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The Profile Analysis of Lactic Acid Bacteria (LAB) from Sumbawa White Honey and Its Potential Producing Antibacterial Compounds

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Abstract

Honey acts as an antibacterial without side effects, and also contains antiseptic substances which function to inhibit bacterial growth. This study aimed to isolate the Lactic Acid Bacteria (LAB) in Sumbawa white honey and the bioactive compounds produced as pathogenic antibacteria. The 1st stage in this study was the isolation of lactic acid bacteria (LAB) in Sumbawa white honey, then continued with a grading test, morphological test, catalase test, methyl red test, and the last test, namely the antimicrobial test against 5 pathogenic bacteria (*Salmonellatyhposa*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter ludwigii*, and *Leclerciaadecarboxylata*). Data analysis was performed using the Analysis of variance (ANOVA) test at a confidence level of 0.05 with SPSS 24. Based on the results of sequencing analysis, it was found that the 5 selected isolates were *Enterococcus faecium* species. The *Enterococcus faecium* species obtained from the sequencing results had different strains. The accession numbers of the 5 *Enterococcus faecium* were: Isolate-03 with a percentage of 97.29 % (accession number: KU324920.1), Isolate-07 has a percent identity of 97.36 % (accession number: MF108201.1), Isolate-09 of 97.73 % (accession number: CP041261.3), Isolate-20 with a percentage of 96.40 % (accession number: MN511819.1), and Isolate-24 with a percentage of 98.61 % (accession number: KM495938.1). These isolates can inhibit the growth of all tested pathogenic bacteria treated with 100 % LAB metabolites and were not significantly different ($p > 0.05$) compared to a positive control (Ampicillin).

Keywords*:* Antibacterial compounds, Lactic Acid Bacteria (LAB), Sumbawa white honey

Introduction

Sumbawa is one of the honey-producing areas in Indonesia, one of the types of honey produced is Sumbawa White Honey, which is produced by *Apis mellifera*. These bees are found around Mount of Tambora, in Dompu Regency. Sumbawa white honey produced by *Apis mellifera* also has very good benefits, the same as other Sumbawa honey, such as the black honey from Sumbawa produced by *Trigona* spp [1], and also honey produced by *Apisdorsata*. But until now, research on white honey is still rare.

LAB is a type of microbe that can live in acidic conditions, especially in food ingredients. LAB is a group of gram-positive bacteria that do not produce spores. LAB can produce lactic acid as a result of the fermentation of food materials such as sugar and carbohydrates [2]. In general, LAB has the characteristic of reacting negatively to the catalase test and having a positive reaction to gram staining [3]. Several genera of known lactic acid bacteria are *Lactobacillus, Streptococcus, Enterococcus, Pediococcus, Tetragenococcus, Leuconostoc*, and *Lactococcus* [4]. In addition, to producelactic acid, LAB is also known to be able to produce antimicrobial compounds such as bacteriocins, hydrogen peroxide, and organic acids (citric, lactic, *etc*.) [5].

Bacteriocins are compounds used as preservatives, while the acetic acid, lactic acid, and propionic acid are antimicrobial agents that are widely used in the food industry [6]. LAB can inhibit pathogenic microbes. Naturally, LAB can be found in various habitats such as fermented food and the human digestive tract. So far, it is known that LAB is not pathogenic and safe for consumption so that it can be used to improve human health. The availability of LAB isolated from domestic sources is still lacking, so exploration of lactic acid bacteria is needed to increase the collection of lactic acid bacteria isolates. Lactic acid bacteria can be obtained by utilizing sources containing lactic acid bacteria.

Materials and methods

Isolation of LAB

The isolation of LAB from Sumbawa white honey refers to Manguntungia *et al*. [7]. A total of 1 mL of white honey was homogenized into 5 mL of MRS broth medium (de Man, Rogosa and Sharpe medium; Merck, USA) and incubated for 24 h at 37 °C. After 24 h, a serial dilution of up to 10^{-5} was made, samples with a dilution of 10^{-3}, 10^{-4}, and 10^{-5} were spread on MRS agar medium and incubated for 24 h at 37 °C. The representative colonies were then randomly selected to be purified on the same medium and maintained at 4 °C.

