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Antimicrobial Activity of Goat Colostrum against Bacterial Strains Causing Food Poisoning Diseases

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Abstract. The study was aimed to investigate the antimicrobial activity of bacterial isolates *L.plantarum* 3CT7 and 20CT8 from goat colostrum. The antimicrobial activity of cell-free supernatant was tested using a well-diffusion method on several indicators: temperature, time of storage, and pH. Antimicrobial activity was recorded in both isolates at pH 2.0; 4.0; 6.0 and 8.0, temperature at 0, 50 and 100^{o}C, and in cold storage for 0, 15, 30, 45 and 60 days. *L.plantarum* 7CT3 and *L.plantarum* 20CT8 have a bigger zone of inhibition than that of *Pseudomonas spp.* as compared to other bacteria. Testing the cell-free activity was aimed to investigate the metabolite inhibition by *L.plantarum*. The isolates were capable of inhibiting all pathogenic bacteria in the experiment (*S. thypimurium, E. coli, and S. aureus*) as evidenced from the similar zone of inhibition from 15.83 to 16.06 mm. Isolates (*L.plantarum* 7CT3 dan 20CT8) exhibit inhibitory properties against *S.thypimurium, S.aureus, Pseudomonas spp.*. and *L.monocytogenes* at 0, 50 and 100^{o}C. *L.plantarum* 7CT3 and *L.plantarum* 20CT8 exhibit antimicrobial activity during cold storage. Both isolates grown in the range of pH from 2 to 8 could inhibit S*.thypimurium, E.coli, S. aureus* and *Pseudomonas spp.* In general, the two isolates are the potential antimicrobial activity with broad ranges of pH, temperature and storage time.

Keywords: isolate, antimicrobial activity, temperature, pH, storage time

Abstrak. Penelitian ini bertujuan untuk mengetahui aktivitas antimikroba isolat bakteri *L.plantarum* 3CT7 dan 20CT8 dari kolostrum kambing. Aktivitas antimikroba dari supernatan bebas sel diuji menggunakan metode difusi pada beberapa indikator yaitu : suhu, waktu penyimpanan, dan pH. Aktivitas antimikroba diamati pada kedua isolat tersebut pada pH 2,0; 4,0; 6,0 dan 8,0, suhu 0, 50 dan 100^{o}C, dan pada penyimpanan dingin selama 0, 15, 30, 45 dan 60 hari. Isolat *L.plantarum* 7CT3 dan *L.plantarum* 20CT8 memiliki zona penghambatan lebih besar pada bakteri *Pseudomonas spp.* dibandingkan dengan bakteri uji lainnya. Pengujian aktivitas bebas sel bertujuan untuk mengamati kemampuan daya hambat metabolit yang dihasilkan *L.plantarum*. Isolat yang diujikan mampu menghambat semua bakteri patogen (*S.thypimurium*, *E.coli*, dan *S.aureus*) dan zona penghambatan yang diperoleh yaitu 15,83 sampai dengan 16,06 mm. Isolat (*L.plantarum* 7CT3 dan 20CT8) menunjukkan sifat penghambatan terhadap *S.thypimurium, S.aureus, Pseudomonas spp.* dan *L.monocytogenes* pada suhu 0, 50 and 100^{o}C. Kedua isolat yaitu *L.plantarum* 7CT3 dan *L.plantarum* 20CT8 menunjukkan aktivitas antimikroba selama penyimpanan dingin. Kedua isolat yang tumbuh dalam kisaran pH dari 2 hingga 8 dapat menghambat *S.thypimurium, E.coli, S.aureus* dan *Pseudomonas spp.* Secara umum, kedua isolat tersebut berpeluang sebagai antimikroba potensial dengan rentang pH, suhu, dan waktu penyimpanan yang luas.

Kata kunci: isolat, aktivitas antimikroba, temperatur, pH, lama penyimpanan

Introduction

The major cause of poisoning in Indonesia during 2017 is instant foods. According to Indonesia Health Authority, the biggest poisoning case happened to women aged 15-34 years old, i.e. 163 cases. (Arisanti et al., 2018) reported that the major cause of food poisoning in Indonesia is pathogenic bacteria (*Escherichia coli)* from the consumed food product.

