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METABOLITE ACTIVITY OF LACTIC ACID BACTERIA FROM SUMBAWA HORSE MILK, WEST NUSA TENGGARA, INDONESIA

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ABSTRACT

Sumbawa horse milk is one of the local products in Sumbawa (West Nusa Tenggara). LAB strains from Sumbawa horse milk have the potential to be used as antimicrobial and probiotics for type 2 diabetes mellitus patients. So far there has been no research that discusses the potential extract of LAB metabolite compounds from Sumbawa horse milk as antimicrobials and probiotics for sufferers of type 2 diabetes mellitus. This study aims to obtain an antimicrobial activity test and test the inhibitory activity of the α-glucosidase enzyme by extracting LAB metabolite compounds that have been successfully isolated and identified from Sumbawa horse milk. There are tree methods in research that preparation of LAB metabolite extract, antimicrobial activity test and α-glucosidase inhibition activity test. The result antimicrobial activity test has found that metabolites extracted from LAB SKP K.9 (*Enterococcus thailandicus*) have the highest antimicrobial activity against *S. aureus* (ATCC 6538) (2.5 cm), *S. typhimurium* (2.5 cm), *L. monocytogenes* (BTCC B693) (2.53 cm) and EPEC K.1.1 (2.7 cm). The extract of LAB SK 1.5 metabolite compound (*Enterococcus faecium*) has the highest inhibitory activity against the α-glucosidase enzyme (78.57%) compared to other LAB isolates. All the extracts of LAB metabolites from Sumbawa horse milk can inhibit the growth of pathogenic bacteria and inhibit the α-glucosidase enzymes with various abilities. Therefore, the potency of LAB strains from Sumbawa horse milk can be an alternative to antimicrobial agents and probiotics for treating Diabetes Mellitus Type 2 through α-glucosidase inhibition.

Introduction

Sumbawa horse milk is one of the local products in Sumbawa. The results extraction of active compounds from Sumbawa horse milk produced an organic compound as protein (galactoequin or galactoferrin) that has strong antimicrobial activity with bacteria pathogens (Hermawati, 2005). Also,

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Sumbawa horse milk has a LAB that can ferment milk into acids and produce antimicrobial compounds such as bacteriocin, lactic acid, and organic acids (acetate and hydrogen peroxide) (Sujaya et al., 2008; Zacharof and Lovitt, 2012; Hakim et al., 2013). Sumbawa horse milk contains several types of LAB (*Lactobacillus bulgaricus*, *Streptococcus lactis*, *Tarula* sp., and as yet unidentified). LAB isolated from Sumbawa horse milk has an antimicrobial activity to *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, and *Shigella flexneri* (Manguntungi et al., 2008; Sujaya et al., 2008). Also, LAB contained in Sumbawa horse milk such as *L. rhamnosus* SKG34 and *L. rhamnosus* SKG49 has the potential to be developed into probiotics (Sujaya et al., 2008). Increasing the amount of LAB in Sumbawa horse milk is caused by variations in storage duration so that the milk has a sour taste (Hakim et al., 2013).

LAB belongs to Gram-positive bacteria that can anaerobically with no spores characteristic, and they can produce lactic acid during the fermentation process (Mustopa et al., 2010). LAB has been used for thousands of years in the fermented food production process where LAB contributes to the taste, quality, texture, and safety of these products (Mayo et al., 2010). LAB is capable of producing natural preservatives in the form of bacteriocin (Da-Silva et al., 2014). The use of LAB and its metabolites are GRAS (generally considered safe or generally recognized). Also, antimicrobial compounds of LAB to food spoilage-causing bacteria have been proven beneficial (Zacharof and Lovitt, 2012).