The profile analysis of LAB

All bacterial colonies obtained were then identified based on morphological and physiological parameters. Identification of LAB profiles was based on morphological characteristics, including bacterial shapes, colony colors, colony margins, and elevation. Microscopic analysis was performed using gram stain and observed under a light microscope at 100× magnification. Physiological tests include the catalase test and methyl red test [8]. Methyl Red (MR) test aims to detect the ability of organisms to produce and maintain stable acids and products from glucose fermentation.

Antimicrobial analysis

Some isolates were selected based on the results of morphological, biochemical, and physiological tests, namely Isolate-03, Isolate-07, Isolate-09, Isolate-20, and Isolate-24 grown on 50 mL of liquid MRS media. The bacterial isolates were then cultivated at 37 °C for 3 days with a shaking rate of 1 g. The antimicrobial test used the agar well diffusion method. The bacteria test used were pathogenic bacteria *Salmonella tyhposa*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter ludwigii*, and *Leclerciaadecarboxylata*. The antimicrobials used were secondary metabolites produced by lactic acid bacteria from Sumbawa white honey. Positive control of test bacteria using ampicillin (50 µg/mL). A total of 3 mL (dilution has been carried out) of test bacteria was added to 20 mL nutrient agar media then poured into a sterile petri dish, waiting to solidify. After compacting a well is made with a diameter of 6 mm. The secondary metabolites were put into the well as much as 30 µL and then incubated at 37 °C. Obstacle zone observations were carried out at 16 h [9].

DNA isolation

1.5 mL of liquid culture was taken in a microtube and centrifuged at 6000×*g* for 10 min at 4 °C. 1.5 mL of liquid culture was added to the pellet and re-centrifuged at 6000×*g* for 10 min at 4 °C. The supernatant was discarded and the pellet was added with 500 µL TE buffer pH 8 and 40 µL Lysozyme (60 mg/mL), then incubated at 37 °C for 60 min. After incubation, 200 µL 10 % SDS, 100 µL 5 M NaCl, 80

μL 10 % CTAB were added to a microtube and then incubated at 60 °C for 30 min (invert microtube every 10 min). 700 μL of chloroform 1:1 was added and centrifuged at 13000×*g* for 10 min at 4 °C. The supernatant was transferred to a new microtube and added 0.6× volume of isopropanol, then incubated for 2 h at −20 °C. After incubation, the microtube was centrifuged at 13000×*g* for 10 min, at 4 °C. The supernatant was discharged. Pellets were added with 1 mL of ethanol 70 % and centrifuged at 13000 ×*g* for 10 min, 4 °C. The supernatant was discarded and the pellet is dried overnight. The pellets were added 30 μL ddH2O and 5 μL RNAse (1 mg/mL) and then incubated at 37 °C for 60 min. After that, the results of DNA isolation were stored at a low temperature to avoid DNA degradation [10] and analyzed with gel electrophoresis on 2 % (w/v) agarose gel by using ×TAE buffer. Then the gel was stained in ethidium bromide solution and using a UV transilluminator to analyze it.

16S rRNA PCR and sequencing analysis

PCR16S rRNA is a step to identify LAB strains using a 16S rRNA universal primer. The primer sequences used are 8F (5'-AGAGTTTGATCATGGCTCAG-3') and 15R (5'-AAGGAGGTGATCCAACCGCA-3'). Positions 1541 to 1522 bp are used to amplify the overall fragment length of 16S rRNA bacteria [11]. The reaction mixture of PCR includes ddH_2O 38.5 μL, 5x MyTaq Green 7.5 μL, 1 μL of each primer, DNA templates (LAB genome) 2 μL so that the total volume is 50 μL. The PCR conditions were 96 °C for 5 min; 35 cycles consisting of 96 °C for 1 min, 55 °C for 30 s, and 72 °C for 1 min; and 72 °C for 7 min. The PCR products were subjected to electrophoresis gel on 1 % agarose gel, followed by ethidium bromide staining. Sequencing results were analyzed using the Basic Local Alignment Search Tool (BLAST) in the NCBI and MegaX software.

Data analysis

The test was carried out with a completely randomized design (CRD) with 3 replications. Data were tested by Analysis of variance (ANOVA) with a confidence level of .05 and SPSS 24.0 software.