One of the measures to prevent food poisoning due to pathogenic bacteria is by using natural preservatives for food processing. Lactic acid bacteria (LAB) are well-recognized for their potential as natural food preservatives. Antimicrobial activity as the natural preservatives is harnessed during food processing and in the digestive tract. LAB produce metabolite with antimicrobial properties that prevent the growth and distribution of pathogenic bacteria and food spoilage. LAB produce lactic acid (Setyawardani et al., 2017) and other organic acids, hydrogen

peroxide and bacteriocin-like substances (Çadirci and Çitak, 2005).

LAB can be obtained from cow and goat colostrum. Previous study reported that cow colostrum exhibits antimicrobial activity against *E.coli*, *E.aerogenes*, *K.pneumonieae*, *B.subtilis* and *S.aureus*. Early presumption of natural preservatives is tested by measuring the inhibition of antimicrobial activity of LAB metabolites. The test was conducted using an agar well diffusion assay method (Khay et al., 2013) to measure the inhibitory properties against the growth, pH and temperature of the medium (Zouhir et al., 2011)

Metabolite obtained from goat colostrum LAB exhibits antimicrobial properties. Previous study indicated that the antimicrobial activities of LAB from goat milk were successful in inhibiting several gram-positive and gram-negative pathogenic bacteria (Setyawardani et al., 2017). Metabolite produced by goat colostrum LAB is predicted to have the excellent antimicrobial activity to be used as natural food preservatives for food material and food processing. Temperature, storage time, and pH are the key factors in food processing (Assefa et al., 2008).

The novelty of this study is the production of LAB metabolite from native Indonesian goat colostrum that exhibits an excellent antimicrobial activity as the candidate for natural food preservatives. The study aimed to investigate the inhibition of the antimicrobial activity of native Indonesian goat colostrum LAB based on different factors including temperature, storage time, and pH of the *L.plantarum* 3CT7 and *L.plantarum* 20CT8 isolates.

Materials and Methods

Testing the Antimicrobial Inhibitory Properties

L.plantarum 7CT3 and *L.plantarum* 20CT8 were isolated from native Indonesian goat colostrum (unpublished data). Both isolates were grown in MRSB (deMann *Rogose Sharp Broth,* Oxoid, USA) and incubated at 37^{o}C for 24h. Cell growth was indicated by the turbidity in the test tube. LAB count was 10^{8} CFU/ml, prepared for inhibitory test against pathogenic and spoilage bacteria. Indicator pathogenic bacteria and spoilage bacteria were cultured in NB media (*Nutrient Broth* Oxoid, USA) and incubated at 37^{o}C for 24h until the media turned turbid. Cell count was 10^{8} CFU/ml. An amount of 20 µl bacteria was put in petri dish and added with 20 ml of *Mueller Hinton Agar* 20 ml. The agar was let to solid, then wells of 5 mm diameter were punched into the agar, filled with 20 µl isolates for antimicrobial activity test and allowed to diffuse at low temperature (5-8^{o}C) for 60 minutes. The petri dish was incubated at 37^{o}C for 24h. The clear zone forming in the well was measured using calipers. Measurement was conducted three times at different locations, and the mean value of the result was obtained.

Antimicrobial Cell-Free Supernatant

L.plantarum 7CT3 and *L.plantarum* 20CT8 isolates were cultured in 10 MRSB media and incubated at 37^{o}C for 20h until the media turned opaque which indicated the growth of bacteria. *L.plantarum* 7CT3 and 20CT8 isolates were collected and centrifuged at 7000 rpm speed for 20 minutes at 4^{o}C. Cell-free supernatant was filtered using a 0.22 µm diameter membrane and neutralized. NaOH 1N was added to the supernatant to yield pH 5,8 – 6,2. Antimicrobial test was conducted using a well diffusion method.

Antimicrobial Testing at Different Temperature

The cell-free supernatant that was obtained from the previous step was tested for temperature durability. Antimicrobial durability was tested at 0, 50 and 100^{o}C for 20 minutes. Inhibition was tested using a well diffusion method.

