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Lactic Acid Bacteria (LAB) with Probiotic Potential from Dengke Naniura

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Abstract:

Dengke Naniura is a traditional food from Sumatera Utara, Indonesia, that is produced through fermenting process, and this food is believed to contain high probiotics. The objective of the current research is to determine the potential of LAB as a probiotic that has been obtained from Dengke Naniura. Dengke Naniura was traditionally prepared from *Cyprinus carpio*. Four LABs have been successfully isolated from Dengke Naniura, such as D7DA3, D7B3, D7DBF and D7DN3. Those four LAB isolates were identified as *Lactobacillus sp.* This result has been confirmed by the non-spore forming bacterium, non-motile, and Gram-positive. Also, it has been supported by biochemical test, for the example Voges Proskauer, catalase test, Methyl Red test, and carbohydrate fermentation. Several tests have been performed for determining the properties of the isolated LABs as probiotic, for the example: physiological properties, acid and bile tolerance and antimicrobial activities. As a probiotic, the fermentation profile of the isolated LAB was evaluated, including titratable acidity, pH, and organoleptic test. The all four isolates show the ability to survive in the MRS broth medium at pH 2 and 3. At the pH 3, the isolates of D7B3 show the highest ability to survive; it is about 100%, after 2 hours of incubation time. This data is followed by D7DBF4 with value 90 and 24% at pH 3 and 2, respectively. Otherwise, the isolate of D7DN3 and D7DA3 shows the lowest value, it is about 55-58% and 52-58% at pH 3 and 2, respectively. The fermented milk has been successfully made from LAB that has been isolated from Dengke Naniura. The fermented milk prepared using D7DA3 and D7DN3 has consistence and odor as similar as yoghurt, otherwise the isolate of D7DB3 and D7DBF4 produced fermented milk that is too thick. The fermented milk prepared from these isolates, D7DA3, D7DBF4 and D7DN3 has a normal taste. The better taste has been obtained in the fermented milk that was prepared using D7B3 isolate. The fermented milk prepared using LAB and obtained from Dengke Naniura has titratable acidity of 0.92-1.15% with pH 4.03-4.60.

Key words: Dengke Naniura, Fermentation, Lactic Acid Bacteria, Probiotic potential.

Introduction:

Probiotic is classified as a microorganism that can bring advantages to humans, especially in human health (1, 2). Several researches that focused on the effect of probiotic reported that this microorganism can prevent and reduce the indication of acute diarrhea, diabetes, hypertension, and inflammatory disease(3, 4). As the impact of technology advancement, nowadays probiotic can be easily found in our daily food and it is called as functional food. In the form of functional food, it has many therapeutic advantages to human, i.e. antioxidant, anticancer, immunomodulator, and

antihypertension. The interest of the researchers and industries to isolate new strain of probiotic and promote it in dietary supplement is increasing nowadays(5).

A consortium of LABs with bifidobacterial can be categorized as the most popular probiotic that was investigated for the last decades (1, 6). Lactic acid bacteria in functional food bring health advantages and it is also involved in the fermentation process of functional food. To evaluate the presence of new probiotic LAB in the isolate, there are several criteria that have been

used, i.e. tolerance to acid and bile salt conditions, has antimicrobial properties. Also, it should be able to survive in the fermentation conditions, to evaluate its potency in food industries (7).

Dengke Naniura or marinated fish is a type of traditional Batak Toba's food in North Sumatra which is consumed without cooking as a source of protein. Dengke Naniura is traditionally made from carp (*Cyprinus carpio*) fresh from Lake Toba. This food is made by marinating the fish in jungga lime (*Citrus jambhiri* Lush.) juice and spices until the fish becomes soft and ready to be consumed without cooking. The method of processing Dengke Naniura varies in time spent marinating and the amount of seasoning used subject to individual taste. As a result of investigation in literature review, several researches have observed the effect of LAB to the growth of carp (8-10), but study that focused on the isolation of LAB from derivative food product of carp, especially Dengke Naniura has yet to be found. According to the explanation above, the aim of this research is to isolate and investigate the probiotic properties of LAB from Dengke Naniura.

Materials and Methods:

Sample and enrichment technique

Carp (*Cyprinus carpio*) used in this research has been taken from Lake Toba area in the Haranggaol. Carp weighting approximately 1 kg each was taken to the laboratory using a closed plastic container that contains water and oxygen. Carp was cleaned, deboned and deskinning. The carp meat was washed with cool boiled water and drained. The carp meat that had been drained was placed in a glass container and then marinated with jungga lime juice with a ratio of 1g of jungga lime juice to 1.8 g of carp meat and added 3% of salt. Marination in jungga lime juice was carried out in a room at 18°C within a period of 7 hours. The mixture of spices was then put in a carp marinating one hour before the marinating time finished. The fish soaking container was sealed tightly during the process.

