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# Potency of Probiotic Bacteria from Noni Fruit (*Morinda citrifolia* L.) as Anti-*Helicobacter pylori* Agent

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**Abstract.** *Helicobacter pylori* is often found in patients with chronic gastritis despite standard medical treatment with antacids and antibiotics. Traditionally, noni fruit used for gastritis treatment and as a source of probiotics are able to compete with pathogenic bacteria *H. pilory*. This study was conducted to characterize the probiotics from noni fruit (*Morinda citrifolia* L.) as an anti *H. pylori* caused gastritis and gastric cancer with two stages: I. isolation and identification of candidate probiotic bacteria; and II. characterization of potential probiotic candidates as anti *H. pylori*. Identification of bacteria is conducted with the API test and analysis of 16S rDNA, while the characterization of anti *H. pylori* probiotics included acidic pH resistance tests, bile salts resistance test, antimicrobial test (inhibition zone) and the probiotic bacteria adhesion test against *H. pylori*. The study was descriptive and experimental. The research design used was a completely randomized design factorial pattern and the results were analyzed by ANOVA ( $\alpha = 0.05$ ) and significant results were tested with Duncan's multiple ranges ( $\alpha = 0.05$ ). The parameters measured were the adhesion ability of probiotic bacteria against *H. pylori* and the width of the inhibition zone (mm). The results were obtained as a potential probiotic anti *H. pylori* are *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii* and *Bacillus licheniformis*. It can be concluded that all probiotics candidates were able to agglomerate *H. pylori* but *L. mesenteroides* inhibition of *H. pylori* was  $\emptyset$  33.0 mm greater than others.

**Keywords:** Gastritis, *Helicobacter pylori*, *Leuconostoc mesenteroides*, *Morinda citrifolia* L.. probiotic.

## INTRODUCTION

*Helicobacter pylori* is often found in patients with chronic gastritis despite standard medical treatment with antacids and antibiotics. *H. pylori* is a gastric pathogen which is present in approximately half of the world's population. It is a major cause of chronic gastritis and ulcer disease and it is an important risk factor for the development of gastric malignancies [1]. Probiotic bacteria are found to be potentially useful in managing lactose intolerance, lowering of cholesterol and blood pressure, *H. pylori* infection, antibiotic-associated diarrhea, inflammation, irritable bowel syndrome, colitis, gastroenteritis in children, ulcerative colitis, crohn's diseases, pouchitis, intestinal cancer, mammary gland cancer and colorectal cancer [2]. Probiotics are dietary supplements containing potentially beneficial bacteria needed for the betterment of gastrointestinal tract [3]. Probiotics can be defined as the preparations or product containing viable, defined microorganisms in sufficient numbers, which alter the microflora by implantation or by colonization in the host and exert beneficial health implications on their host [4] Traditionally, noni fruit used for gastritis treatment and as a source of probiotic are able to compete with pathogenic bacteria *H. pylori* The representative species of probiotics viz., *Lactobacillus* and *Bifidobacterium* include *L. acidophilus*, *L. plantarum*, *L. johnsonii*, *L. gasseri*, *L. casei*, *L. rhamnosus*, *B. longum*, *B. breve*, *B. infantis*, *B.thermophilum*, *B. infantis*, *B. pseudolongum* and others. In addition to these, dairy product comprising *L.bulgaricus*, *Streptococcus thermophilus* and *Leuconostoc* could be used as probiotics [5].



FIGURE 1. *Morinda citrifolia* L.

