IOSR Journal Of Pharmacy www.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 8, Issue 8 Version. I (August 2018), PP. 13-17

The Isolation and Selection of *Lactobacillus Sp*. Can Inhibite *Helicobacter Pylori*

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ABSTRACT: In this newspaper, we want to isolate *Lactobacillus* sp. which have the ability to inhibite *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 adittion, 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 100^0C, the rate of input pump is 5 ml/min.

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

Date of Submission: 20-07-2018 Date of acceptance: 04-08-2018

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 islolate *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^{o}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 37^0C under microaerophilic conditions.
- *H.pylori* is dilluted to 10^6 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 37^0C 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 immobize at 5%, the input temperature is 100^0C, the output temperature is constant at 45^0C. 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 dilluted, spread on MRS agar plates and after incubation for 72h at 37^0C
- Caculate colony forming units (CFU/ml) *L.plantarum*.

-Estimate the input temperature and the output temperature is constant at 45^oC to the survival of *L.plantarum*

The input temperature is processed at 90^0C, 100^0C, 110^0C, 120^0C, 130^0C. The output temperature is constant at 45^0C and the whey protein isolate is used to immobize at 5%, the rate of pump is 5ml/min. We determine the optimum input tempearature

- 1g bioproduct is soaked in 9ml buffer phosphate solution pH7.
- Sample solution is dilluted, spread on MRS agar plates and after incubation for 72h at 37^0C.
- Caculate 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*.

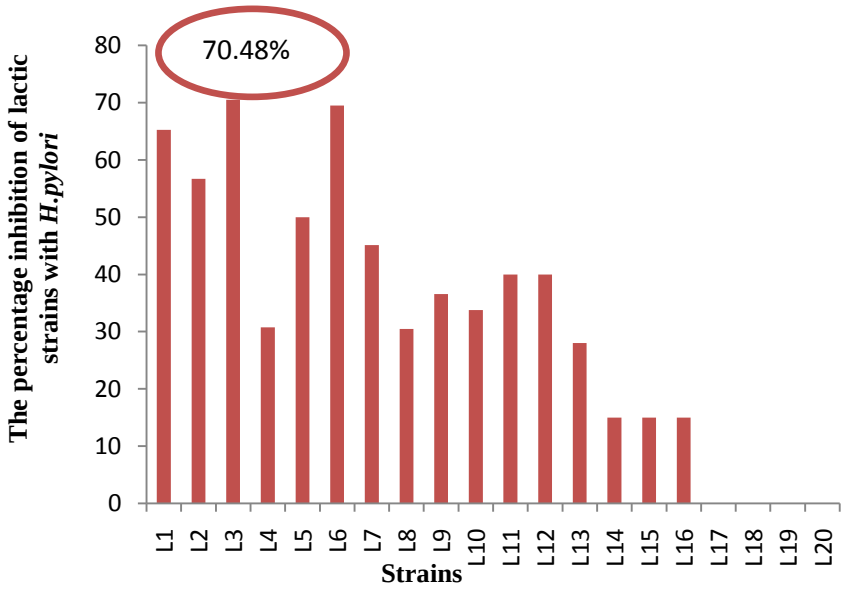

Figure 3.1. The percentage inhibition of lactic strains with *H.pylori*

According to figure 3.1**,** 16 lactic strains **(**L1-L16**)** inhibite *H.pylori*, 4 lactic strains (L17-L20) can not inhibite *H.pylori*. L3 has the biggest percentage inhibition of lactic strain with *H.pylori* (70.48%). According to the previous studies, lactic strains also inhibite *H.pylori*, the percentage inbition 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 stomarch. Therefore, *H.pylori* can not neutrolize acid in the stomarch, so *H.pylori* is killed in the stomarch.

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

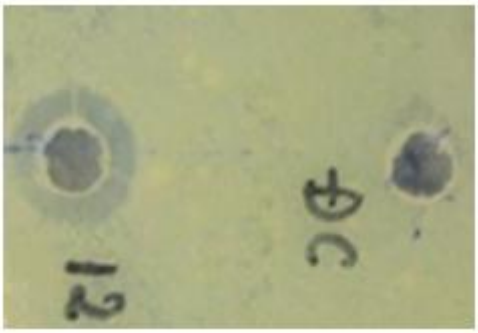

Figure 3.2. The inhibition of lactic strains (L3) with *H.pylori*

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

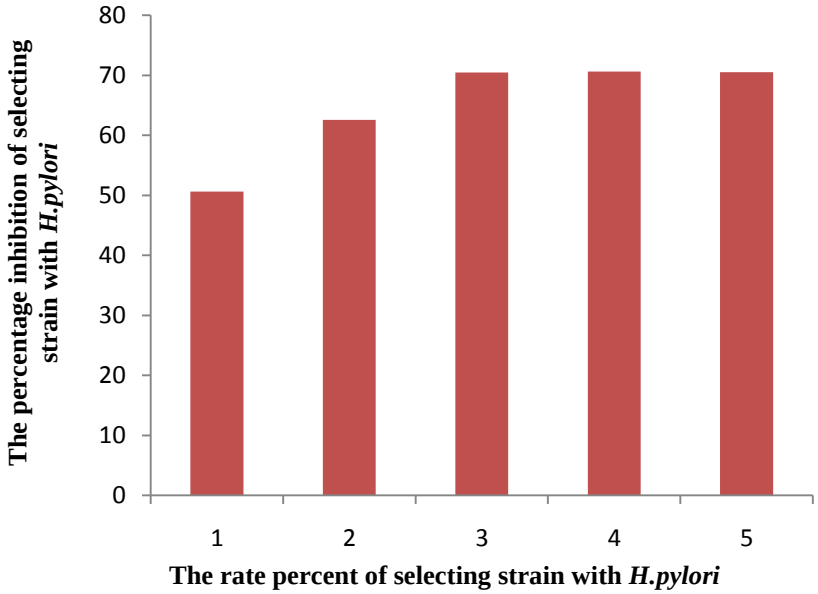

Figure 3.3. Estimate the rate percent of selecting strain with *H.pylori*

According to figure 3.3, the selecting strain can inhibite *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*).

According to figure 3.3, the percentage inhibition of selecting strain with *H.pylori* increases when the rate percent of selecting strain increases. Howerver, 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. Estmate 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

The rate of input pump (ml/min)	**4,5**	**5**	**5,5**	**6**	**6,5**
***L.plantarum* (log CFU/g)**	10,21±0,01[a]	**10,85±0,01[b]**	10,96±0,05[b]	11,32±0,05[c]	11,43±0,02[c]
Moisture (%)	6,01±0,04[a]	**6,71±0,03[a]**	9,15±0,03[b]	10,86±0,02[c]	12,01±0,01[d]

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

Temperature (^oC)	**90**	**100**	**110**	**120**	**130**
***L.plantarum* (log CFU/g)**	11,52±0,11[a]	**11,45±0,06[a]**	10,84±0,02[b]	9,30±0,01[c]	8,12±0,01[d]
Moisture (%)	11,2 ± 0,04[a]	**6,72± 0,02[b]**	6,01±0,04[b]	5,95±0,05[c]	5,78±0,02[d]

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 130^0C, 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, 100^0C 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 100^0C.

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Dang Dinh Hiep ." The Isolation and Selection of *Lactobacillus* Sp. Can Inhibite *Helicobacter Pylori.*" IOSR Journal of Pharmacy (IOSRPHR), vol. 8, no. 08, 2018, pp. 13-17.