Isolation of LAB

The isolation process of LAB was performed according to the method from Abushelaibi et al.(1). LAB of Dengke Naniura was isolated using spread-plate on MRS agar and it was incubated at 30°C for 48 h. The obtained colonies then continued to Gram stain and catalase test.

Antagonistic test

The antagonistic properties of the isolated LAB from Dengke Naniura were determined using disk paper method against *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and

Escherichia coli. The obtained inhibition zone was measured after incubation for 24 h at 37 °C(11).

Tolerance to acid and bile salt

Acid and bile salt tolerances of LAB isolates were evaluated using a modified method from Lee et al.(12). Acid tolerance test was performed in MRS medium and the pH of medium was adjusted to pH 3.0 and 2.0 by adding HCl 0.1 M. The colonies were observed for 2 h. Bile salt tolerances of isolates were determined using sodium deoxycholate (1-3%) during 4 h of incubation at 37°C.

Fermentation profile

The fermentation activity of isolated LAB was evaluated using a method form Angmo et al. (2). The isolated LAB with concentration of 3.0% was inoculated in the sterilized bovine skim milk (10% w/v) and it was incubated at 37°C for 24 h. the obtained fermented milk then was stored at 37°C for 18 h. After the incubation time, samples were taken for pH measurement, determining titratable acidity and organoleptic test.

Results and Discussion:

Four bacteria colonies (D7DA3, D7B3, D7DBF and D7DN3) were isolated from the Dengke Naniura were identified as *Lactobacillus sp.* As in Table 1, all the isolates of bacteria colonies were produced bacilli-cocci shape (Fig. 1) and supported with the all the isolates were found as a non-spore forming bacterium, non-motile, and Gram-positive. Also, it was supported by biochemical test, for the example Voges Proskauer, catalase test, Methyl Red test, and carbohydrate fermentation (13-15).

Table 1. Bacterial identification.

Characteristic	D7DA3	D7B3	D7DBF4	D7DN3
Motility	Non-motile	Non-motile	Non-motile	Non-motile
Gram	+	+	+	+
Cell shaped	Bacilli-cocci	Cocci	Bacilli	Bacilli
Catalase	-	-	-	-

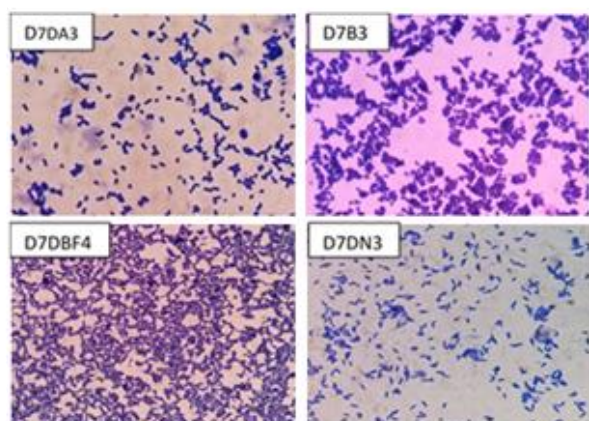


Fig. 1. Microscopic view after gram staining

Figure 1. Microscopic view after Gram staining.

The antagonistic test of D7DA3, D7DBF4, and D7DN3 against Gram-negative pathogens in Table 2 shows a significant difference ($p \leq 0.05$).

Table 2. Screening of antibacterial activity against eight pathogenic bacteria.

Bacteria	D7DA3	D7B3	D7DBF4	D7BN3
Gram-negative Pathogen				
<i>E. coli</i>	9.45 ± 0.45	6.30 ± 0.18	12.00 ± 0.5	10.43 ± 1.60
<i>P. aeruginosa</i>	7.43 ± 0.06	6.10 ± 0.08	9.30 ± 0.40	9.30 ± 0.75
<i>S. typhi</i>	9.37 ± 0.21	6.00 ± 0.00	9.33 ± 0.81	9.27 ± 0.64
<i>E. faecalis</i>	8.40 ± 0.10	6.08 ± 0.12	9.37 ± 0.47	10.25 ± 0.25
Gram-positive Pathogen				
<i>S. aureus</i>	8.80 ± 0.10	6.07 ± 0.09	10.40 ± 0.3	10.13 ± 0.25
<i>B. cereus</i>	7.63 ± 0.15	6.17 ± 0.12	12.97 ± 1.3	8.43 ± 0.61
<i>B. subtilis</i>	7.73 ± 0.59	6.90 ± 0.15	12.27 ± 0.1	12.93 ± 0.42

The all four isolates show the ability to survive in the MRS broth medium at pH 2 and 3 (Fig. 2). At the pH 3, the isolates of D7B3 show the highest ability to survive, it is about 100%, after 2 hours of incubation time. This data is followed by D7DBF4 with value 90 and 24% at pH 3 and 2, respectively. Otherwise, the isolate of D7DN3 and D7DA3 shows the lowest value, it is about 55-58% and 52-58% at pH 3 and 2, respectively. It can be shown that the bacterial colonies were decreased at the certain value when pH increased (18).