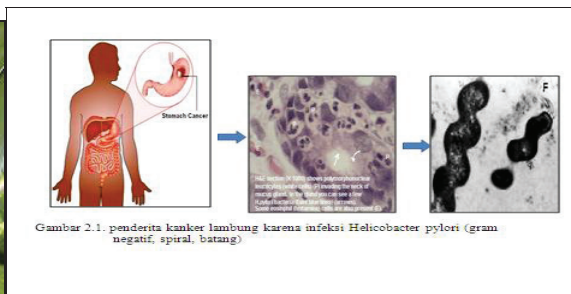


FIGURE 2. Patient with ulcer contains *Helicobacter pylori*

## MATERIAL AND METHODS

### Isolation and Identification of Candidate Probiotic Bacteria

The noni fruit juice was serially diluted by  $10^{-5}$  to  $10^{-7}$  using sterile distilled water and 0.1 mL was plated onto sterile deMann Rogosa and Sharpe (MRS) agar. The MRS plates were maintained in microaerophilic condition and incubated at 37 °C for 48 h. After incubation, well-isolated typical colonies were picked up, transferred to MRS broth and incubated at 37 °C for 48 h. The isolates were identified using standard morphological, cultural and biochemical reactions [6].

### Acid and Bile Salt Tolerance

Isolated probiotics bacteria candidate were inoculated into MRS medium of varying pH, i.e. pH 2, pH 3, pH 4 and pH 5; as well as broth with varying concentrations of bile salt (0.5 %, 1.0 % and 1.50 %) and incubated at 37 °C for 48 h, then 0.1 mL inoculums were transferred to MRS agar by pour plate method and incubated at 37 °C for 48 h. The growth of LAB on MRS agar plate was used to designate isolates as acid or bile salt tolerant.

### Detection of Antagonistic Activities

The antagonistic properties of isolated LAB species were determined by modifying the disc diffusion method. Sterile blotting paper discs (6 mm) were dipped into 48 hours incubated probiotic bacteria culture broth and then placed on solidified nutrient agar seeded with the 3 h old culture of test pathogens, which included *Escherichia coli* (MTCC 443) and *H. pylori*. The plates were kept at 4 °C for 1 h diffusion and then incubated at 37 °C for 24 h. Zones of inhibition were measured [7]. The parameters measured were the adhesion ability of probiotic bacteria against *H. pylori* and the width of the inhibition zone (mm).

### Identification of Bacteria with the API Test

The probiotic bacteria candidates which resulted tolerant to bile salts, acidity and inhibited *H. pylori* were then identified with API test.

## RESULT AND DISCUSSION

The results were obtained as a potential probiotic anti *H. pylori* are *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii* and *Bacillus licheniformis*.

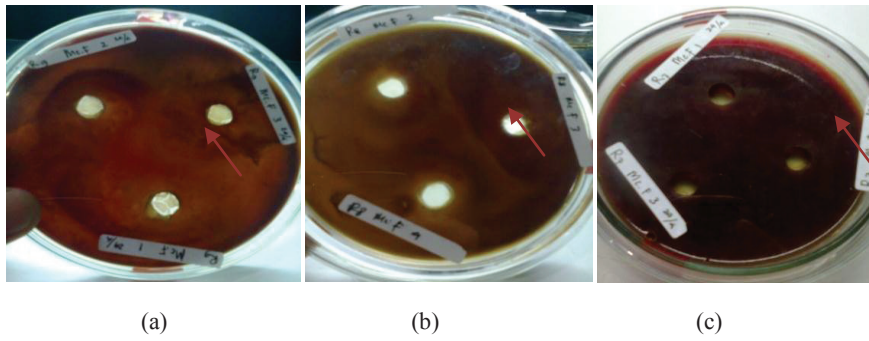


FIGURE 3. Inhibition zone *L. mesenteroides* isolate (a) and *B. licheniformis* (b) compared with negative control *E.coli* (c).

TABLE 1. Result of antagonistic test probiotic isolate from noni fruit (*Morinda citrifolia* L.) against *Helicobacter pylori*

No	Cell Density ( $\times 10^8$ cfu $\cdot$ mL <sup>-1</sup> )	Diameter of Inhibition Zone			
		3.0	6.0	9.0	12,0
1	<i>Leucostoc mesenteroides</i>	16 mm	21 mm	24 mm	33 mm
2	<i>Bacillus licheniformis</i>	8 mm	10 mm	21 mm	28 mm
3	<i>Lactobacillus delbrueckii</i>	10 mm	12 mm	22 mm	30mm
4	<i>Escherichia coli</i>	-	-	-	-