Result and discussion

Morphological characterization of LAB from Sumbawa white honey

The LAB isolation confirmed some bacteria presenced in the samples, and the high survival LAB was identified by the existence of colonies that grow even at 10^{-5} dilutions (**Figure 1**). Unlike other bacteria, the presence of LAB is generally not pathogenic. Olofsson *et al*. [12] stated that honey bees have formed an interaction with some microbial communities and this can be found in all parts of the body, especially the gut (gut microbiota), hive, and honey bees. Although honey is known to have antimicrobial activity, some microbes can adapt and survive in honey, especially LAB [13]. European honey bees (*Apis mellifera*) are known to be host microbial communities which mostly consist of 3 main phyla (*Firmicutes, Proteobacteria,* and *Actinobacteria*) [14]. The presence of these bacteria is thought to have originated from flowers visited by bees [15]. *Lactobacillus* sp. is an example of bacteria associated with pollen flowers that can be found in honey [16]. The characteristics of honey which have a high sugar content plus a pH ranging from 3.2 - 4.5 make pathogenic bacteria and fungi impossible to grow [17]. When compared with conditions in fermented foods, this characteristic of honey is a suitable environment for several lactic acid bacteria that require a low pH environment [18]. Furthermore, several LAB species are known to be capable of producing bioactive compounds such as organic acids, hydrogen peroxide, diacetyl, bacteriocins, antimicrobial peptides, and antibiotics.

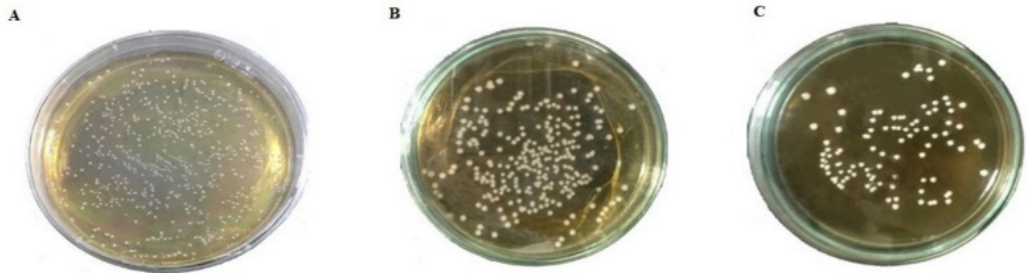

Figure 1 Colonies of isolated bacteria grown on MRS medium in order from several dilutions. A) 10^{-3} dilution; B) 10^{-4} dilution; C) dilution 10^{-5}.

Some bacteria that grow from isolation are believed to be lactic acid bacteria. The use of MRS medium selectively eliminates the growth of bacteria other than LAB due to low pH conditions plus the presence of inhibitors such as sorbic acid [19,20]. From the screening results, 35 bacterial isolates were obtained. Morphological analysis was carried out to determine the characteristics of each isolate, and the results showed that all isolates had the same colony characteristics. Further testing was carried out to determine the microscopic appearance of the morphology of each isolate. Gram staining showed that the isolates consistently showed relatively the same morphological characteristics, gram-positive in the form of rods. The morphological appearance of the 35 isolates selected as the desired LAB candidates is shown in **Table 1**.

Microscopic observation strengthens the test using MRS medium for the selection of LAB in honey samples. In general, LAB has characteristics; Gram-positive, non--spore-forming, stem and cocci shaped, and catalase-negative [21,22]. However, further testing needs to be done to identify all the isolates obtained.

Table 1 Morphological test results on bacteria from Sumbawa white honey.

