th>Air permeability (facultative bacteria)</th>
<th>Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eug 6017</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 0352</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 8126</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 6713</td>
<td>(+)</td>
<td>Coccobacillus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 6123</td>
<td>(+)</td>
<td>Short rod</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 16842</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 8756</td>
<td>(+)</td>
<td>Short rod</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 4416</td>
<td>(+)</td>
<td>Short rod</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 4419</td>
<td>(+)</td>
<td>Short rod</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 0618</td>
<td>(+)</td>
<td>Coccobacillus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 0317</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 0315</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 0412</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 6404</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Eug 4047</td>
<td>(+)</td>
<td>Streptococcus</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
</tbody>
</table>
As shown in Table 4, one kind of *Leuconostoc* species strain identified through the BIOLOG system and 21 kinds of *Lactobacillus* species strains identified through the identification method of Bergey’s manual have properties of inhibiting attachment of *Helicobacter pylori* to the cells.

(Quaternary selection)

For the tertiary selected 22 kinds of strains, tertiary reproduction tests were conducted and 9 strains having high inhibiting activities were selected. At this stage, selection was not made according to sequence of high inhibiting activity, but bacteria were divided and selected according to their shapes, and the results are shown in Table 5.

[Table 5]

<table>
<thead>
<tr>
<th>Bacteria name</th>
<th>Number of attached Hp bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eug 6017</td>
<td>&lt; 10⁴</td>
</tr>
<tr>
<td>Eug 8126</td>
<td>1.2 ×10⁵</td>
</tr>
<tr>
<td>Eug 6713</td>
<td>1.4 ×10⁵</td>
</tr>
<tr>
<td>Eug 4419</td>
<td>&lt; 10⁴</td>
</tr>
<tr>
<td>Eug 4427</td>
<td>1.2 ×10⁵</td>
</tr>
<tr>
<td>Eug 6404</td>
<td>&lt; 10⁴</td>
</tr>
<tr>
<td>Eug 4047</td>
<td>&lt; 10⁴</td>
</tr>
<tr>
<td>Eug 4048</td>
<td>&lt; 10⁴</td>
</tr>
<tr>
<td>Eug 4066</td>
<td>&lt; 10⁴</td>
</tr>
</tbody>
</table>
(Final selection)

For the selected 9 lactic acid strains, quaternary reproduction tests were conducted and 1 strain was selected for each shape of bacteria, and finally, 5 strains were selected as shown in Table 6.

<table>
<thead>
<tr>
<th>Bacteria name</th>
<th>Number of attached Hp bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eug 6017</td>
<td>$&lt; 10^4$</td>
</tr>
<tr>
<td>Eug 4427</td>
<td>$2.5 \times 10^5$</td>
</tr>
<tr>
<td>Eug 6404</td>
<td>$2.6 \times 10^4$</td>
</tr>
<tr>
<td>Eug 4048</td>
<td>$&lt; 10^4$</td>
</tr>
<tr>
<td>Eug 4066</td>
<td>$&lt; 10^4$</td>
</tr>
</tbody>
</table>

For the final 5 strains shown in Table 6, the *Leuconostoc* species strain was named *Leuconostoc* species EUG4427 (KCCM-10274), and 4 kinds of *Lactobacillus* species strains were respectively named *Lactobacillus* species EUG6017 (KCCM-10273), *Lactobacillus* species EUG4066 (KCCM-10270), *Lactobacillus* species EUG4048 (KCCM-10271), and *Lactobacillus* species EUG6404 (KCCM-10272). The 5 strains were deposited with the Korean Microbial Preservation Center on 2001, 5, 16 under the aforementioned names.

Example 4: Inhibition of growth of *Helicobacter pylori* by Kimchi lactic
acid bacteria culture broth

*Leuconostoc* species EUG4427 (KCCM-10274), *Lactobacillus* species EUG6017 (KCCM-10273), *Lactobacillus* species EUG4066 (KCCM-10270), *Lactobacillus* species EUG4048 (KCCM-10271), and *Lactobacillus* species EUG6404 (KCCM-10272) selected in Example 3 were respectively cultured on a MRS liquid medium at 37 °C for 48 hours and then centrifuged to obtain supernatants.

