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# Characterisation of Lactic Acid Bacteria from *Dengke Naniura* of Common Carp (*Cyprinus carpio*) with α-Glucosidase Inhibitory Activity

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**Keywords:** Lactic acid bacteria; Traditional food products; Dengke naniura

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**Abstract**

**BACKGROUND:** Fermented foods were favourable because of its properties in enhancing the shelf life, safety, function, sensory and nutrition. There are many fermented foods tested *in vitro* as an α-glucosidase enzyme inhibitor. *Dengke naniura* is one of Indonesia's traditional food made using fermentation.

**AIM:** To identify lactic acid bacteria (LAB) strains in *dengke naniura* and its properties in inhibiting the α-glucosidase enzyme.

**METHODS:** The carp were sacrificed, and soaked with rough lemon for 6 hours then spices added to it for another 1 hour. Then the isolation of LAB conducted using a serial dilution of the samples. The selected isolates of the LAB were then characterised by its morphology under the microscope, gram staining, growth at 15°C and 45°C and biochemical identification. The isolates were then tested for its inhibiting properties against the α-glucosidase enzyme.

**RESULTS:** The isolates (DL-109 and DL-107) were a gram-positive, nonspore-forming and non-motile rod. The Physiological and biochemical properties of the isolates confirm its LAB properties. On the test against α-glucosidase enzyme activity inhibition, isolate DL-109 LAB (4) showed dominant activity with very low $IC_{50}$ compared to Acarbose ($IC_{50}$ = 128.06 ppm) and DL-107 (46.32 ppm) while at the lowest dosage of 25 µg/ml DL-109 showed activity as much as 54.76%.

**CONCLUSION:** These findings concluded that the isolates were LAB by its properties and can be used for lowering blood glucose in term of inhibition of the α-glucosidase enzyme.

## Introduction

The research of LAB (probiotics) in food is blooming, not only because of the health it served, but also its properties which obstruct the growth of pathogen [1] and food spoiling bacteria, so that the LAB improve the quality of the food [2]. Nowadays, the traditional way of serving food products has been chosen over the modern way because of long-proven safety while being served for many centuries [3]. The Fermentation is playing an important role in serving the safety of traditional food [4]. The basic technique, using maintaining the low pH using organic acids will serve not only as protein hydrolytic but also preventing the growth of spoiling bacteria [5] while promoting acid-resistant bacteria like the LAB to grow [6]. The famous dengke naniura among Batak people served without additional heat along the process and undergo the fermentation process, while the fish used is common carp (*Cyprinus* carpio). The common carp was acidified with an organic acid (*Citrus jambhiri* extract) and then added to it spices, and it stands for 3-9 hours without cooking process [7]. Not only the spices but the carp also possess *Bacilli* that may serve as the source of the LAB in *dengke naniura* [8]. The LAB especially *Lactobacillus* as the living microorganism being unknown by the ancient people, but after much exhaustive research, had brought an

intention when it was proved to be improving the immune system [9], intestine microflora modification and antipathogenic effect [10] and particularly help the diabetes type 2 by inhibiting the α-glucosidase in intestine [11].

This work aimed to characterise the strains of LAB isolated from *dengke naniura* a traditional food from Batak tribe in Indonesia; we embarked on an effort to isolate and identify candidate probiotic lactobacilli from it; along with its properties in inhibiting the α-glucosidase enzyme.

## Material and Methods

The carp fish (*Cyprinus carpio*) was purchased in the traditional market of Parapat, Simalungun regency, Sumatera Utara, Indonesia. All the standard spices for naniura (*Citrus jambhiri*, andaliman, cayenne, red onion, white onion, turmeric, rias, candlenut and table salt) were purchased in the traditional market of Balige, Tobasa regency, Sumatera Utara, Indonesia. The de Man, Rogosa and Sharpe (MRS agar medium and MRS broth) were obtained from Himedia lab, India. Acarbose, yeast α-glucosidase, p-nitrophenyl-α-D-glucopyranoside were obtained from Sigma (MO, USA). Other chemical and reagents used were of the pure and analytical grade.

The live carp fish with 1 kg weighted were sacrificed, washed with tap water followed by removing the gut, then were cutted from the centre axis of the fish into two identic part. The flesh then washed with sterilised water to remove extra blood. Then it was soaked with the juice of 300 g rough lemon (*Citrus jambhiri*) and an additional of 3% of salt weight per weight of the samples, allowed to stand for 6 hours. Then all the spices were added into it (45 g red onion, 12 g garlic, 45 g powdered candlenut, 56 g turmeric, 20 g Sichuan pepper, 50 g cayenne, 162 g powdered torch ginger) and allowed to stand for 1 hour covered along with the soaking [7].