Isolate species	Bacteria shape	Color	Colony shape	Margin	Isolate species	Bacteria shape	Color	Colony shape	Margin
1	Cocci	White	Circular	Entire	19	Cocci	White	Circular	Entire
2	Cocci	White	Circular	Entire	20	Cocci	White	Circular	Entire
3	Cocci	White	Circular	Entire	21	Cocci	White	Circular	Entire
4	Cocci	White	Circular	Entire	22	Cocci	White	Circular	Entire
5	Cocci	White	Circular	Entire	23	Cocci	White	Circular	Entire
6	Cocci	White	Circular	Entire	24	Cocci	White	Circular	Entire
7	Cocci	White	Circular	Entire	25	Cocci	White	Circular	Entire
8	Cocci	White	Circular	Entire	26	Cocci	White	Circular	Entire
9	Cocci	White	Circular	Entire	27	Cocci	White	Circular	Entire
10	Cocci	White	Circular	Entire	28	Cocci	White	Circular	Entire
11	Cocci	White	Circular	Entire	29	Cocci	White	Circular	Entire
12	Cocci	White	Circular	Entire	30	Cocci	White	Circular	Entire
13	Cocci	White	Circular	Entire	31	Cocci	White	Circular	Entire
14	Cocci	White	Circular	Entire	32	Cocci	White	Circular	Entire
15	Cocci	White	Circular	Entire	33	Cocci	White	Circular	Entire
16	Cocci	White	Circular	Entire	34	Cocci	White	Circular	Entire
17	Cocci	White	Circular	Entire	35	Cocci	White	Circular	Entire
18	Cocci	White	Circular	Entire					

Physiological characterization of LAB from Sumbawa white honey

Physiological tests of all isolates mostly showed the biochemical characteristics of LAB. Catalase testing is carried out to determine the ability of bacteria to produce the catalase enzyme which plays a role in the hydrolysis of hydrogen peroxide. The enzyme is produced by aeoro-tolerant bacteria. Stiles and Holzapfel [22] stated that one of the characteristics of LAB is a false negative, the enzyme produced is not catalase but peroxidase. However, from the test results obtained, some isolates showed catalase activity (**Table 2**). Whittenbury [23] stated that some LAB species such as *Lactobacillus, Leuconostoc, Streptococcus,* and *Pediococcus* showed catalase activity. It is lead by their ability to synthesize apoenzyme, but unable for catalase prosthetic groups. Some other LAB groups were also reported to have the ability in producing pseudo catalase activity.

Table 2 The result of physiological tests on bacteria of Sumbawa white honey.

Isolates Code	Gram staining	Catalase test	Methyl red	Isolates code	Gram staining	Catalase test	Methyl red
01	+	+	+	19	+	−	−
02	+	−	+	20	+	−	+
03	+	−	+	21	+	+	+
04	+	+	−	22	+	−	−
05	+	+	+	23	+	−	+
06	+	+	+	24	+	−	+
07	+	−	+	25	+	−	+
08	+	+	+	26	+	−	+
09	+	−	+	27	+	−	+
10	+	−	+	28	+	−	+
11	+	−	−	29	+	−	+
12	+	+	+	30	+	−	−
13	+	−	+	31	+	+	+
14	+	+	+	32	+	−	−
15	+	−	+	33	+	+	+
16	+	+	+	34	+	−	−
17	+	−	+	35	+	+	+
18	+	+	−				

Glucose fermentation activity by LAB was detected using the methyl red test. The basic principle of the test is to detect glucose degradation products during fermentation. Some bacteria can utilize glucose and convert it into stable acids such as lactic acid, acetic acid, or formic acid as end products. The initial stage of this process is the conversion of glucose to pyruvic acid, which is then metabolized via the mixed acid pathway to produce a stable acid. The type of acid produced is different for each species, this depends on the specific enzymatic pathway present in bacteria. The resulting acid lowers the pH to 4.5 or lower, which is indicated by the color change of methyl red from yellow to red. The test in this study showed that almost all isolates gave positive results, while 8 isolates showed negative results. This indicates that the LAB species obtained from Sumbawa white honey are able to utilize the glucose molecule to form various stable acid compounds. As a composition, honey contains 4 types of sugar, namely, glucose, fructose, sucrose, and maltose [18,24]. Although it can utilize glucose as a carbon source, it is suspected that the fermentation activity by LAB is not as efficient as other fermentation products, considering that some components in honey are known to be bacteriostatic and possibly have an effect in inhibiting bacterial fermentation activity. However, this is sufficient to produce organic acids and lactic acid components which not only add to the distinctive taste of honey, especially Sumbawa honey but also possibly give additional properties to honey with bioactive activities.

