BIODIVERSITAS
Volume 22, Number 8, August 2021
Pages: 3098-3104

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220802

Antibacterial activity of lactic acid bacteria isolated from Dengke Naniura of Carp (*Cyprinus carpio*) against diarrhea-causing pathogenic bacteria

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Manuscript received: 31 May 2021. Revision accepted: 5 July 2021.

Abstract. *Nasri, Harahap U, Silalahi J, Satria D. 2021. Antibacterial activity of lactic acid bacteria isolated from Dengke Naniura of Carp (*Cyprinus carpio*) against diarrhea-causing pathogenic bacteria. Biodiversitas 22: 3098-3104.* Diarrhea is the discharge of liquid or watery stools 3 to 4 times a day caused by a bacterial infection. Treatments for diarrhea are probiotics, which have a beneficial effect on the health of the host such as antibacterial. Traditional Batak Toba fermented food, Dengke Naniura, is a source of probiotics. This study aimed to determine the minimum inhibitory concentration, minimum bactericidal concentration, and leakage of DNA and protein from lactic acid bacteria against pathogens. Isolation of LAB was obtained from Dengke Naniura by pour plate method on deMann Rogosa and Sharpe Agar + $CaCO_3$ 1%. In this study, Characterization and analysis of bacterial sequencing used Polymerase Chain Reaction. Determination of MIC used the agar diffusion method. The MBC test used the streaking method which was a stroke from the inhibition zone formed. DNA and protein leakage was measured using spectrophotometry UV-VIS (260nm and 280nm). The isolation results obtained were *Lactobacillus fermentum*, the characterization showed that the bacteria were Gram-positive, bacilli, non-sporing, catalase-negative, and able to ferment sugar. The MIC determination was obtained at a concentration of 10%$^{v}/_{v}$ with a clear zone diameter. Determination of MBC against pathogens was obtained at different concentrations. The results of DNA and protein leakage showed an increased absorption (260nm and 280nm).

Keyword: Antibacterial, Dengke Naniura, lactic acid bacteria, leakage of DNA and protein

INTRODUCTION

Diarrhea is a disease that often occurs among people, especially in developing countries. Acute diarrhea is the occurrence of liquid or watery feces 3 to 4 times a day. Bacterial etiology can be caused by non-infectious causes, but some bacteria can become infectious agents, such as *Vibrio cholera*, Enterotocxigenic *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, *Shigella*, non-typhoidal *Salmonella*, *Vibrio parahemolyticus*, *Clostridium difficile*, and *Campylobacter*. Bacterial agents secrete toxins that act on the small intestine where the fluid is secreted into the lumen. It is also possible for mucosal damage, especially to the ileum and colon, due to ulcerative colitis (Tejan et al. 2018).

The prevalence of diarrhea in developing countries is reported annually to be around 2.5 billion cases of diarrhea in children under 5 years, of which there are as many as 1400 deaths (Sanyaolu et al. 2020). In some parts of the world, the mortality rate due to diarrhea is around 63%, which is the second leading cause of death in infants in developing countries (Ugboko et al. 2020). Based on the results of the Riset Kesehatan Dasar report (RISKESDAS 2007) diarrhea is the leading cause of death in infants and children. A total of 31.4% mortality rate in infants (aged 0-12 months) and 25.2% mortality rate in children (aged 0-59 months) were due to diarrhea (Sari and Budyanra 2017).

Probiotics are microorganisms that can provide beneficial effects on human health. Several studies reported the effect of probiotics to prevent and reduce acute diarrhea, inflammation, hypertension, and diabetes (Manik et al. 2021). The traditional food of Batak Toba is Dengke Naniura which is served without cooking, only by fermentation using Jungga acid (*Citrus jambhiri*) with the addition of other spices (Hutahaean et al. 2019). Lactic acid bacteria are a source of probiotics that can be found in fermented foods such as Dengke Naniura. By turning common carp into Dengke Naniura with the addition of Jungga acid, it can kill and inhibit the growth of pathogenic bacteria that cannot survive in acidic pH, while LAB works and survive in acidic pH (Haro et al. 2020). Research showed several *Lactobacillus* (lactic acid bacteria/LAB) antibacterial mechanisms such as competing for receptors, nutrients, and boosting immunity. LAB can produce organic compounds such as formic acid, lactic acid, acetic acid, and other acids that can lower the pH of the intestine. Another mechanism of LAB is secreted antimicrobial compounds such as ethanol, hydrogen peroxide, fatty acids, and bacteriocins (Chen et al. 2019).