*Dengke Naniura* of common carp (*Cyprinus carpio*) were used for the isolation of lactic acid bacteria. Ten grams of sample was homogenised with 90 mL sterile NaCl solution (0.85%, w/v) to a homogenous suspension and then a tenfold serial dilution in NaCl solution (0.85%, was carried out, plated on MRS agar containing 1.0% $CaCO_3$ and incubated at 37°C for two days. Isolated colonies of different appearances and surrounded by a halo were picked up and tested for catalase and Gram staining. Pure isolated colonies were tested for cell morphology (based on colour, shape, size) by phase-contrast microscopy, rough or smooth surface, Gram and catalase reaction. The strains with Gram-positive and catalase-negative were selected. The pure colonies were kept as stocks in MRS broth supplemented with 10% (v/v) glycerol at-18°C [12]. Before the test, all frozen stocks were recultivated in MRS broth and were incubated at 37°C. Growth of the isolates was observed in MRS broth after incubation at 15°C and 45°C [13].

This study was used 20 µl α-glucosidase (0.5 unit/ml) and 120 µl 0.1 M phosphate buffer pH 6.8. As the substrate were used p-nitrophenyl-α-D-glucopyranoside (p-NPG) 5 mM in the same buffer. As much as 10 µl of the samples dissolved in DMSO in various concentration, were mixed with enzymes solution in 96-well microplate and incubated at 37°C for 15 minutes. During the preparation and test, the enzyme must be taken care of under 2-8°C. Then were added 20 µl substrate solution and were incubated again at 37°C for 15 minutes. Enzymatic reaction halted with the addition of 80 µl Sodium carbonate 0.2 M. The test was done in triplicate. Samples were measured using a microplate reader at 405 nm [14]. Enzyme inhibitory effect was calculated by the formula:

$$\%\ \text{Inhibition} = \frac{[(A\ control - A\ sample)]}{[A\ control]}\ x\ 100\%$$

Linear regression equation between the samples and inhibition activity were used in determining $IC_{50}$ number. The activity of inhibiting α-glucosidase enzyme by inhibitor concentration as much as 50% were called $IC_{50}$ [15].

## Results

The two isolates (DL-109 and DL-107) were a gram-positive, nonspore-forming and non-motile rod. Its physiological and biochemical characteristics, as shown in Figure 1, Figure 2 and Table 1.

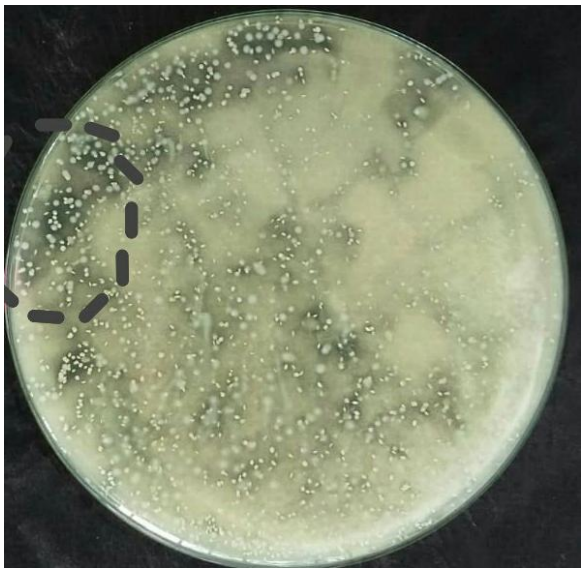

*Figure 1: Lactic acid bacteria in MRSA growth media*

Characterisation of bacterial isolates was carried out morphologically by observing the shape of the colonies on the media visually and Misco-microscopically after gram staining. The biochemical characterisation was carried out by catalase test, Triple Sugar Iron Agar (TSIA), fermentation type test, growth at low pH and growth of isolates at low and high temperatures. Observation results can be seen in Table 1. On the microscope of bacterial isolates from dengke naniura showed that the bacteria were gram-positive, which was characterised by purple bacterial cells with round and rod shapes as shown in Figure 1 and Figure 2 [16].