Molecular identification and clustering of phylogenetic trees of LAB in Sumbawa white honey

Among 35 LAB candidates based on morphological and physiological analysis, 5 isolates were selected (Isolate-03, Isolate-07, Isolate-09, Isolate-20, and Isolate-24) which would be further analyzed. The analysis is in the form of molecular identification as well as secondary metabolite production, and pathogenic antibacterial activity tests of the resulting LAB secondary metabolites. The molecular analysis begins with the isolation of the bacterial genome, followed by detection with 16S rRNA, sequencing analysis, and analysis of phylogenetic trees. Following are the results of PCR detection on 5 randomly selected isolates (**Figure 2**).

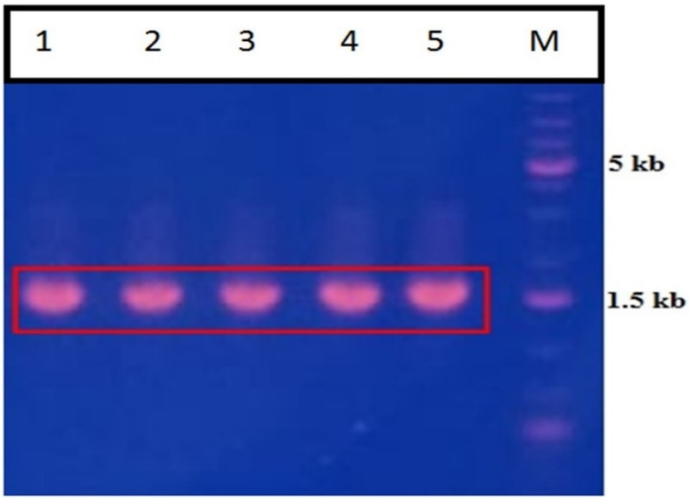

Figure 2 PCR results for 16S rRNA 5 selected isolates (Isolate-03: 1, Isolate-07: 2, Isolate-09: 3, Isolate 20: 4, Isolate24: 5 and M: DNA Marker)

A further test is LAB genome sequencing to determine the specific type of isolate by 16s rRNA bacterial marker. Based on the results of sequence analysis, it was found that the 5 selected isolates were *Enterococcus faecium*, and gave different strains from each selected LAB genome (**Table 3**). Furthermore, phylogenetic analysis was carried out to see the closeness of the selected isolates from other strains. Taken together, the identified LAB strains in this study have to be proven on the next parameter analysis to support the previous study that suggested various strains of LAB could produce bacteriocins or antibacterial proteins on pathogenic microbes, and not limited to specific strain [25]. The presence of some LAB in honey products is thought to have originated from the polluting activity and this has formed an association with honey bees throughout evolutionary history. It appears that the honey bee and plants form a co-evolution. LAB obtains a niche where nutrients are available, and honey bees receive protection from pathogenic microbes [26].

Table 3 The results of 16S rRNA molecular identification.

No	Isolates code	Species (strain)	Identity	Accession number
1	Isolate-03	*Enterococcus faecium* Strain BagHom4	97.29 %	KU324920.1
2	Isolate-07	*Enterococcus faecium* Strain CAU9510	97.36 %	MF108201.1
3	Isolate-9	*Enterococcus faecium* Strain VVEswe-R	97.73 %	CP041261.3
4	Isolate-20	*Enterococcus faecium* Strain AA4	96.40 %	MN511819.1
5	Isolate-24	*Enterococcus faecium* Strain gp21	98.61 %	KM495938.1

Moreover, Olofson *et al.* [13] stated that the diversity of LAB in bees did not come from flowers but from the gut microbiota, which was found in phylotypes of 6 species including the genus *Lactobacillus* and 4 species including *Bifidobacterium*. Environmental habitats may also facilitate the transfer of bacteria across the colony. The environments that are behaviorally connected, such as the nurse gut and the brood cells, may act as microbial hubs through which honey bees obtain, deposit, and propagate LAB within the hive to the next generation. Rokop *et al.* [15] stated that based on the results of the 16S rRNA analysis, some associated with honey bees and possibly transferred to honey and other products produced by bees, all belong to the *Pasteurelaceae, Lactobacillus,* and *Bifidobacteriaceae*.

The existence of various LAB strains in Sumbawa white honey seems to be influenced by the natural habitat of plants which are a source of nectar for bees, and indirectly this is thought to have contributed greatly to the diversity of lactic acid compounds and the products of LAB excretion in Sumbawa white honey. The correlated microbiome diversity in the Sumbawa white honey bee and preserved in the resulting honey product has the potential for further development to determine the efficacy of the fermented LAB isolate compounds obtained.