Those cultures have a higher value of inhibition zone than D7B3. Isolate of D7DBF4 has shown the highest inhibition zone in antagonistic test against Gram negative pathogens with 12 (0.5) mm in diameter inhibition zone against *E. coli*, 9.3 (0.4) mm in diameter against *P. aeruginosa*, 9.33 (0.81) mm in diameter against *S. typhi* and 9.37 (0.47) mm in diameter against *E. faecalis*. On the other hand, the isolates of D7DBF4 and D7DN3 have shown the significance difference activities compared to D7DA3 and D7B3. The highest activity against Gram positive pathogen is also shown by D7DBF4 with 10.40 (0.26) mm in diameter against *S. aureus*, 12.97 (1.31) mm in diameter against *B. cereus* and 12.27 (0.12) mm in diameter against *B. subtilis* (Table 2). This result was relevant to the previous results (16-18).

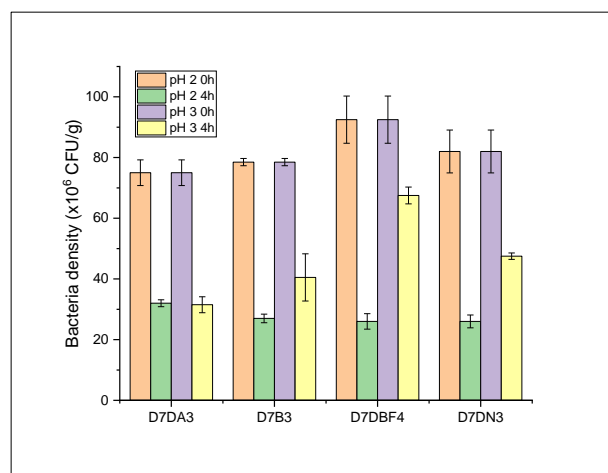


Figure 2. Effect of pH on growth of identified isolates at 0 and 4 h.

Bile salt is one of several tests to determine the ability of LAB to survive in the gastrointestinal system. In the previous research, the obtained LAB was tested in the 2.0% of bile salt and it was able to survive. In the present research, the bile salt concentration was set to be 1.0, 2.0 and 3.0% and it was incubated for 4 h. Fig. 3 shows that D7B3 has

the highest tolerance to the bile salt test; it was observed from the colonies number after 4 h treatment. This ability that was showed by isolated LAB can be associated with the presence of S-layer protein. This protein can help to enhance the cell viability of microflora in the gastrointestinal system. Based on the result of de Valdez and Taranto [19] and Gilliland et al. [20], the isolated LAB may have a potency as probiotic due to its ability to survive in the high bile salt concentration.

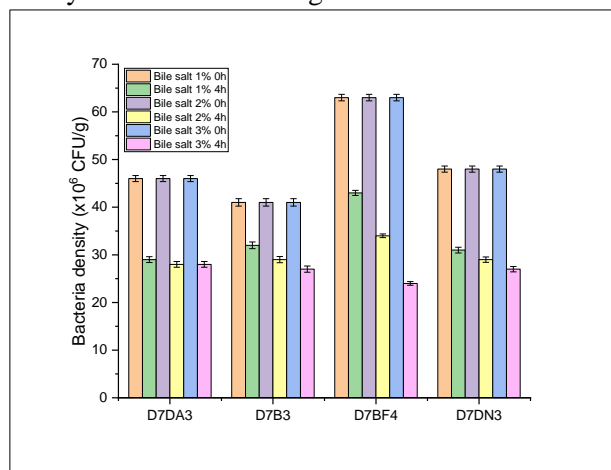


Figure 3. Bile salt tolerance test of identified isolates at 0 and 4 h.

The obtained LAB isolates with the probiotic potency has been applied in the production of

fermented milk. During the production of fermented milk, the isolates were cultured in 10% skim milk to obtain the starter culture. The number of bacterial cells in the starter can be seen in Table 3.

Table 3. Bacterial cell growth in starter.

Isolate	Bacterial Cells (cfu/ml)
D7DA3	11 x 10 ⁷
D7DB3	12 x 10 ⁷
D7DBF4	14 x 10 ⁷
D7DN3	14 x 10 ⁷

The fermented milk was made by inoculating the 3% of LAB isolate in the starter culture. The fermented milk has been successfully made from LAB that was isolated from Dengke Naniura and the characteristic of the fermented milk can be seen in Table 4. The fermented milk that was prepared using D7DA3 and D7DN3 has consistence and odor as similar as yoghurt, otherwise the isolate of D7DB3 and D7DBF4 produced fermented milk that is too thick. The fermented milk that was prepared from these isolates, D7DA3, D7DBF4 and D7DN3 has a normal taste. The bitter taste was obtained in the fermented milk that was prepared using D7B3 isolate. The fermented milk that was prepared using LAB obtained from Dengke Naniura has titratable acidity of 0.92-1.15% with pH 4.03-4.60.

