The Isolation and Selection of Lactobacillus Sp. Can Inhibit Helicobacter Pylori

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ABSTRACT: In this newspaper, we want to isolate Lactobacillus sp. which have the ability to inhibit H.pylori. As a result, 20 lactic strains are isolated from fermented food and choose a strain from 20 strains has the biggest inhibition with H.pylori. In addition, selecting strain is determined Lactobacillus plantarum by 16S rDNA and its inhibition with H.pylori is the strongest (the rate percent of inhibition is 70.48%). Then, L.plantarum is used to create bioproduct by miniB 290 and some parameters are determined: the input temperature is 100°C, the rate of input pump is 5 ml/min.

KEYWORDS: Helicobacter pylori, inhibition, Lactobacillus plantarum, lactic strains, fermented food.

I. INTRODUCTION
Helicobacter pylori is a spiral shaped, gram negative, acid tolerant, it is found in the human stomach and duodenum. Helicobacter pylori infection causes gastrointestinal diseases such as chronic gastritis, peptic ulcers and may lead to gastric cancer. The mechanisms by H.pylori infection lead to gastric mucosal damage include direct effects of virulence factors produced by H.pylori such as urease, oxidative stress and induction of apoptosis in infected gastric epithelial cells [1,2].

The current eradication protocol for H.pylori infections includes 7-14 days of triple therapy consisting of omeprazole, clarithromycin or amoxicillin and metronidazole. However, this triple therapy is not successful and it causes negative affects. Therefore, in order to reduce antibiotic side effects, there is a clear need for the development of new treatment approaches replacing antibiotics. Recent reports show that the combination between probiotic and antibiotics is promising alternatives to antibiotics [5].

In this newspaper, our research is performed to isolate Lactobacillus sp which have the ability to inhibit H.pylori and then first steps to create bioproducts by mini B290 [5].

II. MATERIALS AND METHODS
2.1. Materials
- Fermented food were obtained from supermarkets in HCM city. These samples are analyzed during 24 hours and they are stored at 5-8°C in laboratory (HCM Polytechnique University ).
- 20 lactic strains (L1-L20) are stored at Biotechnology Department in HCM Polytechnique University.
- Helicobacter pylori (PH9) is obtained from Pasteur and it may resistance to clarithromycin.

2.2. Research methods
2.2.1. H.pylori growth inhibition
- H.pylori was activated in pylori broth medium, then incubated for 72 h at 37°C under microaerophilic conditions.
- H.pylori is diluted to 10⁶ and spread on the plates to count the colony, then caculate colony forming units (CFU/ml) H.pylori and colony forming units (CFU/ml) H.pylori in control sample is also determined. Then, we caculate colony forming units (CFU/ml) H.pylori at 1%,2%,3%,4%,5% the volume of lactic strains over H.pylori.
- x= colony forming units (CFU/ml) H.pylori (1%,2%,3%,4%,5%)/ colony forming units (CFU/ml) H.pylori in sample control (x is the rate of survive H.pylori)
- The percentage of inhibition =100-x (x is the rate of survive H.pylori).
2.2.2. Agar well diffusion method
Antibacterial activity was evaluated using the modified method described by Gavidson and Parish (1989), where antimicrobial activity was determined by measuring the clear zones formed around the 6 mm. The selecting lactic strain was cultured for 24h, a suspension was prepared, 80 µl was spread within each well contained H.pylori. After incubation for 72h at 37°C under microaerophilic conditions, the antimicrobial activity was assessed by measuring the clear zone that formed around each disc. Control sample only contain H.pylori.

2.2.3. Estimate the rate percent of selecting strain with H.pylori
After choosing a selecting strain has the biggest inhibition with H.pylori from 20 lactic strains, we estimate the its inhibition with H.pylori at 1%, 2%, 3%, 4%, 5% the volume of selecting lactic strain with H.pylori. The rate percent of inhibition is determined by culture-spread on plates and method is showed in 2.2.1. As a result, the optimum rate percent of selecting strain with H.pylori is determined.