Figure 3 Results of phylogenetic tree analysis of 5 selected isolates.

Production of secondary metabolites of LAB from Sumbawa white honey

Some isolates were selected based on the results of biochemical and physiological tests reproduced on the MSA broth medium. Cultivation carried out at 37 °C for 3 days with a shaking rate of 1 g is expected to be able to produce lactic acid products during fermentation. Some organic acids will be released into the medium during the fermentation process, then testing for antibacterial activity can be carried out using a fermented supernatant. Abdel-Rahman *et al*. [27] stated that the optimal conditions for LAB growth in the fermentation stage depend on the procedure used, but in general LAB can grow in the pH range 3.5 - 10 and temperatures of 5 - 45 °C. Based on the final product produced, LAB can be classified as homofermentative or heterofermentative. Homofermentative LAB has aldolase and produces lactic acid as the main end product. Whereas heterofermentative LAB produces side products other than lactic acid, therefore the maximum yield of lactic acid to glucose only reaches 0.5 g/g or 1.0 mol/mol [27].

Heterofermentative LAB uses the pentose monophosphate pathway, this pathway converts 6-carbon sugar (hexose) into carbonated sugar (pentose) and carbon dioxide which is catalyzed by several enzymes. Then, the resulting pentose is broken down into glyceraldehyde 3-phosphate and acetyl phosphate by phosphocytolase. Most heterofermentative LAB strains convert pentose sugar into lactic acid and by-products (for example, acetic acid) through the phosphocytolase pathway with lactic acid yield [27]. Reddy *et al*. [28] stated that most LAB species require complex nutrients, including vitamins, minerals, amino acids, peptides, and nucleotides to support their growth because LAB does not have varied biosynthetic capabilities. This is thought to be correlated with why LAB species can grow on honey, especially Sumbawa white honey, considering that honey grains a large number of components both minerals and vitamins as well as various sources of sugar. LAB can produce lactic acid as a result of the fermentation of food materials such as sugar and carbohydrates. Besides producing lactic acid, LAB is also known to be able to produce antimicrobial compounds such as bacteriocins, hydrogen peroxide, and organic acids such as citric acid and lactic acid. Bacteriocins are compounds used as preservatives, while the acetic acid, lactic acid, and propionic acid are antimicrobial agents that are widely used in the food industry.

Antibacterial bioprospection of secondary metabolite compounds of LAB against food pathogenic bacteria

LAB can inhibit the growth of pathogenic bacteria, both as probiotics and through the resulting fermentation products which have roles as bacteriostatics and bacteriocides. Shamala *et al*. [29] stated that LAB activity as a probiotic is related to competition for adhesion receptors in the intestinal epithelium, competition for nutrients, antibacterial production and stimulation of the immune system. Lactic acid bacteria that grow in the intestine produce metabolites that will inhibit the growth of pathogens. Various organic acids produced also reduce intestinal pH which will inhibit other pathogenic bacteria from colonizing the intestine.

Supernatant testing of 5 selected LAB isolates was carried out on pathogenic microbes to see their effectiveness in inhibiting the growth of the tested bacteria. Some pathogenic bacteria used are common bacteria in antimicrobial testing, namely *Salmonella typhi, Staphylococcus aureus, Escherichia coli*. Besides that, testing was also carried out on the bacteria *Enterobacter ludwigii* and *Leclercia adecarboxylata*. *E. ludwigii* is known to be a common cause of infection in hospitals, apart from that it is a cause of stomach, urinary tract, and meningeal infections [30]. Meanwhile, *Leclercia adecarboxylata* is usually isolated as part of the polymicrobial culture in immunocompetent patients. Although these bacteria are generally sensitive to most antibiotics there have been several reports of antibiotic-resistant strains [31]. Through initial testing with the agar well diffusion method, it was found that in general the supernatants of the 5 isolates were able to inhibit the growth of the tested bacteria (**Table 4**).

Table 4 Inhibitory activity of pathogenic bacteria from the supernatant of selected isolates from MRS broth medium after 3 days of fermentation.