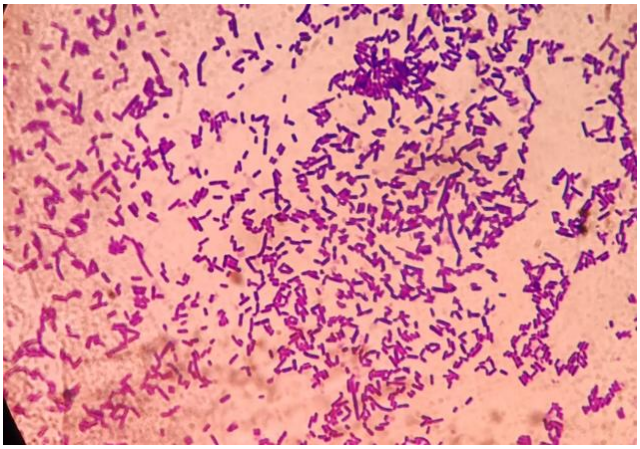

*Figure 2: Cell form in gram staining from an isolate of dengke naniura*

Lactic Acid Bacteria have limited biosynthetic abilities. The acquisition of energy is solely dependent on fermentative metabolism that is carried out in its place. The catalytic test results on the four bacterial isolates from dengke naniura showed negative results, as shown in Table 1. In the review of bacterial isolates, there was no visible gas bubble when dripping with hydrogen peroxide ($H_2O_2$). Lactic Acid Bacteria can produce a variety of other metabolite compounds in addition to lactic acid, including hydrogen peroxide (H2O2) [17].

**Table 1: Morphology characteristic and biochemistry isolate from *dengke naniura***

| Characteristic | DL-109 | DL-107 |
|---|---|---|
| Colony Morphology | | |
| Colony Form | Small oval | Moderate spheric |
| Periphery colony form | Flat | Flat |
| Height of surface | Flat | Flat |
| Colony colour | White | White |
| Cell Morphology | | |
| Gram | + | + |
| Cell Form | Basil | Basil |
| Biochemistry | | |
| Catalase | - | - |
| TSIA | + | + |
| Gas | - | - |
| $H_2S$ | - | - |
| Fermentation Type | Homo | Homo |
| Growth on Temperature | | |
| 15°C | - | - |
| 37°C | ++ | ++ |
| 45°C | +++ | + |
| Growth on PH | | |
| PH 2 | + | + |
| PH 3 | ++ | +++ |

Catalase test is a test to identify microbes that are capable of producing catalase enzyme which can cleavage the hydrogen peroxide that is formed from aerobic respiration and is toxic to bacteria, to dihydrogen oxide ($H_2O$) and oxygen ($O_2$) which are no longer toxic [16] Triple Sugar Iron Agar (TSIA) test results in table 1 show positive results for all isolates. All isolates were able to ferment glucose, sucrose, and lactose contained in TSIA medium and produce acid as indicated by the colour changes in the slant and butt parts to yellow. Heterofermentative lactic acid, besides producing lactic acid is also ethanol and other acids such as acetic acid and $CO_2$ gas [16]. TSIA test is a biochemical test to determine the ability of microbes to ferment glucose, sucrose and lactose contained in the medium.

From the test results, it was shown that all isolates did not produce gas and did not produce $H_2S$, according to Freeman (1979) which stated that the genus Lactobacillus did not produce $H_2S$. The fermentation type testing results show that four isolates are homofermentative because they do not produce gas. Homofermentative Lactic Acid Bacteria only produce lactic acid as the main product of fermentation. Growth test at 15°C, 37°C and 45°C for the four bacterial isolates from dengke naniura showed different growth at each temperature variation. At a temperature of 15°C, there is no visible growth of bacteria in the test tube. At a temperature of 37°, C DL109 and DL107 grew by producing moderate amounts of sediment. At a temperature of 45°C, DL109 can grow best with many deposits below the tube, DL107 grow not optimally with the fewest deposits. Based on the optimum temperature of growth, Lactic Acid Bacteria were grouped into two groups, namely mesophilic (optimal temperature of 25°C growth and maximum temperature of 40°C) and thermophilic (optimal temperature of 37°C growth and maximum temperature of 52°C) [16].

The activity of the isolates in inhibiting the α-glucosidase enzyme can be seen in Table 2.