A agar plate was prepared with 25 mL of brucella agar medium to which 10% horse serum was added and then dried, and 0.5 mL of *Helicobacter pylori* suspension (1x10^8 CFU/MI) was spread thereon and dried for 5 minutes.

The medium was punched with a sterilized cork borer, and 100 μl of centrifuged supernatant of each strain was introduced and cultured under 10% CO₂ partial pressure at 37 °C for 72 hours to determine the size of the produced transparent ring. For a control, a MRS liquid medium was used instead of a supernatant. Results of inhibition of growth of *Helicobacter pylori* by lactic acid bacteria in vitro are shown in Table 7.
[Table 7]

<table>
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<tr>
<th>Strain name</th>
<th>Radius of transparent ring (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Leuconostoc species EUG4427 (KCCM-10274)</td>
<td>3.0</td>
</tr>
<tr>
<td>Lactobacillus species EUG6404 (KCCM-10272)</td>
<td>4.5</td>
</tr>
<tr>
<td>Lactobacillus species EUG4048 (KCCM-10271)</td>
<td>4.0</td>
</tr>
<tr>
<td>Lactobacillus species EUG6017 (KCCM-10273)</td>
<td>2.5</td>
</tr>
<tr>
<td>Lactobacillus species EUG4066 (KCCM-10270)</td>
<td>4.0</td>
</tr>
</tbody>
</table>

As shown in Table 7, as results of culturing the 5 kinds of Kimchi lactic acid bacteria supernatant separated in Example 3, the radius of the produced transparent rings were 2.5 to 4.5 mm, indicating that the Kimchi lactic acid bacteria of the present invention have superior *Helicobacter pylori* growth inhibition activities.

**Example 5 : Inhibition of attachment of* Helicobacter pylori *to gastric carcinoma cells**

Human Kato III gastric carcinoma cells were cultured on a RPMI1640 medium containing 10% FBS at 37 °C under 10% CO₂. *Helicobacter pylori* were cultured on a brucella agar medium containing 10% horse serum at 37 °C.
under 10% CO₂ for 48 hours, and then colonies were collected and suspended in saline water, and 0.5 mL of 1x10⁶ of the Kato III suspension and 0.25 mL of lactic acid bacteria culture liquid were reacted at 37 °C for 30 minutes while slowly shaking. For a control, a MRS liquid medium was used instead of a culture liquid.

0.25 mL of the *Helicobacter pylori* suspension (1x10⁶ CFU/mL) were added thereto and reacted again for 90 minutes, and then washed with saline water three times at 1,000 rpm for 5 minutes each time. The number of *Helicobacter pylori* attached to Kato III was then measured by a urease test.

Urease decomposes urea to produce ammonia, and produced ammonia was quantified using phenol/nitroprusside and alkaline hypochlorite. If urease exists, the reactant liquid changes from colorless to light blue, and the activity of urease is determined by measuring absorbency at 625 nm. The number of *Helicobacter pylori* is calculated by urease activity, and a standard curve for this test showed a linear area when the number of *Helicobacter pylori* is 10⁴~10⁷.

For the 5 kinds of Kimchi lactic acid bacteria selected from Example 3,
activities of inhibiting attachment of *Helicobacter pylori* to human gastric carcinoma cells are shown in Table 8.

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Number of attached <em>Helicobacter pylori</em> (CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>&gt; 10^7</td>
</tr>
<tr>
<td>Leuconostoc species EUG4427 (KCCM-10274)</td>
<td>2.5 × 10^5</td>
</tr>
<tr>
<td>Lactobacillus species EUG6404 (KCCM-10272)</td>
<td>2.6 × 10^4</td>
</tr>
<tr>
<td>Lactobacillus species EUG4048 (KCCM-10271)</td>
<td>&lt; 10^4</td>
</tr>
<tr>
<td>Lactobacillus species EUG6017 (KCCM-10273)</td>
<td>&lt; 10^4</td>
</tr>
<tr>
<td>Lactobacillus species EUG4066 (KCCM-10270)</td>
<td>&lt; 10^4</td>
</tr>
</tbody>
</table>

From Table 8, it can be confirmed that the 5 kinds of Kimchi lactic acid bacteria selected in Example 3 have a small number of *Helicobacter pylori* attached to Kato III human gastric carcinoma cells, indicating that the 5 kinds of lactic acid bacteria separated from Kimchi according to the present invention inhibit attachment of *Helicobacter pylori* to the gastrointestinal tract.