According to the background, this study aims to isolate the LAB from Dengke Naniura and analyze the antibacterial mechanism (MIC, MBC, DNA, and protein leakage) against diarrhea-causing pathogenic bacteria, Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*), and Gram-negative (*Escherichia coli* and *Salmonella typhi*).

MATERIALS AND METHODS

Materials and apparatus

Diarrhea-causing pathogenic bacteria were Gram-negative (*Escherichia coli* ATCC 25922, and *Salmonella typhi* ATCC 19430) and Gram-positive (*Bacillus cereus* ATCC 14579 and *Staphylococcus aureus* ATCC 6538). Lactic acid bacteria isolated were obtained from Dengke Naniura. The medium used were deMann Rogosa and Sharpe Agar (MRSA), deMann Rogosa and Sharpe Broth (MRSB), Nutrient Broth (NB), Nutrient Agar (NA), Peptone Dilution Fluid (PDF), $CaCO_3$, Triple Sugar Iron Agar (TSIA), Tryptic Soy Agar (TSA), Gram staining kit, H_2O_2 3%, phosphate buffer pH 7.0, and Presto™ Mini gDNA Geneaid Biotech Ltd. The apparatus used were an incubator, microscope, vortex, centrifuge, Polymerase Chain Reaction (PCR), spectrophotometry UV-Visible, and glassware from the Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. Medan, Indonesia.

Isolation and characterization of lactic acid bacteria

Isolation of LAB from Dengke Naniura was using the dilution method with MRSB medium. 25 g of Dengke Naniura was mixed with 225 mL of MRSB then homogenized and incubated at 37ºC for 24-48 hours. 1 mL of the incubated suspension was put into 9 mL PDF media and homogenized. A series of dilutions were carried out from 10^{-2} to 10^{-10}. From 10^{-10} dilution 1 ml pipettes and transferred into sterile Petri dishes then added 15 ml of MRSA + $CaCO_3$ 1% 15 ml, and incubated at 37ºC for 24-48 hours. Isolation results showed a clear zone around the colony. Then it was taken using ose and cultured repeatedly 3-4 times to get pure bacterial colonies (Haro et al. 2020). LAB obtained were examined for bacterial morphology (color, shape, and size), Gram-staining of bacteria, catalase-test, fermentation type, gas formation, H_2S, and TSIA test (Hutahaean et al. 2019).

Extraction and amplification of DNA

DNA extraction was carried out based on the procedure available in the extraction tool from Presto™ Mini gDNA from Geneaid Biotech Ltd. The extracted DNA template was amplified with the 16S rRNA gene with fD1_rP2 Reverse primer (5'-ACG GCT ACC TTG TTA CGA CTT-3') and Forward (5'- GAG TTT GAT CCT GGC TCA-3') with a concentration of 1nM/µL. The 50µL PCR mixture contained 2µL of Primer R (1µM), 2µL of Primer F (1µM), and 19µL of nuclease-free water. The process of denaturation, annealing, and elongation consists of 30 cycles. The stages of each cycle comprised of pre-denaturation at 94°C for 3 minutes, denaturation at 94°C for 30 seconds, annealing at 52°C for 1 minute, elongation at 72°C for 30 seconds, and finalization at 72°C for 5 minutes then sample temperature at 4ºC for heat preservation. Amplification is performed on an automatic temperature cycler (Kawthar et al. 2018). The PCR product was followed by electrophoresis using 1% agarose (agarose gel was added with ethidium bromide). Electrophoresis was carried out at 80 volts for 60 minutes, then DNA visualization was performed using a UV transilluminator and documented with gel documentation (Fitri et al. 2017).

DNA base sequencing and identification of bacterial species

The PCR product was then purified and sequenced at the 1st Base Laboratory, Singapore. Nucleotide sequences were aligned with GenBank data using the BLAST software from NCBI (National Center for Biotechnology Information) and Sequence Scanner Software 2 for DNA sequence consensus, where FASTA was compared against data in the most recent NCBI databases. Bacteria with homologs greater than 97% were selected as identified bacteria (Fitri et al. 2017).