LAB has the potential as a probiotic for type 2 diabetes mellitus patients because of consumption probiotic can reduce glucose and diabetes in patients (Yun et al., 2009; Farida et al., 2019). *Bifidobacteria*, *Lactobacilli*, *Lactococci*, and *Streptococci* are the most common LAB bacteria applied as probiotics (He and Shi, 2017). Several species and strains of *Lactobacilli*, including *L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. helveticus*, have been extensively studied in terms of their purpose in preventive medicine (Azad et al., 2018). Some research on reducing the consumption of probiotics can reduce cholesterol levels and expenditure on diabetics is the study of Yun et al. (2009), about giving probiotics to animal models (mice) which shows that LAB (*Lactobacillus*) is effective for preventing diabetes. Research by Sari et al. (2017), particularly the administration of *L. casei* fermented milk as diabetes therapy in mice can reduce blood calcium levels. Research by Sangwan and Singh (2014) also reports that the consumption of significant probiotic fermented milk of LGG and *L. casei* NCDC 19 supports various factors that require type 2 diabetes. Research by Farida et al. (2019), is related to research on the consumption of LAB metabolites (*L. fermentum* S21209), *L. plantarum* MB427, *L. plantarum* Pi28a, *L. delbrueckii* W24802, and *L. plantarum* 2W22409) have inhibitory activity against the α-glucosidase enzyme.

Previous research succeeded in getting Sumbawa horse milk collected from Penyaring village and Lennanguar village contains several types of LAB i.e: SK 1.5, SKP K.3, SKP K.5, SKP K.9/SKP K.7/M.SKP K.3, SKL K.4, M.SKL K.1/ M.SKL K.5, and SKP K.4 belong to the species of *Enterococcus faecium*, *Weissella confusa*, *Lactococcus garvieae*, *Enterococcus thailandicus*, *Lactobacillus fermentum*, *Enterococcus faecalis*, and *Lactococcus petauri* (Kusdianawati et al., 2020). The identification of LAB from Sumbawa horse milk successfully identified three closely related type strains of bacteria from *Enterococcus* sp. (M.SKP.K3), *Lactococcus garvieae* (SKP K.5), and *Lactococcus petauri* (SKP K.4) as a novel bacteria in Sumbawa horse milk compared to the previous findings reported by Mulyawati 2019 and Sujaya et al. 2008 (Kusdianawati et al., 2020). LAB strains from Sumbawa horse milk have the potential to be used as antimicrobials and probiotics for

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type 2 diabetes mellitus patients. So far there has been no research that discusses the potential extract of LAB metabolite compounds from Sumbawa horse milk as antimicrobials and probiotics for sufferers of type 2 diabetes mellitus. Therefore, this study aims to conduct an antimicrobial activity test and inhibitory activity test of the α-glucosidase enzyme from extracting LAB metabolite compounds from Sumbawa horse milk.

Materials and Methods

Tools and Materials

The tools used in this study are an incubator, shaker incubator, centrifuge machine (Hitachi, CR 21GIII), rotary evaporators (Hei-VAP Core, Rotary Evaporators, Heidolph), freeze dryers (CoolSafe 4-15L), petri dish, test tube, micropipette, ELISA reader (Thermo).

The materials used in this study are de Man Rogosa Sharpe agar (MRS) media, nutrient broth, agar bacteriological, glucose, ethanol, paper disc (Filtres Fioroni, France), alcohol 70% and 95%, α-glucosidase enzyme, acarbose, M phosphate buffer, PNPG substrate, ampicillin antibiotics, bacteria pathogen (*S. aureus* (ATCC 6538), *S. typhimurium*, *L. monocytogenes* (BTCC B693), and EPEC K.1.1), and LAB isolates from Sumbawa horse milk. The main ingredients in the form of LAB isolate from Sumbawa horse milk SK 1.5, SKP K.3, SKP K.5, SKP K.9, SKL K.4. The LAB isolate was isolated from two different villages from Sumbawa Regency. LAB isolates SK 1.5, SKP K.3, SKP K.5, and SKP K.9 originated from horse milk in the Penyaring village. While LAB SKL K.4 isolates were from horse milk in Lenangguar village. The 5 species of LAB isolates from Sumbawa horse milk (Table 1).