Antimicrobial Durability Test at Different pH

The cell-free supernatant of the two isolates was tested for durability at pH 2, 4, 6 and 8. The

pH of cell-free supernatant was adjusted according to treatment by adding 1N HCl and 1N NaOH. The test was conducted using a well diffusion method.

Antimicrobial Test in Cold Storage

A cold storage is pivotal in investigating microbial durability and activity at specific temperature and duration. Cell-free supernatant was stored for 0, 15, 30, 45 and 60 days at 4^{o}C. The test was conducted using a well diffusion method.

Data Analysis

The obtained data was calculated to obtain the mean value and standard deviation. A further test was conducted using a Duncan's Multiple Range Test (DMRT).

Result and Discussion

Antimicrobial Activity of Crude Extract

Table 1 shows that *L.plantarum* 7CT3 and *L.plantarum* 20CT8 have a bigger zone of inhibition in *Pseudomonas spp.* compared to other bacteria, indicating a significant difference ($p<0.05$). The smallest zone of inhibition in *L.plantarum* 7CT3 and *L.plantarum* 20CT8 isolates was *S.aureus*, measuring 13.00 and 13.67 mm, respectively. In general, both isolates showed inhibitory activity against pathogenic and spoilage bacteria. Antimicrobial activity of the isolates from *L.plantarum* 7CT3 and 20CT8 was produced from metabolite in form of acid. The acid lowered pH and lysed the indicator bacteria, namely *S.thypimurium, E.coli, S.aureus,* and *Pseudomonas spp.* One of the inhibitory mechanisms was pH reduction by LAB metabolites, i.e. lactic acid and acetic acid (Tejero-Sariñena et al., 2012). The neutralizing process in the supernatant culture using alkali did not reduce the antimicrobial activity. It is evidenced that the metabolites yield were the potential candidates for bacteriocin. *L.plantarum* isolates are the group of Lactobacili with a high production of acid that can lower pH faster than other isolates, such as *L.fermentum* (Tejero-Sariñena et al., 2012). The cell-free supernatant contains active metabolites which function as the initial indicator of bacteriocin (Hartmann et al., 2011).

Antimicrobial activity is generated by lactic acid and other organic acids (Teneva et al., 2017) (Teneva et al., 2017). *L.plantarum* TW 4 exhibits antimicrobial properties against some pathogenic and spoilage bacteria (Setyawardani et al., 2014). The inhibitory properties of *L.plantarum* against pathogenic and spoilage bacteria are organic acids which reduce pH value and created an inhibition zone. The organic acids produced by *L.plantarum* are mainly lactic acid. Some strains of *L.plantarum* are the potential natural preservatives for food industry, and therapy for microbial infection (Dinev et al., 2018).

Antimicrobial Activity of Cell-Free

Figure 1 shows that the mean inhibition zone created by *L.plantarum* 7CT3 and *L.plantarum* 20 CT8 is 12.94 to 18.67 mm. *L.plantarum* 7CT3 isolates shows a higher inhibition that that of *L.plantarum* 20 CT8 against pathogenic bacteria but not against spoilage bacteria (*Pseudomonas spp.*). The cell free activity was tested to observe the

Table 1. Inhibition zone diameter of *L.plantarum* crude extracts in different bacteria indicators

Indicator bacteria	Inhibition zone diameter (mm)	
	L.plantarum 7CT3	*L.plantarum* 20CT8
S. thypimurium	15.33±0.58^{b}	15.33±0.58 ^{b}
E. coli	15.00 ±0.00^{b}	14.67±0.58 ^{bc}
S.aureus	13.00±0.00^{c}	13.67±0.58 ^{c}
Pseudomonas spp.	18.00±0.00^{a}	18.00±0.00 ^{a}
L.monocytogenes	14.33±0.58^{b}	15.00±1.00^{b}

Values bearing different superscripts within column show significant difference ($P<0.05$)