Determination of minimum inhibitory concentration

MIC determination against pathogenic bacteria causing diarrhea was carried out by agar diffusion method (wells method) on MRSA and NA bilayer media. Suspension of LAB (10^8 CFU/mL) with various concentrations (5%, 10%, 20%, 40%, 60%, 80%, 100% and Lacto B 100%, $\%^{v}/_{v}$) of 50 µL dropped on each well then left for 15 minutes and incubated at 35 ± 2ºC for 18-24 hours, repeated 3 times (Haro et al. 2020). In this study, 0.2% injection of ciprofloxacin antibiotic was used as a positive control and aquadest as a negative control. After incubation, the inhibition zone around the wells which showed clear zones was observed. The zone of inhibition was measured using a digital caliper in mm. The activity index was calculated using the formula below: (Kuspradini et al. 2019)

$$\text{Activity Index} = \frac{\textit{inhibitor zone of LAB}}{\textit{inhibitor zone of positive-control (antibiotic)}}$$

Determination of minimum bactericidal concentration

MBC determination was taken from the clear zone of the MIC determination and subcultured onto Tryptic Soy Agar (TSA) media (Mostafa et al. 2018). It was incubated at 35 ± 2ºC for 18-24 hours. The MBC value was determined by the lowest concentration which reduced 98%-99.9% viability of the initial bacterial population (negative-control) (Balouiri et al. 2016). The reduction percentage and log reduction can be calculated by the formula: (Ashakirin et al. 2017)

$$\text{Percent reduction} = \frac{(B-A)}{B} x100\%$$

$$\text{Log Reduction} = \text{Log (B-A)}$$

Where:

A: number of bacterial colonies at each concentration

B: number of colonies in negative control (Yang et al., 2019).

Determination of leakage DNA and protein

The suspension of the test pathogenic bacteria that had been grown for 24 hours in 10 mL NB medium was taken and centrifuged at 3500 rpm for 20 minutes. The supernatant was discarded then the pellets were washed with phosphate buffer pH 7.0 for 2 times, then suspended in 10mL phosphate buffer pH 7.0. Furthermore, the metabolites of lactic acid bacteria were added with a concentration of ½ MIC, 1 MIC, 2 MIC, and 4 MIC. Subsequently, it was incubated with an incubator at a temperature of 35 ± 2ºC for 24 hours. The suspension was centrifuged at 3500 rpm for 20 minutes. Separate the supernatant with pellets. The absorbance of the supernatant was measured using a spectrophotometer UV-VIS at a wavelength of 260nm and 280nm. The 260nm wavelength is used to measure the nitrogen content of nucleic acids, while the 280nm wavelength is used to measure the nitrogen content of cell proteins (Asriani et al., 2007).

Statistical analysis

Each test was carried out in three repetitions and the values were presented as mean and standard deviation. The statistical analysis software used was SPSS v.22. Data were analyzed using variance (ANOVA) analysis followed by Post Hoc LSD test when required with a level of significance of $p<0.05$.

RESULTS AND DISCUSSION

Isolation and characterization of lactic acid bacteria

Isolation of LAB from Dengke Naniura was using selective media deMann Rogosa and Sharpe Agar + $CaCO_3$ 1%. 1 lactic acid bacteria isolate was obtained which was marked by the presence of a clear zone around the colony after 24-48 hours incubation. The characterization of LAB can be seen in Table 1.

The characterization of LAB showed oval colony shape, rounded-edge shape, flat surface height, and white colony color. With Gram stain, it was seen that Gram-positive bacteria were characterized by purple bacteria and the shape of bacilli cells. Catalase-test by dropping 3 drops of H_2O_2 3% did not show the formation of gas bubbles. Tests using TSIA media showed that lactic acid bacteria were able to ferment the sugars contained in TSIA media (lactose, sucrose, and dextrose) characterized by a change in the color of the media to yellow. LAB did not produce H_2S. Furthermore, the type of fermentation shows homofermentative because the test using the Durham tube LAB did not show any gas bubbles.