**Table 2: Inhibition Activity and $IC_{50}$ of the LAB**

| Test solution concentration (µg/mL) | Absorbance (A) $S_1$ | $S_0$ | $S_1$-$S_0$ ($A_{sample}$) | % Inhibition | $IC_{50}$ (ppm) |
|---|---|---|---|---|---|
| Blank | 0.016 ($A_{blank}$) | | - | - | - |
| Control | 0.294 ($A_{control}$) | | - | - | - |
| Acarbose | | | | | |
| 100 | 0.240 | 0.080 | 0.160 | 45.58 | 128.06 |
| 75 | 0.258 | 0.085 | 0.173 | 41.16 | |
| 50 | 0.277 | 0.092 | 0.185 | 37.07 | |
| 25 | 0.290 | 0.094 | 0.196 | 33.33 | |
| Isolate DL-109 | | | | | |
| 100 | 0.146 | 0.019 | 0.127 | 56.80 | (-) |
| 75 | 0.139 | 0.010 | 0.129 | 56.12 | |
| 50 | 0.136 | 0.005 | 0.131 | 55.44 | |
| 25 | 0.135 | 0.002 | 0.133 | 54.76 | |
| Isolate DL-107 | | | | | |
| 100 | 0.205 | 0.064 | 0.141 | 52.04 | 46.32 |
| 75 | 0.164 | 0.020 | 0.144 | 51.02 | |
| 50 | 0.154 | 0.007 | 0.147 | 50.00 | |
| 25 | 0.152 | 0.003 | 0.149 | 49.32 | |

Note: (-) for DL-109 means that the inhibition under 0 ppm based on calculation.

The results showed that the LAB isolated from *dengke naniura* possessed inhibitory activity better than the positive control (acarbose). The isolate

DL109 have an unmeasured $IC_{50}$ (below 0) and showed dominant inhibition compared to acarbose (128.06 ppm) and isolate (L107 (46,32 ppm). While the lowest concentration of DL109 (25 µg/ml) showed inhibition as much as 54.76%, which is higher than the highest concentration (100 µg/ml) of acarbose and DL107.

## Discussion

The results shown above revealed the fermentation conducted by the LAB in *dengke naniura*. From the results, we have four isolates that have morphology and biochemical properties of lactic acid bacteria. Lactic acid bacteria have the ability to grow under low pH and by addition of 3% of salt favour the growth of lactic acid bacteria in this spontaneous fermentation [18]. The sources of LAB could be from the fish as it was reported having *Bacilli* (belonging to the phylum *Firmicutes*) by using pyrosequencing of 16S rRNA gene amplicons method revealed the bacteria including Lactobacillus and Leuconostoc bacteria [8]. *Dengke naniura* in its preparation also included many fresh spices which could also be the sources of the LAB, as it was reported in many vegetable fermentations that contains Lactobacillus and Leuconostoc as dominant species [18].

The viability of the LAB in fermented foods like *dengke naniura* depend not only by the pH level and salt, but also by the utilisation of bonded sugar creating an aglycon, and the aglycon may become one of the therapeutic ingredients produced by the LAB [19]. Under the stress condition such as low pH, the LAB synthesised a polyphosphate compound to protect itself under that condition. Some of our pure LAB colonies isolate were unable to grow under subsequent cultivation; this growth failure might because of particular nutrient needed by the LAB isolates [20].

All of the LAB isolates were having inhibition activity against the α-glucosidase enzyme, where the enzyme has the role in degrading polysaccharide into monosaccharide [21]. Thus inhibition makes the isolates can be an alternative way of treating type 2 diabetes. The study for LAB α-glycosidic breaking activity was not many, but this ability replaced by its ability to form exopolysaccharide from lignin (β-glycosidic bond compound). That exopolysaccharide produced by the LAB, protect the LAB from a hard condition such as dehydration and acidity even by bile acid [22].

Carp fish is the uncommon raw material for being fermented foods, but in Batak tribe of Indonesia, this food is delicacies to the local people. From the findings mentioned above, it showed that *dengke naniura* not only serve us the nutrition but also health especially to the grown-up people for the treatment of some health issues like diabetes since the LAB can reduce the sugar bonded or not and synthesising metabolites that benefit human health [19]. The *dengke naniura* can also benefit children with the lack of protein because dengke naniura have more protein that comes from its raw material. The nutritional value of *dengke naniura* not only come from the fish itself but also the spices added to it. The herbs and spices may serve special nutrient that can be utilized by the LAB to synthesised compound that brings benefit to us.

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