Note: (-) no inhibition zone; yellow color is potential isolate

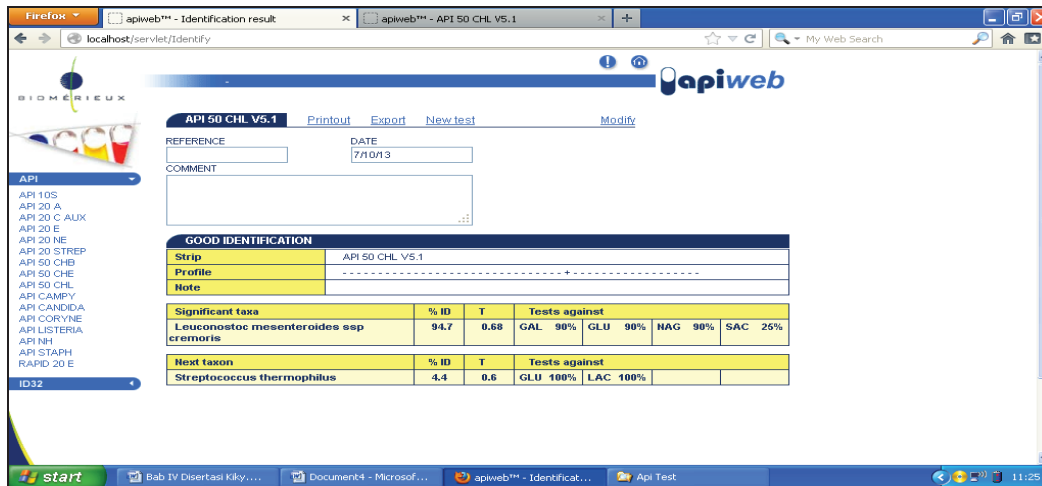


FIGURE 4. API test to *Leuconostoc mesenteroides* (94.7 %)

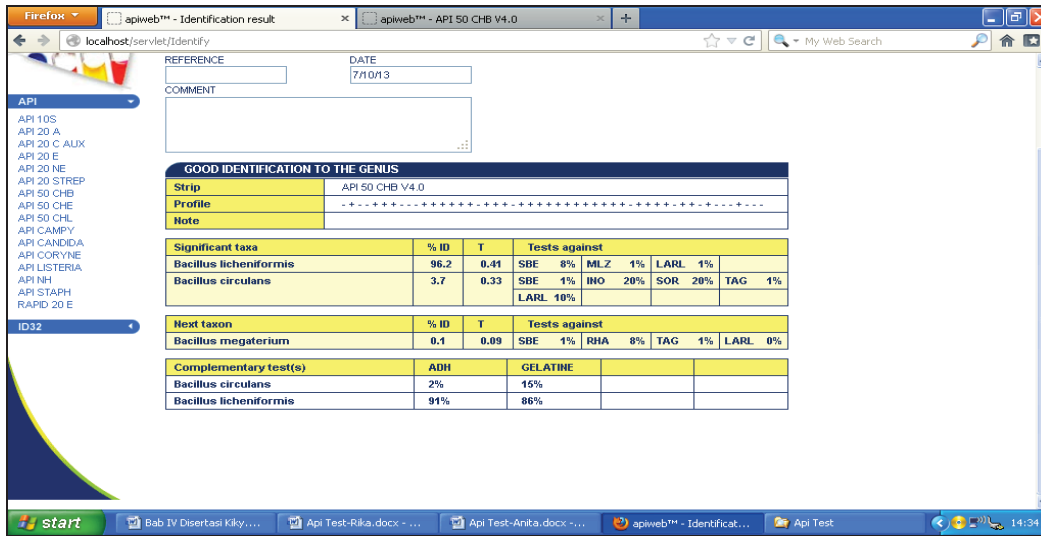


FIGURE 5. API test to *Bacillus licheniformis* (96.2 %)