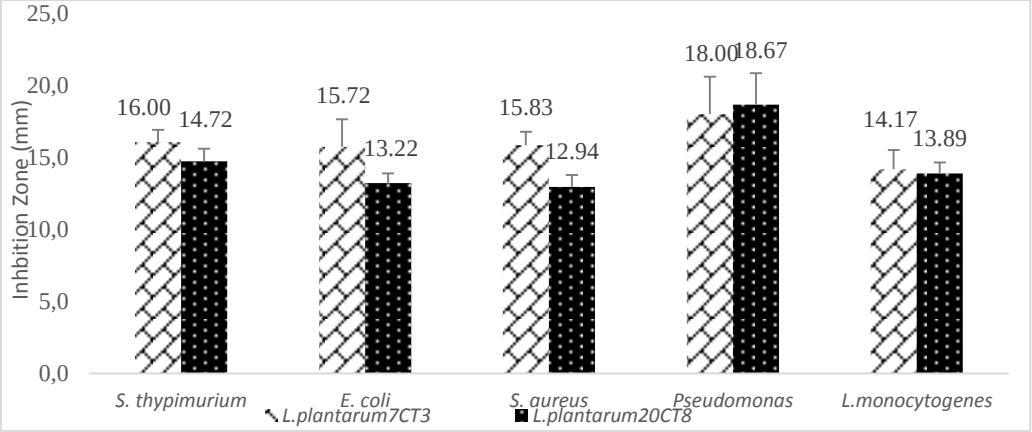

Figure 1. Average zone of inhibition (mm) of *L.plantarum* cell-free

Table 2. Zone of inhibition of *L.plantarum* isolates at different temperatures

Indicator bacteria	*L.plantarum* 7CT3			*L.plantarum* 20CT8		
	0°C	50°C	100°C	0°C	50°C	100°C
S. thypimurium	13.3±0,5^{b}	12.3±0,5^{c}	15.0±0,0^{a}	1.3±1.5^{c}	16.0±0.5^{b}	17.7±1.1
E. coli	13±0.0^{b}	0±0.0^{d}	11.67±1.5^{b}	16±0.0^{ab}	16±0.0^{b}	17±0.0
S. aureus	13.0±1.0^{b}	12.3±0,5^{c}	14.7±0,5^{a}	17.0±0,0^{ab}	15.3±0.5b	17.3±0.5
Pseudomonas spp.	20.0±0,0^{a}	15.7±0.0^{a}	15.3±0.5^{a}	15.0±0.0^{bc}	18.0±0.0^{a}	18.0±0.0
L.monocytogenes	11.3±0.5^{c}	13.3±0.5^{b}	13.0±0.0^{b}	15.0±0.0^{bc}	15.3±0.5^{b}	17.0±0.0

Values bearing different superscripts within column show significant difference (P<0.05)

metabolite inhibition by *L.plantarum*. The two isolates showed different inhibitory properties (P<0.05).*L.plantarum* 7CT3 and *L.plantarum* 20 CT8 show a higher inhibition against *Pseudomonas spp.,* indicating a significant difference from the inhibition against pathogenic bacteria (P<0.05). *L.plantarum* 7CT3 isolates had the lowest zone of inhibition in *L.monocytogenes*, namely 14.17 mm. The isolates were capable of inhibiting all pathogenic bacteria in the experiment (*S.thypimurium, E.coli, and S.aureus*) as evidenced from the similar zone of inhibition (P>0.05) from 15.83 to 16.06 mm. *L.plantarum* 20 CT8 showed the smallest zone of inhibition against *S.aureus* namely 12.94 mm which is not significantly different (P>0.05) from that of *E.coli.* The isolates showed a relatively similar zone of inhibition against *L.monocytogenes* and *E.coli* except for *S.thypimurium*.

The same isolates with different strains created different zones of inhibition. Previous study reported that the result of gene sequencing with 16 S rRNA showed that isolates 7CT3 was included in *L.plantarum* KCCM200656 and isolates 20CT8 was under *L.plantarum* IMAU 40170.