Table 1. LAB isolate Sumbawa horse milk species

No	Isolate	Species	Identity	Accession number
1.	SK 1.5	*Enterococcus faecium*	99,86%	CP032308.1
2.	SKP K.3	*Weissella confusa*	99,39%	MK818759.1
3.	SKP K.5	*Lactococcus garvieae*	99,51%	LC376029.1
4.	SKP K.9	*Enterococcus thailandicus*	100%	AA00296
5.	SKL K.4	*Lactobacillus fermentum*	99,93%	KY249642.1

Sources : Kusdianawati et al., 2020

Methods

Preparation of LAB metabolite extract

LAB isolates SK 1.5, SKP K.3, SKP K.5, and SKP K.9 originated from horse milk in Penyaring village, while isolates SKL K.4 originated from horse milk in Lenangguar village. All species of LAB isolates from Sumbawa horse milk were identified (Kusdianawati et al., 2020) and prepared for the extraction of their metabolite compounds then used for testing antimicrobial activity and inhibition of the α-glucosidase enzyme. The preparation of extracts metabolite LAB method was done according to a previous study by Baipai et al. (2016) with modification. LAB isolates (SK 1.5, SKP K.3, SKP K.5, SKP K.9, SKL K.4) were grown in MRS media and incubated at 28 and 37°C for 24 hours under anaerobic conditions. Then as many as 0.25 mL cultures were grown to 25 mL of MRS media (5% glucose) and incubated at 28 and 37 °C for 38 hours. The LAB isolate culture was then extracted using 50 mL of 96% ethanol and incubated using a shaker incubator for 4 hours. LAB isolates were centrifuged (Centrifuge Machine, Hitachi, CR 21GIII) at 5000 rpm at 4 °C for 30 minutes to obtain supernatant and pellets. The supernatant taken for evaporation uses rotary evaporators (Hei-VAP Core, Rotary Evaporators, Heidolph) at 40 °C. LAB extract produced from the evaporation results is then followed by freeze-drying (CoolSafe 4-15L

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Freeze Dryers) to produce extracts of LAB metabolite compounds.

Antimicrobial activity test

Antimicrobial activity tests using the diffusion method use a paper disc (Arief et al., 2013; Mustopa et al., 2018). This method is based on the principle that metabolite extract-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture, and the extract metabolite LAB diffuses radially outward through the agar medium producing a metabolite LAB concentration gradient. An antimicrobial activity using *S. aureus* (ATCC 6538), *S. typhimurium*, *L. monocytogenes* (BTCC B693), and EPEC K.1.1. The pathogen bacteria was added to NB for 24 hours, incubated at 37 °C with a shaker incubator. The concentration of pathogenic bacteria is 1 × 10^8 CFU / mL (Mustopa et al., 2018). Then 3 mL of pathogenic bacteria are mixed with 17 mL of NA, and poured into a petri dish until solid. The extract of LAB isolate metabolite compound freeze-dried was pipetted as much as 30 µL and was spotted in a 6 mm paper disc (Filtres Fioroni, France), then incubated for 1 hour at 4 °C. Next, it was incubated at 37 °C for 24 hours. The diameters of the inhibition zones around the paper disc were then measured.