DNA extraction and amplification

The extracted DNA was amplified using a thermal cycler and tested for quantity and quality of DNA by electrophoresis and nano spectrophotometer (DNA concentration 5.720 ng/µL with A260/280 value is 2.014). From the electrophoresis results, the best amplification optimization was obtained at an aneling temperature of 52ºC by showing a very clear band at 1500bp which can be seen in Figure 1, where this band indicated the amplicon of bacteria (Klindworth et al. 2013).

DNA base sequencing and identification of bacteria species

The results of 99% identical bacterial DNA sequencing were derived from *Lactobacillus fermentum* bacteria with DNA sequences and the results of homologous database adjustments can be seen in Table 2.

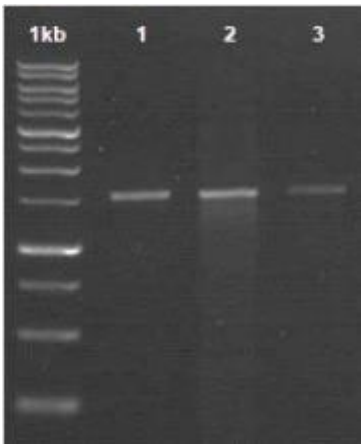


Figure 1. Electrophoresis results from DNA amplification. Note: *aneling optimization of amplification: 1. Temperature 50ºC; 2. Temperature 52ºC; 3. Temperature 54ºC

Table 1. Characteristics of LAB isolated from Dengke Naniura

Characteristics	Isolate LAB
Colony forms	Oval
Edge shape	Round
The height of the surface	Flat
Colony color	White
Gram stain	Gram +
Bacterial cell shape	Basil
Catalase-test	Negative
TSIA	Positive
Gas	Negative
H_2S	Negative
Fermentation Type	Homofermentative

Table 2. NCBI database homologs of DNA sequences

Description	Max score	Total score	Query cover	E value	ID	Accession
Lactobacillus fermentum strain LF 16S Ribosomal RNA gene, partial sequence	2625	2625	100%	0.0	99%	MK245999.1
Lactobacillus fermentum strain LMEM36 16S Ribosomal RNA gene	2625	2625	100%	0.0	99%	MK239985.1
Lactobacillus fermentum strain LMEM19 16S Ribosomal RNA gene	2526	2625	100%	0.0	99%	MK239955.1

DNA sequence:

TTGATTGATGGTGCTTGCACCTGATTGATTTTGGTTGCCAACGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGC
CCAGAAGCGGGGGACAACATTTGGAAACAGATGCTAATACCGCATAACAACGTTGTTCGCATGAACAACGCTTAAAAGATGG
CTTCTCGCTATCACTTCTGGATGGACCTGCGGTGCATTAGCTTGTTGGTGGGGTAACGGCCTACCAAGGCGATGATGCATAG
CCGAGTTGAGAGACTGATCGGCCACAATGGGACTGAGACACGGCCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCA
CAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAAC
ACGTATGAGAGTAACTGTTCATACGTTGACGGTATTTAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA
CGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGAGAGTGCAGGCGGTTTTCTAAGTCTGATGTGAAAGCCTTCGG
CTTAACCGGAGAAGTGCATCGGAAACTGGATAACTTGAGTGCAGAAGAGGGTAGTGGAACTCCATGTGTAGCGGTGGAATGC
GTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTACCTGGTCTGCAACTGACGCTGAGACTCGAAAGCATGGGTAGCGA
ACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGC
TAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTG
GARCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCCAACCCTAGAGATAGGGCGTTT
CCTTCGGGAACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGA
CGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGTCGCGAACTCGCGAGGGC
AAGCAAATCTCTTAAAACCGTTCTCAGTTCGGACTGCAGGCTGCAACTCGCCTGCACGAAGTCGGAATCGCTAGTAATCGCG
GATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTC
GGTGGGGTAACCTTTTAGGAGCCAGCCGCCTAAGGATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGT
ACAACGAGTCGCGAACTCGCGAGGGCAAGCAAATCTCTTAAAACCGTTCTCAGTTCGGACTGCAGGCTGCAACTCGCCTGCA
CGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC
CATGAGAGTTTGTAACACCCAAAGTCGGTGGGGTAACCTTTTAGGAGCCAGCCGCCTAAGGATCATGCCCCTTATGACCTGG
GCTACACACGTGCTACAATGGACGGTACAACGAGTCGCGAACTCGCGAGGGCAAGCAAATCTCTTAAAACCGTTCTCAGTTC
GGACTGCAGGCTGCAACTCGCCTGCACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCG
GGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTCGGTGGGGTAACCTTTTAGGAGCCAGCCGCC
TAAGG

