Evaluation of the Effect of *Lactobacillus planetarium* Probiotics Produced from Broad Bean Seed in Prevention of *Helicobacter pylori* in Stomach Tissue of C57BL/6 Mice

Amin Afshahi¹, Hassan Mahmoudi², Azizalah Ebrahimí¹, Zahra Aeiní¹ and Davoud Esmaeili*³

¹Department of Pathobiology, Shahrekord University, Shahrekord, Iran
²Department of Microbiology, Hamadan University of Medical Sciences, Hamadan, Iran
³Department of Microbiology and Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Abstract

**Background and aims:** *Helicobacter pylori* is one of the most common human infections, which colonizes more than half of the world’s population. This causes chronic stomach inflammation diseases without clinical syndromes, gastric and duodenal ulcer, and stomach cancer. Nowadays, the use of probiotics has received much consideration as one of the common therapeutic methods, which prevents bacterial colonization by creating a balance in the microbial gastrointestinal tract.

**Methods:** This experimental study was conducted on 30 rats in five groups from August 2016 to June 2017 in the Microbiology and Animal Laboratory of Shahrekord University. First, the rats were infected with *H. pylori* bacteria. PCR method was used to confirm the presence of bacteria in the stomach to ensure that the rats were inoculated with *H. pylori*. After inoculation, the infected rats were treated with probiotic product, and then gastric tissue of the infected group was evaluated by haematoxylin and eosin stain.

**Results:** The absence of Cag A and Ure C genes in fecal specimens of the group receiving probiotic products before and after *H. pylori* incubation showed a positive effect for this product on the prevention and treatment of *H. pylori* infection. Also, in stomach histology specimens, the effects of mild inflammation were observed in treated group with the probiotic product before and after *H. pylori* inoculation compared to the control group.

**Conclusion:** The results of this study showed that the addition of probiotic to a non-dairy product (broad bean extract) can be effective in preventing and treating *H. pylori* infection in the animal model.

Keywords: *Helicobacter pylori*, Probiotic; Broad bean; Haematoxylin; Gastrointestinal tract

Introduction

*Helicobacter pylori* is a gram-negative, motility, curved rod or spiral-shaped bacterium. This has been isolated from the human stomach in all parts of the world. Colonization with *H. pylori* is associated with various diseases in the upper gastrointestinal tract such as gastritis, peptic ulcer, gastric cancer, and stomach lymphoma. It seems that the major reservoir of this bacterium is humans [1]. *H. pylori* is currently the only bacterial species that has been classified as a class I carcinogen by the International Agency for Research on Cancer (IARC). This bacterium is a cause of death worldwide. It has been reported that this germ-negative bacterium infects 50% of the world’s population, and 80% of the population in developing countries [2,3]. Many people become infected with this bacterium until adulthood and it is difficult to prevent the infection even with the health observance. However, a vaccine has not been yet made in protecting human against this germ-negative bacterium. *Helicobacter pylori* is a gram-positive and fermentative bacterium. This bacterium is a cause of death worldwide. It has been reported that this germ-negative bacterium infects 50% of the world’s population, and 80% of the population in developing countries [2,3]. Many people become infected with this bacterium until adulthood and it is difficult to prevent the infection even with the health observance. However, a vaccine has not been yet made in protecting human against this germ-negative bacterium.
iron. This plant is a good source of thiamine, which is vital for normal functioning of the nervous system and is also a rich source of fiber. Fiber helps the digestive system to break down food faster. Broad bean is low in calories and has no cholesterol. Its amino acid plays the role of the neurotransmitter in the brain [12,13]. The purpose of this study was to evaluate the effect of probiotic based on Vicia faba plant on the prevention and treatment of \( H. \) pylori infection in \( C57BL/6 \) mice. To our knowledge this is the first study to use probiotic in broad bean.