Testing the cell-free activity was aimed to investigate the inhibitory properties of metabolites without the cell produced by *L.plantarum*. The same isolate with different strains created different zones of inhibition. The previous study reported that 16 S rRNA gene sequencing indicated that isolates 7CT3 were of *L.plantarum* KCCM200656 and isolates 20CT8 were of *L.plantarum* IMAU 40170 (Setyawardani, unpublished data).

Antimicrobial Activity at Different Degrees of Temperature

Table 2 shows that the two isolates (*L. plantarum* 7CT3 dan 20CT8) exhibit inhibitory properties against *S.thypimurium, S. aureus, Pseudomonas spp.* and *L. monocytogenes* at 0, 50 and 100°C. The highest mean value of zone of inhibition in both isolates was observed in *Pseudomonas spp.*. *L. plantarum* 20CT8 isolates

was capable of inhibiting all bacteria in all degrees of temperature and exhibited a larger zone of inhibition than that of *L.plantarum* 7CT3. In the 0^{o}C cold storage, both isolates could inhibit all the indicator bacteria. *L.plantarum* 7CT3 isolates showed the higher inhibitory properties against *Pseudomonas spp.* namely 20 mm, significantly different (P<0.05) from the other indicator bacteria. The lowest inhibition was 11.3 mm against *L.monocytogenes*. *L.plantarum* 7CT3 isolates showed a relatively similar inhibition against *S.thypimurium, E.coli* and *S.aureus. L.plantarum* 20CT8 isolates had a relatively similar inhibition against *E.coli, S.aureus, Pseudomonas spp.* and *L.monocytogenes,* while the lowest was against *S. thypimurium* with a significant difference (P<0.05).

L.plantarum 7CT3 heated at 50^{o}C could inhibit other indicator bacteria except for *E.coli.* The isolates exhibited the highest inhibition (15.7 mm) against *Pseudomonas spp.,* significantly different (P<0.05) from other bacteria. At 50^{o}C, *L.plantarum* 20CT8 isolates showed the higher mean of inhibition than that of *L.plantarum* 7CT3. Inhibitory properties of *L.plantarum* 20CT8 against *S.thypimurium, E.coli,* and *S.aureus* dan *L.monocytogenes* were not significantly different (P>0.05).

The two isolates could inhibit all bacteria during the experiment at 100^{o}C. *L.plantarum* 7CT3 isolates could inhibit *S.thypimurium*, *S.aureus* and *Pseudomonas spp.* with a relatively similar zone of inhibition without significant difference (P>0.05). 20CT8 isolates showed a higher inhibition at 100^{o}C compared to 50 and 0^{o}C across the indicator bacteria. The antimicrobial activity of *L.plantarum* 20 CT8 isolates at 100^{o}C was successful in inhibiting all indicator bacteria with a zone of inhibition of 17.4 mm.

Antimicrobial activity of *L.plantarum* 3CT7 dan 20CT8 isolates was stable at the temperature of 0; 50 and 100^{o}C for 30 minutes, although the 3CT7 isolates could not inhibit *E.coli* at 50^{o}C. It was in line with a previous study (Heredia-Castro et al., 2015) that *Lactobacillus* isolates from Mexican Cocido cheese produced bacteriocin-like substances against *S.aureus, L.innocua, E.coli* and *S.thypimurium* using diffusion method. The antimicrobe produced was stable at 65^{o}C for 30 minutes and exhibited antimicrobial activity at pH between 2 and 8. It showed that bacteriocin produced from antimicrobial activity could be harnessed as the natural preservatives for pasteurized products, particularly dairy products (Heredia-Castro et al., 2015). Antimicrobe with the potential as bacteriocin showed durability in sterilization temperature of 121^{o}C for 15 minutes (Kusmarwati et al., 2014) and could only serve as preservatives for high-temperature food processing. Antimicrobe that endures high temperature is the potential candidate for bacteriocin because bacteriocin is protein consisted of short peptides that remain stable under the heat and endures a wide range of pH value.