Isolate Code	Conc. (ppm)	Inhibitory Zone Diameter of Pathogenic Bacteria (mm)				
		S. thyposa	***S. aureus***	***E. coli***	***E. ludwigii***	***L.adecarboxylata***
07	20	5 ± 0.00^{b}	6.67 ± 1.15^{b}	8 ± 0.00^{b}	8 ± 0.00^{b}	5.33 ± 0.58^{b}
	40	7.67 ± 0.58^{c}	9.33 ± 0.58^{c}	9.67 ± 0.58^{c}	9.67 ± 0.58^{c}	6.33 ± 0.58^{c}
	60	11 ± 1.00^{d}	12.33 ± 0.58^{d}	13 ± 0.00^{d}	11 ± 0.00^{d}	11.33 ± 0.58^{d}
	80	12 ± 1.73^{d}	14.33 ± 1.00^{e}	14.33 ± 0.58^{e}	13 ± 0.00^{e}	13.67 ± 0.58^{e}
	100	14.67 ± 1.53^{e}	17.67 ± 0.58^{f}	18.33 ± 0.58^{f}	15 ± 0.00^{f}	18 ± 0.00^{f}
	K+	15.33 ± 1.53^{e}	19.67 ± 0.58^{g}	18.67 ± 0.58^{f}	19 ± 0.58^{g}	18 ± 0.00^{f}
	K−	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}
09	20	6 ± 0^{b}	5.00 ± 0.00^{b}	5.67 ± 0.58^{b}	5.67 ± 0.58^{b}	5.33 ± 0.58^{b}
	40	8.33 ± 0.58^{c}	7.00 ± 0.00^{c}	9.33 ± 1.15^{c}	7.67 ± 0.58^{c}	7.00 ± 0.00^{c}
	60	13.67 ± 0.58^{d}	9.33 ± 0.58^{d}	10.67 ± 0.58^{d}	9.00 ± 0.00^{d}	9.00 ± 0.00^{d}
	80	14.33 ± 0.58^{d}	14.00 ± 0.00^{e}	14.00 ± 0.00^{e}	14.33 ± 0.58^{e}	13.67 ± 0.58^{e}
	100	17.67 ± 0.58^{e}	18.67 ± 0.58^{f}	16.00 ± 1.00^{f}	18.33 ± 0.58^{f}	17.67 ± 0.58^{f}
	K+	18 ± 0^{e}	19.00 ± 0.00^{f}	18.00 ± 0.00^{g}	18.00 ± 0.00^{f}	18.00 ± 0.00^{f}
	K−	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
13	20	5 ± 0^{b}	5.33 ± 0.58^{b}	5 ± 0^{b}	5 ± 0^{b}	$5.00 \pm 0{,}00^{b}$
	40	7.67 ± 0.58^{c}	7.67 ± 1.15^{c}	6 ± 0^{c}	7.67 ± 0.58^{c}	5.67 ± 0.58^{c}
	60	11.67 ± 0.58^{d}	12.00 ± 1.00^{d}	6.33 ± 0.58^{c}	11.67 ± 0.58^{d}	8.00 ± 0.00^{d}
	80	12.67 ± 0.58^{e}	14.67 ± 0.58^{e}	8.67 ± 0.58^{d}	13 ± 0^{e}	8.67 ± 0.58^{e}
	100	12.67 ± 0.58^{e}	18.67 ± 0.58^{f}	11.33 ± 1.15^{e}	16.33 ± 0.58^{f}	12.00 ± 0.00^{f}
	K+	18.33 ± 0.58^{f}	19.67 ± 0.58^{f}	18 ± 0^{f}	18.33 ± 0.58^{g}	18.00 ± 0.00^{g}
	K−	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
20	20	5 ± 0^{b}	5.33 ± 0.58^{b}	5.67 ± 1.15^{b}	6.33 ± 0.58^{b}	6.33 ± 2.31^{b}
	40	7.67 ± 0.58^{c}	8.00 ± 0.00^{c}	8.67 ± 2.89^{c}	8.67 ± 0.58^{bc}	8.00 ± 1.73^{bc}
	60	9.67 ± 0.58^{d}	11.67 ± 0.58^{d}	13.67 ± 0.58^{d}	13 ± 0^{bcd}	11.33 ± 0.58^{bcd}
	80	14 ± 0^{e}	14.67 ± 1.15^{e}	16.33 ± 0.58^{e}	14.33 ± 0.58^{cd}	13.00 ± 0.00^{cd}
	100	16.67 ± 1.15^{f}	18.00 ± 0.00^{f}	18.33 ± 0.58^{e}	18.67 ± 0.58^{de}	16.33 ± 1.53^{d}
	K+	19.33 ± 0.58^{g}	18.33 ± 0.58^{f}	18.33 ± 0.58^{e}	9.67 ± 7.23^{e}	14.00 ± 6.93^{d}
	K−	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
24	20	5 ± 0^{b}	6.67 ± 1.15^{b}	5.33 ± 0.58^{b}	5 ± 0^{b}	5.33 ± 0.58^{b}
	40	7.33 ± 0.58^{c}	8.00 ± 0.00^{b}	6.33 ± 0.58^{c}	7.33 ± 0.58^{c}	8.67 ± 0.58^{c}
	60	9.67 ± 0.58^{d}	9.00 ± 0.00^{bc}	8.67 ± 0.58^{d}	12 ± 1.73^{d}	13.00 ± 0.00^{d}
	80	13 ± 0^{e}	13.67 ± 0.58^{cd}	13.67 ± 0.58^{e}	13 ± 0^{d}	13.67 ± 0.58^{d}
	100	14.33 ± 0.58^{f}	17.67 ± 0.58^{d}	17.67 ± 0.58^{f}	15.67 ± 1.15^{e}	17.67 ± 0.58^{e}
	K+	18 ± 0^{g}	14.00 ± 6.93^{d}	18 ± 0^{f}	18.33 ± 0.58^{f}	18.00 ± 0^{e}
	K−	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}