Table 4. Organoleptic evaluation

Fermented Milk	LAB isolate from Dengke Naniura			
	D7DA3	D7B3	D7DBF4	D7DN3
Physical Properties				
Consistency	Thick	Too thick	Too thick	Thick
Odor	Yoghurt	Yeasty	Yoghurt	Soft yeasty
Taste	Yoghurt	Bitter	Yoghurt	Yoghurt
Chemical properties				
Acid total	1.13 ± 0.23	0.92 ± 0.07	0.93 ± 0.07	1.15 ± 0.19
pH	4.27 ± 0.21	4.60 ± 0.36	4.57 ± 0.33	4.03 ± 0.09

Conclusion:

The investigation of the presence of lactic acid bacteria and its potency as probiotic sources in a traditional fermented food is based on *Cyprinus carpio*, Dengke Naniura of Bataknese, Indonesia has been conducted. Four isolates of LAB have been obtained, i.e. D7DA3, D7B3, D7DBF4 and D7DN3. All of the isolates have good probiotic properties that were evaluated from antagonistic test, acid and bile salt tolerance.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Sumatera Utara.

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بكتيريا حمض اللاكتيك (LAB) مع إمكانات البروبيوتيك من Dengke Naniura

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الخلاصة:

Dengke Naniura هو طعام تقليدي من Sumatera Utara ، إندونيسيا ، يتم إنتاجه من خلال عملية التخمير ، ويعتقد أن هذا الطعام يحتوي على نسبة عالية من البروبيوتيك. ان الهدف من البحث الحالي هو تحديد إمكانات LAB باعتبارها بروبيوتيك تم الحصول عليها من Dengke Naniura. تم تحضير Dengke Naniura بشكل تقليدي من *Cyprinus carpio* حيث تم عزل أربعة LABs بنجاح من Dengke Naniura، مثل D7DA3 و D7B3 و D7DBF و D7DN3. وتم تحديد عزلات LAB الأربعة على أنها *Lactobacillus* sp. وتم تأكيد هذه النتيجة عن طريق البكتيريا غير البوغية وغير المتحركة وإيجابية الجرام. أيضًا ، تم دعمه من خلال اختبار الكيمياء الحيوية ، على سبيل المثال Voges Proskauer واختبار الكتلاز واختبار Methyl Red وتخمير الكربوهيدرات. تم إجراء العديد من الاختبارات لتحديد خصائص LABs المعزولة باعتبارها بروبيوتيك ، على سبيل المثال: الخصائص الفسيولوجية وتحمل الحمض والصفراء والأنشطة المضادة للميكروبات. باعتباره بروبيوتيكًا ، تم تقييم ملف التخمير الخاص بـ LAB المعزول ، بما في ذلك الحموضة القابلة للمعايرة ، ودرجة الحموضة ، والاختبار الحسي. تظهر جميع العزلات الأربع القدرة على البقاء على قيد الحياة في وسط مرق MRS عند درجة الحموضة 2 و 3. عند درجة الحموضة 3 ، تظهر عزلات D7B3 أعلى قدرة على البقاء ؛ يكون حوالي 100٪ بعد ساعتين من وقت الحضانة. ويتبع هذه البيانات D7DBF4 بقيمة 90 و 24٪ عند الرقم الهيدروجيني 3 و 2 على التوالي. خلاف ذلك ، فإن عزلة D7DA3 و D7DN3 تظهر أقل قيمة ، وهي حوالي 55-58٪ و 52-58٪ عند درجة الحموضة 3 و 2 على التوالي. تم تصنيع الحليب المخمر بنجاح من LAB المعزول من Dengke Naniura. وان الحليب المخمر المحضر باستخدام D7DA3 و D7DN3 له قوام ورائحة تشبه الزبادي ، وإلا فإن عزل D7DB3 و D7DBF4 أنتج لبنًا مخمرًا سميكًا جدًا. اللبن المخمر المحضر من هذه العزلات D7DA3 و D7DBF4 و D7DN3 له طعم طبيعي. تم الحصول على أفضل طعم في الحليب المخمر الذي تم تحضيره باستخدام العزلة D7B3 والحليب المخمر المحضر باستخدام LAB والذي تم الحصول عليه من Dengke Naniura له حموضة معايرة تبلغ 0.92-1.15٪ مع pH 4.03-4.60.

الكلمات المفتاحية: Dengke Naniura ، بكتيريا حمض اللاكتيك، التخمير، إمكانات البروبيوتيك.