### Materials and Methods

#### Bacterial strain and growth condition

The standard strain of \( H. \) pylori was provided from research center from the Gastrointestinal and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The specimens were cultured to the microbiological laboratory, and then were cultured on Brucella agar-based media with 7.5% of sheep blood, 10% of the fetal bovine serum, and supplements containing antibiotics of amphotericin B (2 mg/L), vancomycin (10 mg/L), polymyxin (0.25 mg/L). After 3-4 days, the cultures obtained were placed at 37°C in anaerobic jar or gas pack C, and then were identified using catalase and oxidase, gram staining, observation of gram-negative bacteria with specific morphology, and rapid urease test. After this process, the specimens were tested [14]. The standard strain of \( L. \) plantarum (ATCC8014) was provided in freeze-dried formulation from the collection of fungi and industrial and infectious bacteria in Iran and stored at -80°C in a freezer.

#### Animal experiments

The present study was conducted in 2017 in the laboratory of microbiology. The required rats (30 male \( C57BL/6 \) mice) all were aged 6-8 weeks and weighed between 20-30 gr. All experiments were conducted based on the license number (6572/9/35/16/p) of the University’s Medical Ethics Committee.

#### Preparation and production of fermentation products

Broad bean seeds were soaked in water for 12 h, and then the seeds were crushed, and the resulting slurry was filtered and placed in a glass container. Next, the filtered solution was pasteurized at 70°C for 20 min in a heated bath. After cooling, 1% of the suspension (v/v) at concentration of 10^6 CFU/mL of \( L. \) plantarum was added to the liquid and then incubated at 37°C for 48 h in the presence of 5% of \( CO_2 \) [15].

#### Infecting animals to \( H. \) pylori

After transferring the rats to a laboratory for keeping animals and adapting the animal to the new environment (Three days after transfer), the first fecal specimen (before the experiment) was taken from all animals. Then, the proliferation of the \( cagA \) and the \( ureC \) genes in relation to \( H. \) pylori were investigated using the specific primers (Table 1). The program time and temperature for PCR in 35 cycles were as follows: 3 min of the initial denaturation at 95°C, 45 sec of the second denaturation at 93°C, 60 sec of annealing at 55°C, 60 sec of extension at 72°C, and the final stage of expansion was conducted at 72°C for 5 min. Then, PCR product was observed on agarose gels (1.5%) by using electrophoresis method [16,17].

#### Histological examination of stomach tissue

For histological examination of the stomach samples, an animal was randomly selected from each group on week of 12 after inoculation. After killing animals, their stomach was removed with chloroform and stored at -80°C in a freezer. Then, volumes of 1 mL of the suspension were poured directly into the stomach of the fats, their feeding was discontinued 24 hours before the inoculation, the most common used antibiotics for treating \( H. \) pylori (including a combination of metronidazole, erythromycin and bismuth) was used for 2 weeks.

#### Stool sampling

Fecal samples were ordinarily collected from the rats before and on weeks of 3, 5, 7, and 12 after inoculation, and then the collected samples were stored at 20°C in a freezer.

#### DNA extraction and replication by PCR method

In this study, to extract DNA, the DNA extraction kit (Gene all, South Korea) was used. Then, the proliferation of the \( cagA \) and the \( ureC \) genes in relation to \( H. \) pylori were investigated using the specific primers (Table 1). The program time and temperature for PCR in 35 cycles were as follows: 3 min of the initial denaturation at 95°C, 45 sec of the second denaturation at 93°C, 60 sec of annealing at 55°C, 60 sec of extension at 72°C, and the final stage of expansion was conducted at 72°C for 5 min. Then, PCR product was observed on agarose gel (1.5%) by using electrophoresis method [16,17].

#### Results

#### Identification of bacteria

The electrophoresis of PCR products showed a fragment of the 400-

<table>
<thead>
<tr>
<th>Group Characteristics of the studied group</th>
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<tbody>
<tr>
<td>1 Two weeks before ( H. ) pylori inoculation, they were daily fed the product via gavage and diet.</td>
</tr>
<tr>
<td>2 Two weeks after ( H. ) pylori inoculation, they were daily fed the product via diet and gavage.</td>
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<tr>
<td>3 They were treated with common drugs used to treat ( H. ) pylori for three weeks</td>
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<tr>
<td>4 Control group with ( H. ) pylori inoculation and without treatment</td>
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<tr>
<td>5 Control group without ( H. ) pylori inoculation and without treatment</td>
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Table 1: Characteristics of studied groups.