**Example 6 : Preparation of gastrointestinal-tract-protecting high functional Kimchi**

*Leuconostoc species EUG4427 (KCCM-10274), Lactobacillus species EUG6017 (KCCM-10273), Lactobacillus species EUG4066 (KCCM-10270),*
*Lactobacillus* species EUG4048 (KCCM-10271), and *Lactobacillus* species EUG6404 (KCCM-10272) selected in Example 3 were lyophilized and powdered, one or more kinds of the strains were mixed, and then $10^6$-$10^{11}$ and suitably $10^{10}$ bacteria per kg of Kimchi were mixed with Kimchi seasoning to prepare gastrointestinal-tract-protecting high functional Kimchi by a common Kimchi preparation method.

The Kimchi lactic acid bacteria of the present invention inhibits attachment of *Helicobacter pylori* inhabiting gastric mucous membrane tissue to the gastrointestinal tract and inhibits growth thereof, and thus is very effective for *Helicobacter pylori* extermination and prevention of reinfection therefrom. In addition, the gastrointestinal-tract-protecting high functional Kimchi comprising the Kimchi lactic acid bacteria is very effective for prevention and treatment of gastritis, peptic ulcers, and duodenal ulcers caused by *Helicobacter pylori*.

In addition, lactic acid bacteria are not derived in human intestines or from a fermentation product of milk, but the lactic acid bacteria having the desired functions are selected from Kimchi, which is the main lactic acid
bacteria supply source of Koreans. Therefore, the functions can be added to the traditional food Kimchi as well as other foods, so the supply source of functional lactic acid bacteria can be diversified.
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152, Gun-dong, Yuseong-ku, Taejon 305-333, Republic of Korea

Date of deposit: **May 16, 2001**

Accession Number: **KCTC 10270**

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Date of deposit: May 15, 2001

Accession Number: KCTC 10274

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WHAT IS CLAIMED IS:

1. Kimchi lactic acid bacteria that inhibit growth of Helicobacter pylori and inhibit attachment thereof to the gastrointestinal tract.

2. The Kimchi lactic acid bacteria according to Claim 1, wherein the Kimchi lactic acid bacteria are separated from Kimchi while it is being aged at 1-40 °C up to 90 days after start of preparation, or until its pH becomes approximately 3.0.

3. The Kimchi lactic acid bacteria according to Claim 1, wherein the Kimchi lactic acid bacteria are Leuconostoc species or Lactobacillus species.

4. The Kimchi lactic acid bacteria according to Claim 1 or 2, wherein the Kimchi lactic acid bacteria are one or more kinds selected from a group consisting of Leuconostoc species EUG4427 (KCCM-10274), Lactobacillus species EUG6017 (KCCM-10273), Lactobacillus species EUG4066 (KCCM-10270), Lactobacillus species EUG4048 (KCCM-10271), and Lactobacillus species EUG6404 (KCCM-10272).

5. A functional food for preventing and treating gastritis, peptic ulcers, and duodenal ulcers caused by Helicobacter pylori, comprising the Kimchi lactic acid bacteria according to any one of Claims 1 to 4.

6. A gastrointestinal-tract-protecting high functional Kimchi for preventing and treating gastritis, peptic ulcers, and duodenal ulcers, comprising the Kimchi lactic acid bacteria according to any one of Claims 1 to 4.

7. A medicine for preventing and treating gastritis, peptic ulcers, and duodenal ulcers caused by Helicobacter pylori, comprising the Kimchi lactic
acid bacteria according to any one of Claims 1 to 4.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

**IPC7 C12N 1/20**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)


Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and Applications for Inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubMed, Delphion, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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</table>

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**Date of the actual completion of the international search**

07 SEPTEMBER 2002 (07.09.2002)

**Date of mailing of the international search report**

09 SEPTEMBER 2002 (09.09.2002)

**Name and mailing address of the ISA/KR**

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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

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