Numbers followed by the same letter notation indicate no significant difference based on Duncan's test at $\alpha = 0.05$ and the number of replications = 3. Conc.: Concentration; K+: Positive control; K−: Negative control.

Statistical analysis showed that the whole supernatant tested at a concentration of 20 % (supernatant / sterile distilled water, v/v) was significantly different from the negative control ($p < 0.05$), with the range of inhibition in the test medium as indicated by a burning zone of 5 mm. This result is relative to the positive control, but at a concentration of 100 % almost all the samples tested had the same inhibitory activity as the positive control and were considered statistically not significantly different ($p > 0.05$). The antimicrobial activity of LAB is due to the production of various compounds that can inhibit the growth of pathogenic bacteria such as lactic acid, hydrogen peroxide (H_2O_2), carbon dioxide, diacetil, and bacteriocins**.** The mechanism of inhibiting the growth of pathogenic bacteria by LAB is through a decrease in pH caused by the secretion of organic acid compounds**.** Organic acid molecules can enter through the cell membrane of pathogenic bacteria to change the permeability of the cell membrane and cause protein instability in cells and the material transport system in pathogenic bacteria is still disrupted**.**

H_2O_2 produced by LAB can cause denaturation of enzymes in pathogenic bacteria and produce free radicals such as superoxide and hydroxyl which can damage DNA. In addition, H_2O_2 can oxidize sulfhydryl groups which cause membrane lipid peroxidation, resulting in increased membrane permeability and as a precursor for the production of bactericidal free radicals [8]. Lactic acid has antimicrobial properties because it can interfere with cell membrane work, inhibit transport activity, reduce intracellular pH and inhibit various metabolic functions [32]. Bacteriocins in LAB can change membrane permeability so that they interfere with membrane transport or eliminate proton movement which results in inhibition of energy production and protein or nucleic acid biosynthesis [33]. The antimicrobial compounds produced by LAB such as those produced by *L. plantarum* NS are reported to be able to inhibit the growth of food pathogens such as *E. coli*, *B. cereus*, *L. monocytogenes, S. aureus,* and *S. thypimurium* [5].

Conclusions

In conclusion, this study revealed a potential LAB isolated from Sumbawa White Honey as antimicrobial compounds against pathogens. The 16s rRNA analysis identified *Enterococcus faecium* species with identity above 90 % of 5 isolated LAB. The species identified could ferment carbon sources present in honey, and this data indicated a potential source LAB in the raw material food for alternative antibacterial production. Further improvement is also needed to analyze the metabolite active compound and its activity for antioxidant, antidiabetic, anticancer activity since LAB in raw food is an important source for alternative therapy.

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