2.2.4. First steps try to create bioproduct Lactobacillus plantarum
Estimate the rate of input pump to the survival of L.plantarum
The rate of input pump is processed at 4.5 ml/min; 5ml/min; 5.5ml/min; 6ml/min, the whey protein isolate is used to immobilize at 5%, the input temperature is 100°C, the output temperature is constant at 45°C. We estimate and evaluate the survival of L.plantarum in bioproduct after dry spraying.
• 1g bioproduct is soaked in 9ml buffer phosphate solution pH7
• Sample solution is diluted, spread on MRS agar plates and after incubation for 72h at 37°C
• Calculate colony forming units (CFU/ml) L.plantarum.

Estimate the input temperature and the output temperature is constant at 45°C to the survival of L.plantarum
The input temperature is processed at 90°C, 100°C, 110°C, 120°C, 130°C. The output temperature is constant at 45°C and the whey protein isolate is used to immobilize at 5%, the rate of pump is 5ml/min. We determine the optimum input temperature
• 1g bioproduct is soaked in 9ml buffer phosphate solution pH7.
• Sample solution is diluted, spread on MRS agar plates and after incubation for 72h at 37°C.
• Calculate colony forming units (CFU/ml) L.plantarum

2.2.5. Data analyses
Data are processed by Excel and SPSS.

III. RESULTS AND DISCUSSION

3.1. Estimate the inhibition of lactic strains with H.pylori by culture-spread on plates
After isolating 20 lactic strains from fermented foods, estimate the inhibition of 20 lactic strains with H.pylori. Then, we select lactic strain which has the biggest rate of inhibition H.pylori.

![Figure 3.1. The percentage inhibition of lactic strains with H.pylori](image-url)
According to figure 3.1, 16 lactic strains (L1-L16) inhibit *H.pylori*, 4 lactic strains (L17-L20) cannot inhibit *H.pylori*. L3 has the biggest percentage inhibition of lactic strain with *H.pylori* (70.48%). According to the previous studies, lactic strains also inhibit *H.pylori*, the percentage inhibition of lactic strains with *H.pylori* is 72.46% and these results are similar to the result in this thesis.

Lactic strains produce acid lactic, acid acetic or bacteriocins. These organic compounds prevent *H.pylori* to release urease in stomach. Therefore, *H.pylori* cannot neutralize acid in the stomach, so *H.pylori* is killed in the stomach.

### 3.2. Agar well diffusion method

After selecting lactic strain (L3) has the biggest percentage of inhibition *H.pylori* (70.48%). Then, the inhibition of lactic strain (L3) with *H.pylori* is tested by agar well diffusion method. The result is showed in figure 3.2.

![Figure 3.2. The inhibition of lactic strains (L3) with *H.pylori*](image)

In conclusion, both culture-spread on plates and agar well diffusion method show that lactic strain (L3) inhibit *H.pylori*. In contrast, control sample does not inhibit *H.pylori*.

### 3.3. Estimate the rate percent of selecting strain with *H.pylori*

After choosing a selecting strain has the biggest inhibition with *H.pylori* from 20 lactic strains, we estimate the its inhibition with *H.pylori* at 1%, 2%, 3%, 4%, 5% the volume of selecting lactic strain with *H.pylori*. the result is showed in figure 3.3.

![Figure 3.3. Estimate the rate percent of selecting strain with *H.pylori*](image)

According to figure 3.3, the selecting strain can inhibit *H.pylori* at the different rates. The rate percentage inhibition of selecting strain with *H.pylori* is 70.48% at 3% (the volume of selecting strain with *H.pylori*).
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According to figure 3.3, the percentage inhibition of selecting strain with H.pylori increases when the rate percent of selecting strain increases. However, the percentage inhibition of selecting strain with H.pylori does not have many differences at 3%, 4%, 5%. Therefore, 3% is the optimum rate because it has the economy. Inconclusion, both culture-spread on plates and well agar diffusion methods show that the selecting strain inhibites H.pylori; in contrast, control sample does not inhibite H.pylori. The selecting strain is determined Lactobacillus plantarum by NK-BIOTEK company.