Determination of minimum inhibitory concentration

Determination of MIC against the bacteria *S. aureus*, *B. cereus*, *E. coli*, and *S. typhi* were obtained at 10%$^{v}/_{v}$ concentration with a clear zone diameter of each 9.13 ± 0.51 mm, 7.87 ± 0.15 mm, 7.27 ± 0.25 mm, and 7.13 ± 0.15 mm. The activity index value of each bacteria at 100% concentration were 0.51 ± 0.06 (*S. aureus*), 0.54 ± 0.86 (*B. cereus*), 0.54 ± 0.04 (*E. coli*), and 0.56 ± 0.14 (*S. typhi*). The diameter of the inhibition zone for each bacterium can be seen in Table 3. (sig. 0.000; statistically there was a significant difference between means at $p<0.05$).

Determination of minimum bactericidal concentration

Determination of MBC against pathogens showed that *S. aureus* at a concentration of 40%$^{v}/_{v}$ with a 98.3% reduction percent, *B. cereus* at a concentration of 80%$^{v}/_{v}$ with a 98.0% reduction percent, *E. coli* at a concentration of 80.0%$^{v}/_{v}$ with a 99.0% reduction percent, and *S. typhi* at a concentration of 100.0%$^{v}/_{v}$ with a 99.0% reduction percent. The results of the MBC percentage values can be seen in Table 4. Figure 2 shows that the log reduction graph of the percent reduction indicates that a high percent reduction also influences a high log reduction. The log reduction value for 100%$^{v}/_{v}$ on *S. aureus* is 2.287, *B. cereus* is 2.037, *E. coli* is 2.088, and *S. typhi* is 2.039. This is because the number of bacterial colonies at each concentration showed a significantly different reduction with the number of colonies in the negative control. The percent reduction and log reduction values can be seen in Table 4.

Leakage of DNA and protein

The DNA and Protein leakage test results showed the absorbance of the bacterial cell supernatant at a wavelength of 260 nm and 280 nm which indicated an increase in the compound released by bacterial cells, which can be seen in Figures 3.A–3.D. LSD post hoc results showed sig. 0.000 in the negative control for all concentrations, sig. 0.012 at a concentration of 1/2 MIC to 1 MIC, sig. 0.005 at a concentration of 1 MIC to 2 MIC. It means that there is a significant difference between the negative control with each concentration and the difference between each concentration ($p<0.05$). It can be seen in Figure 3 that the ratio of 260/280 nm of each compound released by bacterial cells increased. (sig. 0.000; statistically there was a significant difference between the means at $p<0.05$).

Discussion

Bacteria isolated from Dengke Naniura produced 1 white oval-shaped bacterial colony and provided a clear zone around the bacteria. Because when MRSA + $CaCO_3$ 1% media reacting with lactic acid which was metabolized from lactic acid bacteria, it will form calcium lactate which dissolves in the media and produces a clear zone around the bacterial colony (Haro et al. 2020) The characteristics of the LAB are similar to those of the *Lactobacillus* sp, namely purple Gram-positive bacteria, bacilli-shaped, non-spore, non-motile, catalase-negative, and able to ferment sugar (Hutahaean et al. 2019; Ismail et al. 2017; Manik et al. 2021).

Table 3. The diameter of the zone of MIC of LAB against *S. aureus*, *B. cereus*, *E. coli*, and *S. typhi*