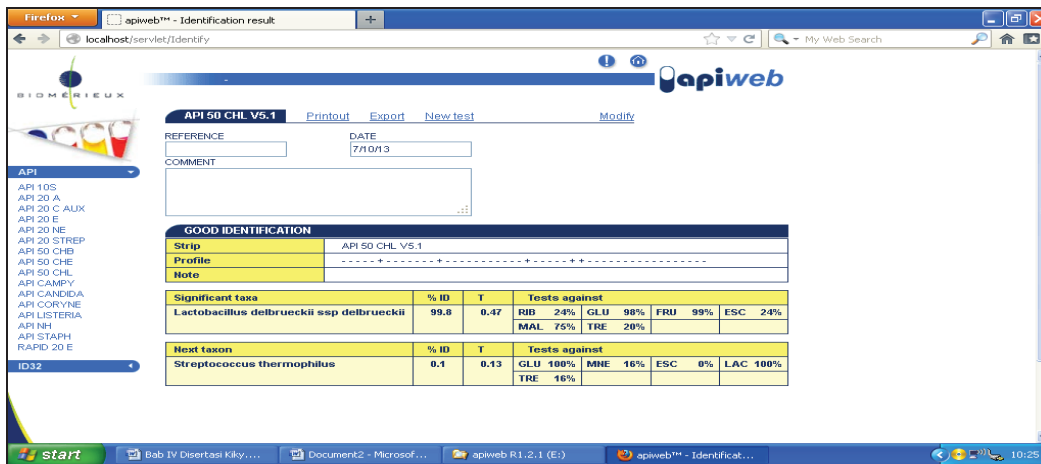


FIGURE 6. API test to *Lactobacillus delbrueckii* (98.8 %)

The lactic acid bacteria (LAB), a group of Gram-positive, non-spore forming, non-motile microorganisms are well known for their probiotic properties. They can produce inhibitory compounds such as lactic acid, bacteriocin and hydrogen peroxide preventing the growth of harmful microbes [8].

Probiotics are live microbial feed supplements that positively affect the host animal by improving its intestinal microbial balance. In recent years, the research on probiotic bacteria has been increased due to their beneficial effects on human health. The bacteria have to fulfill some criteria to behave as probiotic such as adhesion to gastrointestinal tract, resistance to low pH, bile salts and lysozyme [9]. The inhibitory mechanisms of LAB against pathogenic bacteria are primarily due to the production of organic acid and bacteriocins [10], in children using oral microbial feeding with LAB has prevented acute rotavirus associated diarrhea and antibiotic-induced gastrointestinal disorders [11].

*L. delbrueckii* the cell was rod, Gram-positive, non-spore, non-motile, catalase test negative and oxidase test positive, 0.5 µm in diameter and 1 µm length. The bacteria live in acidic (pH 2 to pH 5), bile salt, anaerobic facultative and lactic acid homofermentative (BAL) produced L-lactic acid caused the environment not conducive for pathogen bacteria growth. *L. mesenteroides* is BAL, rod cell, Gram-positive, no spore bacteria, fit in 30 °C to 37 °C, the bacteria can live in lower pH (pH 2 to pH 5), produce CO<sub>2</sub> and acis on glucose medium, decreased pH, so it can inhibit pathogen bacteria growth [3]. *L. mesenteroides* is probiotic as anti cervical cancer and colorectal cancer by adhesion and colonization in intestines, it has resistance in stomach HCL (pH 3), lysozyme (100 µg · mg<sup>-1</sup>) and

bile salt [4]. *B. licheniformis* is rod bacteria, Gram-positive, has terminal spore, grow in lower pH (pH 3 to pH 4) as probiotic characteristic.

Probiotics convert sugars and other carbohydrates into lactic acid and produces a characteristic sour taste to form fermented dairy food and in fortified foods. Some of the probiotic bacteria act as preservative by lowering the pH of the medium and reduce opportunistic for spoilage organism to grow [5].

## CONCLUSION

It can be concluded that all probiotics candidates isolated from noni fruit (*Morinda citrifolia* L.) were able to agglomerate *Helicobacter pylori* but *L. mesenteroides* inhibition to *H. pylori* was  $\Theta$  33.0 mm greater than others.

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