Antimicrobial Activity during Cold Storage

Table 3 shows that the isolates produced by both *L.plantarum* 7CT3 and *L.plantarum* 20CT8 show antimicrobial activity during cold storage. The isolates were stored at 5^{o}C for 60 days and the test was conducted every 15 days. *L.plantarum* 7CT3 isolates had a relatively similar diameter across the indicator bacteria and not significantly different (P>0.05) from condition before storage. *L.plantarum* 20CT8 isolates had a higher inhibition against *Pseudomonas spp.* and significantly different against all other bacteria (P<0.05). During the 15-day cold storage, *L.plantarum* 7CT3 showed the highest inhibition against *Pseudomonas spp.* 17.78 mm and was significantly different (P<0.05) from other indicator bacteria. On the contrary, *L.plantarum* 20 CT8 did not show different inhibitory properties across indicator bacteria. During the 30-day storage, both *L.plantarum* 7CT3 and *L.plantarum* 20CT8 could inhibit all indicator bacteria and showed

Table 3. Zone of inhibition of *L plantarum 7CT3 and 20CT8* isolates during cold storage time

Isolates/Indicator bacteria	Cold storage time (days)				
	0	15	30	45	60
***L.plantarum* 7CT3**					
S.thypimurium	16.78±0.58	12.56±0.96^{b}	16.11±2.55^{b}	11.31±1.15^{b}	12.33±0.58^{d}
E.coli	16.44±2.22	13.78±2.12^{b}	21.67±2.91^{a}	12.22±0.77^{b}	0.00±0.00^{c}
S.aureus	16.11±0.51	15.33±1.15^{ab}	13.55±2.12^{bc}	10.33±0.58^{b}	14.00±0.00^{b}
Pseudomonas spp.p	18.34±2.52	17.78±2.27^{a}	15.00±0.00^{bc}	18.00±0.00^{a}	15.00±0.00^{a}
L.monocytogenes	15.33±0.58	15.00±0.00^{ab}	12.00±0.00^{b}	11.33±0.58^{b}	13.00±0.00^{c}
L.plantarum 20CT8					
S.thypimurium	16.78±1.26^{b}	14.89±0.58	16.33±2.08^{b}	11.83±0.93^{abc}	13.67±1.15^{b}
E.coli	15.00±1.00^{b}	14.89±1.71	20.56±0.96^{a}	14.00±3.00^{ab}	12.67±2.08^{b}
S.aureus	16.11±0.51^{b}	16.89±1.92	16.00±0.00^{b}	11.33±0.58^{bc}	17.00±0.00^{a}
Pseudomonas spp.p	19.34±1.12^{a}	18.33±2.08	16.67±0.58^{b}	14.33±0.58^{a}	14.00±0.00^{b}
L.monocytogenes	15.33±0.58^{b}	17.33±0.58	13.67±0.58^{c}	10.67±0.58^{c}	13.00±0.00^{b}

Values bearing different superscripts within column, either *L.plantarum* 7CT3 or *L.plantarum 20CT8,* show significant differences (P<0.05)

significant difference (P<0.05) in *E.coli* compared to the other bacteria. The highest inhibition was against *E.coli* (21.67 and 20.56 mm) and the lowest was *L.monocytogenes* (12.00 and 13.67 mm). During the 45-day storage, the isolates of both *L.plantarum* 7CT3 and plantarum 20CT8 showed the highest inhibition against *Pseudomonas spp.* 18 and 14.33 mm, respectively, and was significantly different from the other indicator bacteria. *L.plantarum* 20CT8 and *L.plantarum* 7CT3 isolates during the 60-day storage was not capable of inhibiting *E.coli*, but showed inhibitory properties against the other indicator bacteria. *L.plantarum* 20CT isolates showed the highest inhibition against *S.aureus* and was significantly different from other bacteria (P<0.05). In the cold storage, antimicrobial activity survived up to 60 days in *L.plantarum* isolates and most of the bacteria. It showed that antimicrobial activity in both isolates could inhibit the indicator bacteria for 60 days. *E.coli* could not be inhibited during 60-day of cold storage by *L.plantarum* 7CT3 isolates, but inhibited by 20CT8 isolates. Therefore, *L.plantarum* 7CT3 was more effective in inhibiting *E.coli* in cold storage for up to 6 days.