The α-glucosidase inhibition activity test

The α-glucosidase enzyme inhibitory activity was carried out based on the modification of the method of Farida et al. (2019). An *α*-glucosidase inhibitor is one of the compounds for the treatment of diabetes. The sample used was the result of the extract of LAB metabolite compounds. The positive control used in this study was in the form of acarbose. The negative control used is in the form of MRS media. Some components that will be tested consist of a sample extract of LAB metabolite compound as sample (S), control sample (S0), blank (B), and control blank (B0). The sample reaction mixture (S) consisted of 10 µL of a sample, 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL of 0.5 mM PNPG substrate, and 25 µL of α-glucosidase enzyme 0.04 U/mL inserted into the microplate. The reaction mixture for sample control (S0) without using the α-glucosidase enzyme. The reaction mixture blank (B) consisted of 50 µL 0.1 M phosphate buffer (pH 7.0), 25 µL 0.5 mM PNPG substrate, and 25 µL 0.04 U/mL α-glucosidase enzyme inserted into the microplate. The reaction mixture for the control blank (B0) without using the α-glucosidase enzyme. The reaction mixture was incubated at 37 °C for 30 minutes. The reaction was stopped by the addition of 100 µL sodium carbonate 0.2 M. Then the absorbance was measured at a wavelength of 405 nm (Elisa Reader, Thermo). The formula to calculate the percentage of inhibition LAB metabolite compound extracts against α-glucosidase enzyme is:

$$\text{α-glukosidase inhibition (\%)} = \frac{\text{K-(A1-A0)}}{\text{K}} \times 100\ \%)$$

Note:
K : Absorbance blank (B1) - Control blank (B0)
A1 : Absorbance of sample
A0 : Absorbance of sample control

Results and Discussion

Antimicrobial activity of LAB metabolite compound extract Sumbawa horse milk

Antimicrobial activity results from from extracted metabolite LAB Sumbawa horse milk showed that five samples tested inhibit pathogenic bacteria growth (Table 2). This indicates that LAB isolates produce antimicrobial compounds with an inhibition effect on the growth of pathogenic bacteria. Accordingly, Vieco-Saiz (2019) has shown that metabolites produced during LAB growth are the components that inhibited harmful microorganisms. Some LAB strains are known to produce antimicrobial peptides in the form of small proteins called bacteriocin (Da-Silva et al., 2014). This ribosomally synthesized peptide produced by LAB showed inhibitory activity toward diverse

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groups of pathogenic microorganisms (Lopetuso et al., 2019). Also, LAB can produce organic acids and hydrogen peroxide as antimicrobial components (Yahya, 2012). *L. plantarum* S34 is one of the LAB which is isolated from *Bekasam* and can produce plantaricin and has potency as a food preservative. Based on current findings this peptide can be easily degraded by proteolytic enzymes (Kusdianawati et al., 2015; Mustopa et al., 2016).

Table 2. Antimicrobial activity of LAB metabolite compound extracts

No	LAB-derived metabolite extract	Inhibitory zone (cm) 6 hours incubation			
		***Staphylococcus aureus* (ATCC 6538)**	***Salmonella typhimurium* (ATCC)**	***Listeria monocytogenes* (BTCC B693)**	**EPEC K.1.1**
1.	Ampicillin (control +)	1,2	1,4	1,13	1,26
2.	MRS (control -)	1,9	1,9	1,8	1,93
3.	*Enterococcus faecium* (SK 1.5)	2,23	2,3	2,3	2,26
4.	*Weissella confusa* (SKP K.3)	2,33	2,4	2,26	2,56
5.	*Lactococcus garvieae* (SKP K.5)	2,3	2,4	2,4	2,43
6.	*Enterococcus thailandicus* (SKP K.9)	2,5	2,5	2,53	2,7
7.	*Lactobacillus fermentum* (SKL K.4)	2,4	2,5	2,4	2,5

Note: SD (n = 3)