Conc. (% v/v)	*S. aureus*		*B. cereus*		*E. coli*		*S. typhi*	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI
- Control	6.00 ± 0.00[b]	0.17± 0.00	6.00 ± 0.00[b]	0.17± 0.00	6.00 ± 0.00[b]	0.18± 0.00	6.00 ± 0.00[b]	0.19± 0.00
5%	6.00 ± 0.00[bc]	0.17 ± 0.00	6.00 ± 0.00[bc]	0.17± 0.00	6.00 ± 0.00[bc]	0.18± 0.00	6.00 ± 0.00[bc]	0.19± 0.00
10%	9.13 ± 0.51[ab]	0.27 ± 0.34	7.87 ± 0.15[ab]	0.23± 0.12	7.27 ± 0.25[ab]	0.22± 0.09	7.13 ± 0.15[ab]	0.23± 0.04
20%	11.57 ± 0.25[ab]	0.34± 0.16	10.00 ± 0.10[ab]	0.29± 0.08	9.43 ± 0.32[ab]	0.28± 0.12	8.47 ± 0.25[ab]	0.27± 0.07
40%	14.03 ± 0.15[ab]	0.41 ± 0.1	14.07 ± 0.40[ab]	0.41± 0.33	14.20 ± 0.53[ab]	0.43± 0.20	12.93 ± 0.21[ab]	0.42± 0.06
60%	15.70 ± 0.36[ab]	0.46 ± 0.24	16.10 ± 0.17[ab]	0.47± 0.14	15.17 ± 0.25[ab]	0.46± 0.09	14.60 ± 0.40[ab]	0.47± 0.11
80%	16.60 ± 0.17[ab]	0.49 ± 0.11	17.43 ± 0.71[ab]	0.51± 0.59	15.93 ± 0.25[ab]	0.48± 0.09	15.77 ± 0.35[ab]	0.51± 0.10
100%	17.50 ± 0.10[ab]	0.51 ± 0.06	18.33 ± 1.04[ab]	0.54± 0.86	17.63 ± 0.12[ab]	0.54± 0.04	17.13 ± 0.49[ab]	0.56± 0.14
Lacto B	19.63 ± 0.55[ab]	0.58 ± 0.36	19.30 ± 0.82[ab]	0.57± 0.68	18.40 ± 0.78[ab]	0.56± 0.30	18.13 ± 0.25[ab]	0.59± 0.07
+ Control *	33.67 ± 0.15[a]	1.00± 0.10	33.77 ± 0.12[a]	1.00± 0.10	32.60 ± 0.26[ab]	1.00 ± 0.10	30.43 ± 0.35[ab]	1.00 ± 0.10

Note: * Ciprofloxacin injection 0.2% was used as positive control; IZ: Inhibitor Zone; AI: Activity Index. Post Hoc LSD test that shows: [a] Sig (P) < 0.05 there was a significant difference with the negative control (sig. 0.000). [b] Sig (P) < 0.05 there was a significant difference with the positive control (sig. 0.000). [c] Sig (P) > 0.05 there was no significant difference with the negative control (sig. 1.000)

Table 4. The MBC of LAB against *S. aureus*, *B. cereus*, *E. coli*, and *S. typhi*

Conc. (%v/v)	*S. aureus*		*B. cereus*		*E. coli*		*S. typhi*	
	% Reduction	Log Reduction	% Reduction	Log Reduction	% Reduction	Log Reduction	% Reduction	Log Reduction
- Control	0.0%	0,000	0.0%	0,000	0.0%	0,000	0.0%	0,000
5%	18.1%	0.087	17.6%	0.084	57.4%	0.370	4.7%	0.021
10%	25.8%	0.130	21.3%	0.104	66.3%	0.473	23.6%	0.117
20%	97.2%	1,560	95.7%	1,369	70.8%	0.534	92.7%	1,134
40%	98.3%	1,780	97.0%	1,518	96.0%	1,403	93.2%	1,169
60%	99.1%	2,037	97.5%	1,608	97.0%	1,521	94.1%	1,231
80%	99.4%	2,241	98.0%	1,698	99.0%	1,998	94.5%	1,264
100%	99.5%	2,287	99.1%	2,037	99.2%	2,088	99.0%	2,039
Lacto B	99.8%	2,639	99.3%	2,127	99.2%	2,124	99.4%	2,257
+ Control *	100.0%	4,258	99.7%	2,464	99.4%	2,248	99.8%	2,671

Note: * Ciprofloxacin injection 0.2% was used as a positive control

The extracted DNA after amplification and electrophoresis showed a clear band appearance at 1500bp. Where this band indicates the amplicon of the bacteria (Klindworth et al. 2013). Sequencing of amplified DNA obtained 99% identical bacterial DNA, which is a bacterial derivative of *Lactobacillus fermentum*. Several similar studies also obtained the same results from the isolation of traditional foods from various countries. The fermentation process with fast acidification will produce more *L.fermentum* derivatives than the slow acidification process. Rapid acidification techniques are also needed to reduce fermentation time, spoilage contamination, and/or pathogenic microorganisms (Owusu-Kwarteng et al. 2015).