![Figure 1: PCR results of \( cagA \) genes: well 1: Negative control samples, well 2: Positive control samples, wells 3 and 4: Positive samples in terms of the presence of the \( cagA \) gene, well M: Marker is 1kb.](image)
base pair of cagA gene and 294 base pair of the ureC gene associated with H. pylori (Figures 2 and 3).

**Study of the presence of H. pylori in animal stool using PCR method**

The lack of propagation of the specific portion of 400 and 294 base pairs in the first stool sample of animals (before H. pylori inoculation) showed that none of the animals were naturally infected with the bacterium. The second fecal specimen was taken three weeks after H. pylori inoculation. This is due to the fact that before three weeks, the positive PCR response does not indicate the definitive infection with H. pylori, and it may be attributed to the transient bacteria and without deployment of the bacteria in the stomach. The result of the PCR was positive for groups 4 (control group with H. pylori inoculation), and the experimental groups were negative. Moreover, the proliferation of CagA and UreC gene from animal stools in groups 1, 2, 3 and 5 at 3, 5, 7 and 12 weeks after inoculation was also negative, while it was positive in group 4 was positive (Table 2).

**Histopathological examination of the stomach tissue**

The strained gastric slices of 1 animal stomach from each group at week 12 after H. pylori inoculation were studied in term of the degree of inflammation using an optical microscopy. The severity of inflammation for each histology sample was graded from 1 to 4 at

<table>
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<th>Group Type of tests</th>
<th>Inflammation intensity</th>
<th>Pathological changes</th>
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<tr>
<td>1 Intaking probiotic products two weeks before inoculation</td>
<td>1</td>
<td>A small number of leukocytes dispersed in depth of mucus and inflammation and mild degradation of mucus</td>
</tr>
<tr>
<td>2 Intaking probiotic products two weeks after inoculation</td>
<td>2</td>
<td>Average number of leukocytes in depth or middle of the mucus and mild inflammation in the glands and under the mucosa</td>
</tr>
<tr>
<td>3 Intaking the usual treatment 2 weeks after inoculation</td>
<td>1</td>
<td>Mild mucus degradation</td>
</tr>
<tr>
<td>4 Control (bacterial inoculation)</td>
<td>3</td>
<td>Dense leukocyte infiltration in depth or middle of the mucosa in the anthrom tissue</td>
</tr>
<tr>
<td>5 Control</td>
<td>0</td>
<td>Without change</td>
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**Table 2:** The primer used in this study.

### Discussion

This study aimed to investigate the effect of probiotic product produced from broad bean seed fermentation on the prevention and treatment of H. pylori infection in C57BL/6 mice and compare its effect with the standard treatment method. In the present study, the PCR results of all specimens were negative before H. pylori inoculation and this finding showed no naturally infection with this bacterium. The positive result of the specimens after H. pylori inoculation in group 4 (control + bacterium inoculation) confirmed the successful bacterial colonization in the animal stomach. Moreover, in histological examination of stomach sample, the mild degradation was apparent in the anthrome tissue. The negative result of samples in group 5 (controls without inoculation) also showed that there was no environmental contamination and possible error. There were no inflammation and degradation effects in histological examination of stomach samples.