Based on Table 2, the extract of LAB metabolites of Sumbawa horse milk has antimicrobial activity against *S. aureus* (ATCC 6538), *S. typhimurium*, *L. monocytogenes* (BTCC B693), and EPEC K.1.1. The activity of the LAB metabolite compound extract has an optimum inhibitory zone area at the 6th hour which is greater when compared with negative controls (MRS media) and positive controls (Ampicillin antibiotics). The SKP K.9 (*E. thailandicus*) metabolite extract had the highest antimicrobial activity against the four pathogenic bacteria used, *S. aureus* (ATCC 6538) (2.5 cm), *S. typhimurium* (2.5 cm), *L. monocytogenes* (BTCC B693) (2.53 cm) and EPEC K.1.1 (2.7 cm) when compared with other LAB isolates. This is different from the study of da Silva do Nascimento et al. (2010), that *E. faecium* FAIR-E 198 produces bacteriocin which can inhibit the growth of *L. monocytogenes* bacteria. However, none of the *B. cereus* and *S. aureus* strains investigated were inhibited. *Enterococcus* isolated from human normal flora has the potential to be used as an antibacterial against *S. typhi*, *S. flexneri*, and *E. coli* bacteria (Karimae et al., 2016).

LAB SK 1.5 isolate belongs to *E. faecium* which has activity against *S. aureus* (ATCC 6538), *S. typhimurium*, *L. monocytogenes* (BTCC B693), and EPEC K.1.1 (Table 2 and Figure 1). The antimicrobial activity of this LAB has also been overserved by Hadji-Sfaxi et al. (2011) who concluded that bacteriocin-like activity was the factor that dictates the ability of *E. faecium* PC4.1 to kill pathogenic bacteria such as *Listeria* and fungi.

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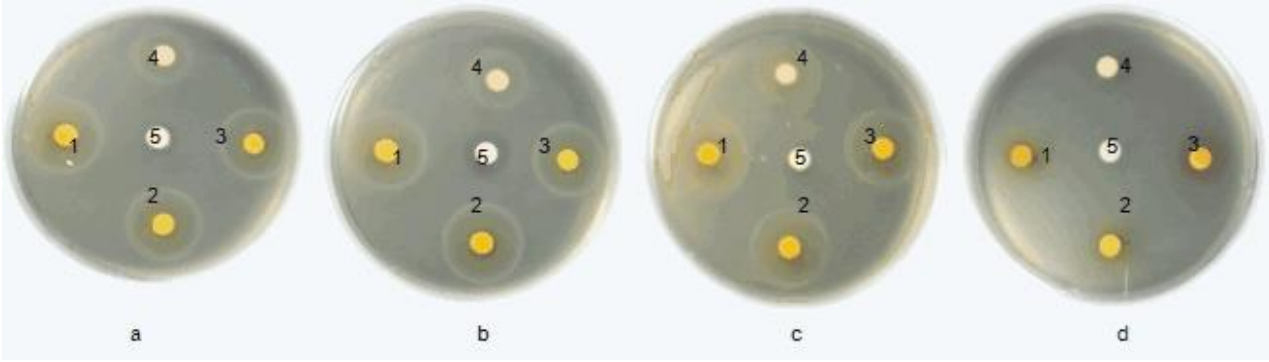

Figure 1. LAB SK 1.5 (6 hours incubation). a.) *Staphylococcus aureus* (ATCC 6538), b.) *Salmonella typhimurium* (ATCC), c.) *Listeria monocytogenes* (BTCC B693), dan d.) EPEC K.1.1. 1 to 3 : LAB-derived metabolite extract, 4: MRS medium (Negative control), 5: Ampicillin (Positive control). SD (n = 3)

LAB SKP K.3 isolate belongs to *W. confusa* which has activity against *S. aureus* (ATCC 6538), *S. typhimurium*, *L. monocytogenes* (BTCC B693), and EPEC K.1.1 (Table 2 and Figure 2). This is following the research of Shah et al. (2016), that *W. confusa* (AI10) produces antimicrobial compounds that can inhibit the bacteria *E. coli* NG 502121 and *S. aureus* AY 507047. Besides, the study of Ye et al. (2018), states that the two strains *W. viridescens* have antimicrobial activity against *L. monocytogenes*. Tenea and Lara (2019) stated that the bacterial compound *W. confusa* Cys2-2 produces bacteriocin which gives a bactericidal effect or kills the growth of Gram-negative bacteria (*E. coli* ATCC 25922, *Salmonella enterica* ATCC 51741 and *Shigella sonnei* ATCC 25931). The bacteriocin produced by *W. confusa* Cys2-2 interferes with the integrity of the target cell membrane of the bacteria which causes the death of the bacterial cell. In this study, the extract of the LAB SKP K.3 metabolite compound (*W. confusa* MK818759.1) has activity against *S. aureus*. This is different from the study of Goh and Philip (2015), in that the bacteriocin produced from the *W. confusa* A3 bacteria cannot inhibit the growth of the *S. aureus* RF122 bacteria.