The antibacterial activity of *L. fermentum* isolates from Dengke Naniura showed antibacterial activity against the four bacteria that cause diarrhea, marked by the formation of a clear zone around the well which was given *L. fermentum* inoculum with various concentrations. Although the resulting inhibition zone is lower than the inhibition zone for positive control, it does not mean that the tested sample does not have antibacterial activity (Kuspradini et al. 2019). The MIC in *S. aureus* has an inhibition zone diameter of 9.13 ± 0.51 mm, in *B. cereus* the inhibition zone diameter is 7.87 ± 0.15 mm, in *E. coli* the inhibition zone diameter is 7.27 ± 0.25 mm, and in *S. typhi* the inhibition zone diameter is 7.13 ± 0.15 mm. Complete results can be seen in Table 3.

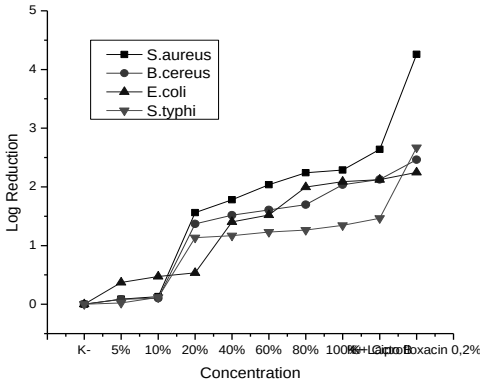
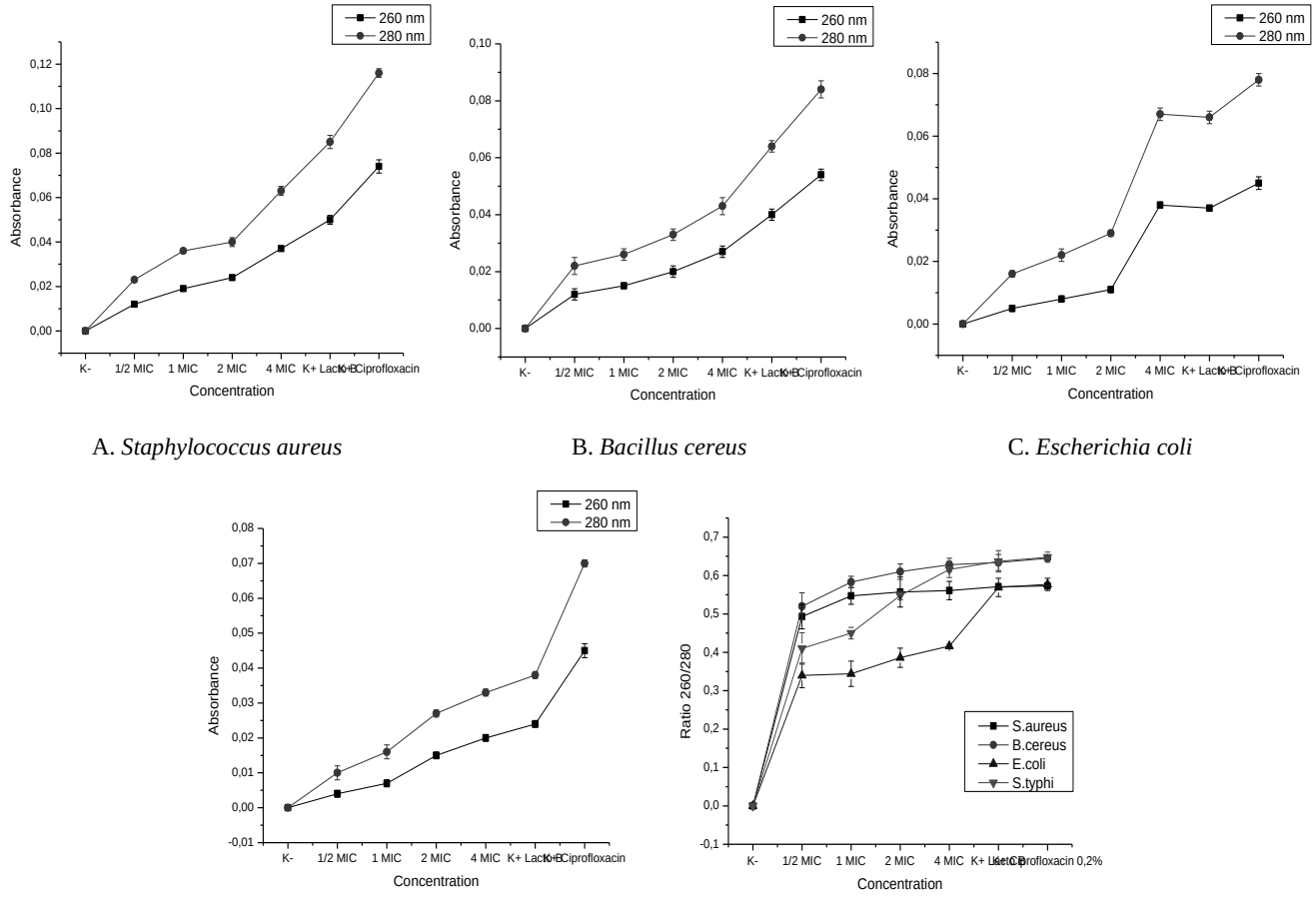