Antimicrobial Activity in Different pH Values

Table 4 shows that both isolates grown in the range of pH from 2 to 8 could inhibit S*. thypimurium, E.coli, S.aureus* and *Pseudomonas spp*. Both isolates did not show inhibition in pH 2. Media with pH 4 showed significantly different (P<0.05) zone of inhibition against the bacteria. *L.plantarum* 7CT3 isolates showed the highest inhibition against *Pseudomonas spp.* (17.78 mm) while *L.plantarum* 20CT8 had the highest inhibition against *L.monocytogenes*.

At pH 8, the highest and lowest average of *L.plantarum* 7CT3 isolates inhibition was against *E.coli* (15.67 mm) and *Pseudomonas spp.* (11.33 mm), respectively. In *L.plantarum* 20CT8 isolates, the highest and lowest average of inhibition was against *Pseudomonas spp.* (18 mm) and *L.monocytogenes* (12 mm), respectively. The highest mean inhibition in isolates 3CT7 was obtained in pH value 4, followed by pH 8. It indicated that both isolates are applicable to different ranges of pH value.

The changing antimicrobial activity was affected by different environmental condition, such as pH. Antimicrobial durability in a wide range of pH value will determine the metabolite application produced by LAB. Studies by Fayol-Messaoudi et al. (2005) and (Zacharof and Lovitt, 2012) reported that antibacterial was more effective in absorbing the surface of the bacteria in pH < 5.0. Research showed that the antimicrobial properties survived in a wide range of pH values from 4 to 10 in all indicator bacteria. It was in line with Mezaini et al. (2009)

Table 4. Antimicrobial activity of *L.plantarum 7CT3 and 20CT8* isolates in a range of pH

Isolates/Indicator bacteria	pH			
	2	4	6	8
***L.plantarum* 7CT3**				
S.thypimurium	7.67±0.58^{b}	14.33±0.96^{b}	13.33±0.58^{a}	15.00±0.00^{ab}
E.coli	10.33±0.58^{a}	13.78±2.12^{b}	10.67±0.58^{c}	15.67±0.58^{a}
S.aureus	10.00±0.00^{a}	15.33±1.15^{ab}	13.00±1.00^{ab}	13.33±0.58^{c}
Pseudomonas spp.p	8.00±0.00^{b}	17.78±2.27^{a}	12.00±0.00^{b}	11.33±1.15^{d}
L.monocytogenes	0.00±0.00^{c}	15.00±0.00^{ab}	7.33±0.58^{d}	14.33±0.58^{bc}
L.plantarum 20CT8				
S.thypimurium	10.67±0.58^{c}	13.33±0.58^{c}	15.33±0.58	16.33±0.58^{b}
E.coli	12.67±0.58^{ab}	14.50±0.71^{ab}	12.67±1.53	16.00±1.73^{b}
S.aureus	12.33±2.08^{bc}	13.67±0.58^{b}	13.00±1.00	13.33±0.58^{c}
Pseudomonas spp.p	14.33±0.58^{a}	13.00±0.00^{c}	13.00±0.00	18.00±0.00^{a}
L.monocytogenes	0.00±0.00^{d}	15.00±1.00^{a}	12.67±1.53	12.33±0.58^{c}

Values bearing different superscripts within column, either *L.plantarum* 7CT3 or *L.plantarum 20CT8,* show significant differences (P<0.05)

that antimicrobial activity could survive in pH between 4.0 and 8.0. Meanwhile, Pinto et al. (2009) stated that *Pediococcus pentosaceus* and *E.faecium* could maintain the antimicrobial activity in pH 2.0 to 8.0 before vanishing in pH 12. Antimicrobial activity in a wide range of pH will accommodate the application of the product.

Conclusion

Isolates *L.plantarum* 3CT7 and *L.plantarum* 8CT20 exhibit antimicrobial activities in a wide range of pH value (2.0-8.0); from low to high temperatures (0^{o}C – 100^{o}C), and under cold storage for 60 days. Both isolates are the potential natural preservatives.

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