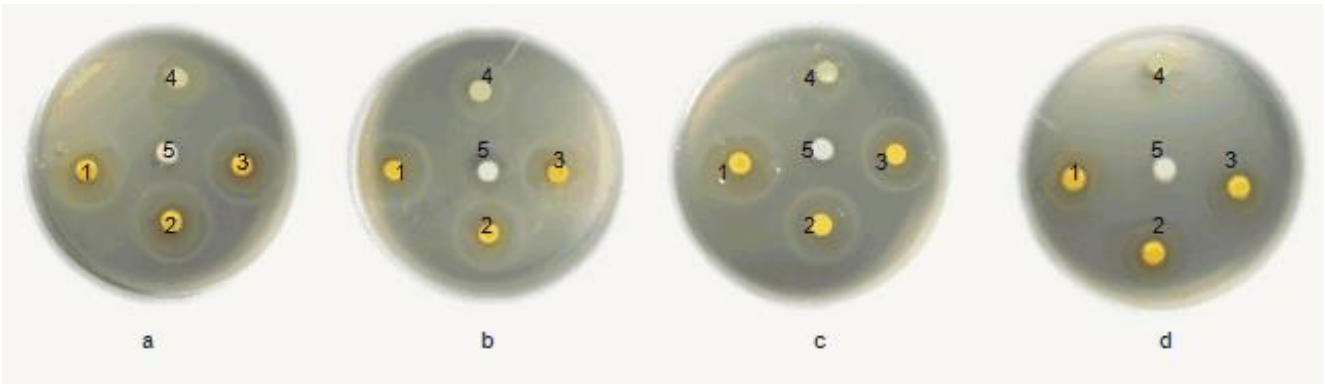

Figure 2. LAB SKP K.3 (6 hours incubation). a.) *Staphylococcus aureus* (ATCC 6538), b.) *Salmonella typhimurium* (ATCC), c.) *Listeria monocytogenes* (BTCC B693), dan d.) EPEC K.1.1. 1 to 3 : LAB-derived metabolite extract, 4 : MRS medium (Negative control), 5: Ampicillin (Positive control). SD (n = 3)

LAB SKP K.5 isolate belongs to *L. garvieae* which has activity against *S. aureus* (ATCC 6538), *S. typhimurium*, *L. monocytogenes* (BTCC B693), and EPEC K.1.1 (Table 2 and Figure 3). This is following the research of Suneel and Basappa (2013), that *L. garvieae* (LAB isolates from cow's milk) produce bacteriocin which can inhibit Gram-positive bacteria (*S. aureus*, *Bacillus subtilis*, and *Bacillus cereus*) and Gram-

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negative bacteria (*Pseudomonas aeruginosa* and *E. coli*). Supported by the research of Abdelfatah and Mahboub (2018), *L. garvieae* isolated from cow's milk produces metabolite compounds in the form of bacteriocin which plays a role in controlling the growth of *S. aureus* in Nile tilapia.