Figure 2. The log graph of the reduction of each concentration for each bacteria



A. *Staphylococcus aureus* B. *Bacillus cereus* C. *Escherichia coli*

D. *Salmonella typhi* E. Ratio 260/280

Figure 3. Leakage of DNA and protein in diarrhea-causing pathogenic bacteria cells that are given the antibacterial compound metabolite *Lactobacillus fermentum*

Davis and Stout (1971) Classification of the zone of inhibition (ZOI) is divided into 4 classifications according to the ZOI diameter, ZOI > 20mm (very strong), 10-20 mm (strong), 5-10 mm (moderate), and <5 mm (no response). The inhibition zone obtained different results depending on the metabolite compounds produced by the test isolate and the response of pathogenic bacteria to it (Ouchari et al. 2019). The activity index measurement aims to determine whether the antibacterial activity produced from the potential sample is comparable to the positive control (antibiotics). If the index activity value is equal to 1.00 then the activity is the same as the positive control activity (Kuspradini et al. 2019). The sample activity index at the highest concentration of 100% each has a value of 0.51 ± 0.06 (*S. aureus*), 0.54 ± 0.86 (*B. cereus*), 0.54 ± 0.04 (*E. coli*), and 0.56 ± 0.14 (*S. typhi*). From the results obtained, it can be considered that the *L. fermentum* has half the positive control potential (antibiotics).

The MBC determination was determined by streaking from each zone of inhibition resulting from the MIC test. From the test results, it was found that the MBC test sample for each bacteria was at different concentrations, 40%$^{v}/_{v}$ (*S. aureus*: 98.3%), 80%$^{v}/_{v}$ (*B. cereus*: 98.0%), 80%$^{v}/_{v}$ (*E. coli*: 99.0%) and 100%$^{v}/_{v}$ (*S. typhi*: 99.0%). The determination of MBC is known to be the lowest concentration of antimicrobial agent needed to kill 98,0% - 99.9% of the final colony number compared to the initial colony number (Balouiri et al. 2016).

Determination of bacterial cells leakage (wall/membrane) was analyzed by measuring the supernatant at a wavelength of 260 (nucleic acid) and 280 (protein) (Mierza et al. 2020). DNA and protein leakage is characterized by an increase in the absorbance of the cell supernatant measured at a wavelength of 260nm and 280nm (Asriani et al. 2007). The increase in absorbance which can be seen in Figure 3.A-3.D shows the release of metabolite compounds in bacterial cells, which can be in the form of RNA and its derivatives such as nucleotides which are absorbed in a wavelength of 260 nm and protein compounds at a wavelength of 280nm (Lin et al. 2000). Based on previous research, the addition of *Artemisia asiatica* essential oil to bacterial cultures, there was a shrinkage of bacteria and an increased release of constituents (Huang et al. 2018).

This research concludes that *Lactobacillus fermentum* isolated from Dengke Naniura has a potent antibacterial activity of MIC, MBC, DNA, and protein leakage.

ACKNOWLEDGEMENTS

The authors wish to thank Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara for their support in this research.

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