The result of the PCR test in group 1 (intake of daily probiotic product, 2 weeks before inoculation) showed a negative presence of Cag A and Ure C genes, and there was also a very slight degree of inflammation (grade 1) in their stomach tissue samples. The results of the PCR test in group 2 (intake of daily probiotic product, 2 weeks after inoculation) and group 3 (intake of common drugs, 2 weeks after inoculation) were also negative, and in histological examination of specimen in group 2 and 3 showed a light (grade 2) and mild (grade 1) inflammation, respectively. When study on the anti-helicobacter effect of 38 strains of lactobacillus showed that L. plantarum strain had the most anti-H. Pylori effect and significantly reduced the activity of

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**Figure 2:** Results of PCR of Ure C genes: well M: Marker is 100 bp, well 1: Positive control samples, well 2: Negative control samples, wells 3 to 4: Positive samples in terms of the presence of ure C gene.

**Figure 3:** Gastric pathology examples of intervention groups: Control group (A): Gastric tissue without pathological changes, Group 1 (B): finding a small number of leukocytes dispersed at depth of mucus, inflammation and mild degradation of mucus, Group 2 (C): finding the average number of leukocytes in depth or middle of the mucosa, and sometimes neutrophils in the glands and mild inflammation in the mucosa and sub-mucosa, group 3 (D): observation of a mild mucosal degeneration, group 4 (E): observation of a dense leukocyte infiltration in depth or middle of the mucosa, and the degradation of the mucosa in the anthrom tissue.
urease urinary vectors [18]. Xiaohua study showed an inhibition effect of two strains of L. gasseri and L. plantarum on H. pylori growth under laboratory conditions [19]. El-Adawi et al. reported that L. bulgaricus, L. acidophilus and L. plantarum had the most anti-helicobacterial effect and prevented adhesion and invasion of the bacteria and reduced the level of TNF-α by 62.13% [20].

In the present study, L. plantarum strain was used to prepare a fermented probiotic product. Our results also confirmed the antibacterial activity of this strain, which is consistent with the findings of the previous studies. Chompoonut et al. showed that daily inoculation of L. plantarum inhibited the growth of H. pylori for a week, reduced inflammation, and significantly improved the gastric tissue of the rat, and these effects were also dependent on the inoculated dosage, which this finding are consistent our results [21]. Rogga study showed the anti-helicobacter effect of various strains of L. plantarum under laboratory conditions. They introduced and proposed the production of a fermentation product containing lactobacillus as a potential and strong tool for the treatment of H. pylori infection [22]. Our results are consistent with the findings of Rogga study, and our study confirmed the hypothesis proposed in this research. Mingfan Pan et al. study indicated that the pre-treatment with L. plantarum prevented the increase of inflammatory cytokines such as IFN-γ in H. pylori infection and played an important role in preventing inflammation of the gastric mucosal tissue. Moreover, they introduced oral treatment with probiotic products of L. plantarum as an alternative and suitable treatment method for the prevention of H. pylori infection [23].

The results of this study also indicated that two weeks before inoculation the intake of probiotic products can prevent H. pylori infection. The Thiraworawong study showed that L. plantarum reduced the inflammation caused by H. pylori infection in the stomach of the studied rats and may result in TNF-α suppression and can be used as an adjunctive therapy in the treatment of this infection [24]. This is in line with the results of our study. In the present study, the extract of broad bean plant was first used to prepare a probiotic product. The results of this study showed that the probiotic produced from broad bean plant can be successfully used for the prevention and treatment of H. pylori infection in the animal model of C57BL/6 rat [25,26]. Therefore, with regard to the growing interest of people around the world for the use of probiotics and the pathogenesis of gastroduodenal infection, it is suggested that future studies focus on the survival time of different probiotics in the broad bean plant extract and producing a non-dairy probiotic product with low cost and higher nutritional value for treating and preventing H. pylori infection.

Conclusion

In this study, the addition of probiotic to non-dairy products (broad bean plant extract) and its successful testing in the prevention and treatment of H. pylori infection in the animal model can be considered as a novelty of this study compared to previous related research. To our knowledge this is the first study to use probiotic in broad bean.

Suggestions

Considering the positive results of this study in the prevention and treatment of H. pylori infection, it is suggested that future studies focus on the survival time of different probiotics in the broad bean plant extract and producing a non-dairy probiotic product with low cost and higher nutritional value for treating and preventing H. pylori infection.

References