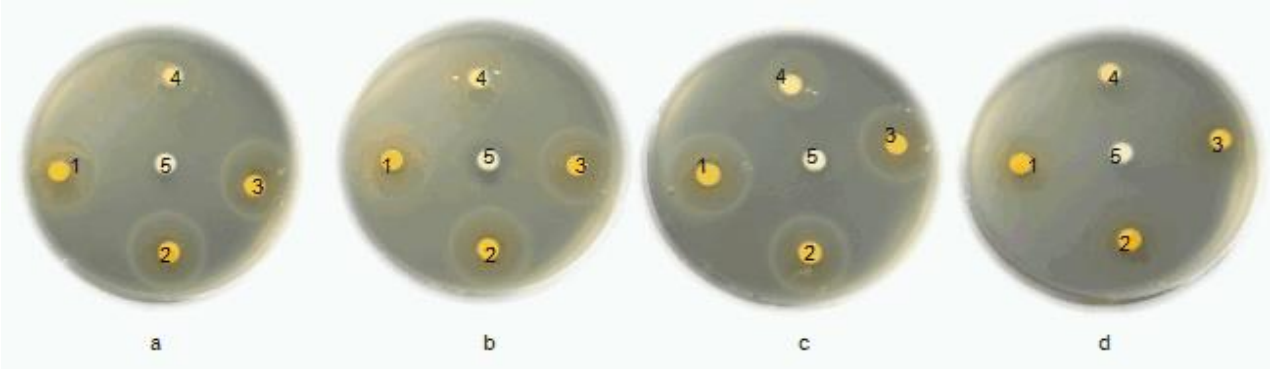

Figure 3. LAB SKP K.5 (6 hours incubation). a.) *Staphylococcus aureus* (ATCC 6538), b.) *Salmonella typhimurium* (ATCC), c.) *Listeria monocytogenes* (BTCC B693), dan d.) EPEC K.1.1. 1 to 3 : LAB-derived metabolite extract, 4 : MRS medium (Negative control), 5: Ampicillin (Positive control). SD (n = 3)

LAB SKP K.9 isolate belongs to *E. thailandicus* which has activity inhibition *S. aureus* (ATCC 6538), *S. typhimurium*, *L. monocytogenes* (BTCC B693), and EPEC K.1.1.1 (Table 2 and Figure 4). *E. thailandicus* is a lactic acid bacteria (Gram-positive) that belongs to the genus *Enterococcus*. *Enterococcus* is usually isolated from livestock products such as cheese and fresh milk (Morandi et al., 2013; Elmoslih et al., 2017). *Enterococcus* is not recommended for GRAS status. Despite the recent advances in molecular biology research and recommended methods for the safety of *Enterococcus* strain evaluations, there are differences between commercial and clinical clades (Hanchi et al., 2018).

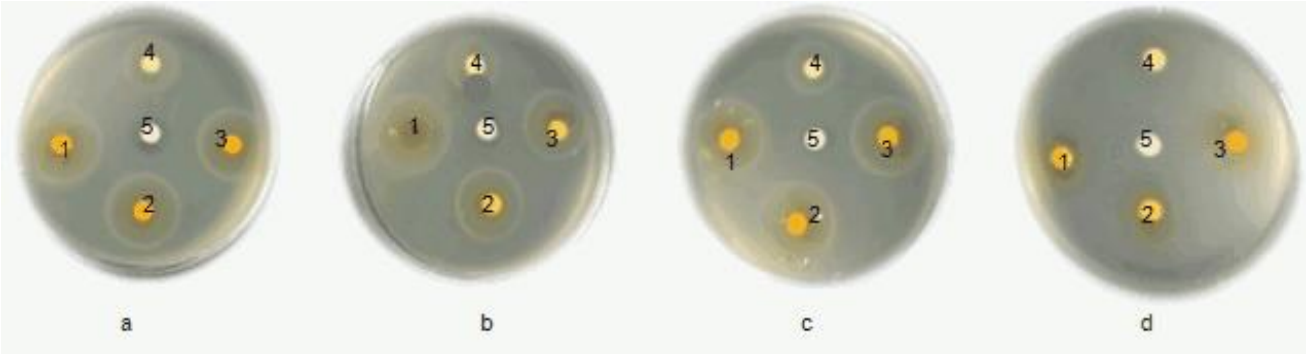

Figure 4. LAB SKP K.9 (6 hours incubation). a.) *Staphylococcus aureus* (ATCC 6538), b.) *Salmonella typhimurium* (ATCC), c.) *Listeria monocytogenes* (BTCC B693), dan d.) EPEC K.1.1. 1 to 3 : LAB-derived metabolite extract, 4 : MRS medium (Negative control), 5: Ampicillin (Positive control). SD (n = 3)