3.4. First steps try to create Bioproduct Lactobacillus plantarum by mini B290
3.4.1. Estimate the rate of input pump
The rate of input pump affects to the bioproduct. The rate of input pump increases, the water exists slowly so the moisture in bioproduct increases and it is not good for bioproduct.

Table 3.1. Estimate the rate of input pump

<table>
<thead>
<tr>
<th>The rate of input pump (ml/min)</th>
<th>4.5</th>
<th>5</th>
<th>5.5</th>
<th>6</th>
<th>6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.plantarum (log CFU/g)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10.21±0.01</td>
<td>10.85±0.01</td>
<td>10.96±0.05</td>
<td>11.32±0.05</td>
<td>11.43±0.02</td>
<td></td>
</tr>
<tr>
<td>Moisture (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.01±0.04</td>
<td>6.71±0.03</td>
<td>9.15±0.03</td>
<td>10.86±0.02</td>
<td>12.01±0.01</td>
<td></td>
</tr>
</tbody>
</table>

According to table 3.1, colony forming units (CFU/ml) L.plantarum and the moisture of bioproduct are affected by the rate of input pump. When the rate of input pump is low, bioproduct has low moisture, colony forming units (CFU/ml) L.plantarum is not as high as the high rate of input pump. The reason is that the suspension L.plantarum is stayed in a long time in dry room, so colony forming units (CFU/ml) L.plantarum is decreased by the temperature of dry room. The rate of input pump increases, the colony forming units (CFU/ml) L.plantarum increases because the suspension L.plantarum is stayed in a short time in dry room. Therefore, the optimum rate of input pump is 5ml/min.

The colony forming units (CFU/ml) L.plantarum in 5ml/min is not as high as 5.5;6 ml/min. However, bioproduct has low moisture at 5ml/min, so L.plantarum is stored in a long time and it has high bioactivities.

3.4.2. Estimate the input temperature
Bioproduct is also affected by the temperature. The colony forming units (CFU/ml) L.plantarum decreases when the temperature is high. Thus, the input temperature is estimated and the result is showed in table 3.2.

Table 3.2. Estimate the input temperature

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
<th>130</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.plantarum (log CFU/g)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>11.52±0.11</td>
<td>11.45±0.06</td>
<td>10.84±0.02</td>
<td>9.30±0.01</td>
<td>8.12±0.01</td>
<td></td>
</tr>
<tr>
<td>Moisture (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.2 ± 0.04</td>
<td>6.72±0.02</td>
<td>6.01±0.04</td>
<td>5.95±0.05</td>
<td>5.78±0.02</td>
<td></td>
</tr>
</tbody>
</table>

It is not good when the input temperature is so high or low. According to the table 3.2, the temperature of dry air affect to the colony forming units (CFU/ml) L.plantarum and the moisture of bioproduct. The input temperature is low, the colony forming units (CFU/ml) L.plantarum and the moisture are still high. On the other hand, the input temperature increases, the colony forming units (CFU/ml) L.plantarum and the moisture will decrease. The input temperature is 130°C, the colony forming units (CFU/ml) L.plantarum decreases quickly because L.plantarum is killed by the high temperature. In addition, the moisture of bioproduct also decreases. Therefore, 100°C is the optimum input temperature because the colony forming units (CFU/ml) L.plantarum is high and the moisture of bioproduct is suitable (it is smaller than 7%).

IV. CONCLUSION
1. Choosing a lactic strain from 10 strains isolated from fermented foods and 10 strains stored at Biotechnology Department. The selecting strain is determined Lactobacillus plantarum can inhibite H.pylori (the percentage inhibition of L.plantarum with H.pylori is 70.48%).
2. First steps try to create Bioproduct Lactobacillus plantarum and determine following parameters:
   - The rate of input pump is 5ml/min.
   - The input temperature is 100°C.
REFERENCES