LAB SKL K.4 isolate belongs to *L. fermentum* which has activity inhibition *S. aureus* (ATCC 6538), *S. typhimurium*, *L. monocytogenes* (BTCC B693), and EPEC K.1.1 (Table 2 and Figure 5). Supported by research Jong-Su et al. (2015), that *L. fermentum* (isolate from Healthy Elderly Korean) has the function of antimicrobial activity to inhibit the growth of six intestinal pathogens (*E. coli* O157: H7, *S. enterica* subsp. *enterica Typhimurium*, *S. enterica* subsp. *enterica Enteritidis*, *E. faecalis*, *S.*

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aureus, and *L. monocytogenes*). This isolate, designated as *L. fermentum* PL9988, has all the characteristics of a good probiotic. *L. fermentum* 1 (cell-free supernatant) has growth-inhibitory activity in *S. aureus* bacteria (Georgieva et al., 2015).

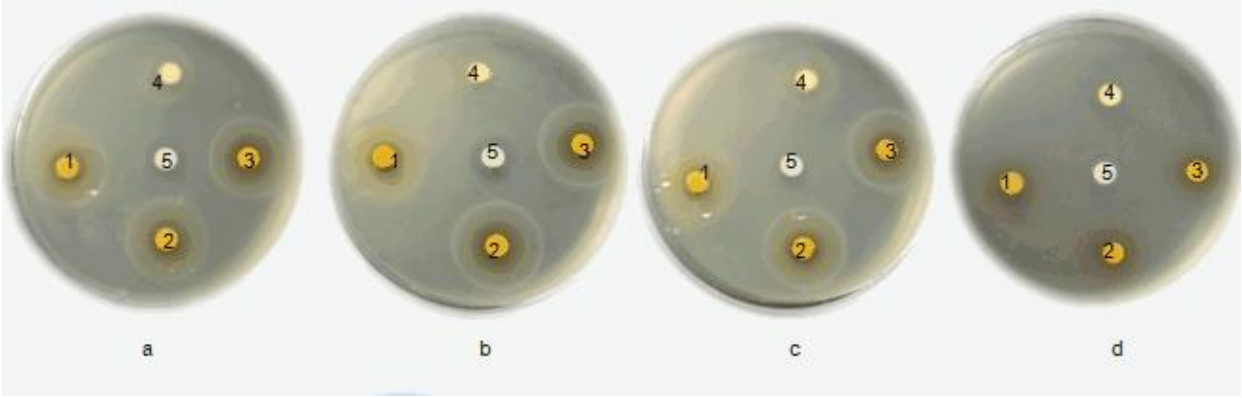

Figure 5. LAB SKL K.4 (6 hours incubation). a.) *Staphylococcus aureus* (ATCC 6538), b.) *Salmonella typhimurium* (ATCC), c.) *Listeria monocytogenes* (BTCC B693), dan d.) EPEC K.1.1. 1 to 3 : LAB-derived metabolite extract, 4 : MRS medium (Negative control), 5: Ampicillin (Positive control). SD (n = 3)

Inhibitory activity of LAB metabolite extract Sumbawa horse milk against α-glucosidase enzyme

Antidiabetic inhibition uses the α-glucosidase enzyme which aims to see the potential extract of LAB metabolite compounds in Sumbawa horse milk to be used as an antidiabetic probiotic. The α-glucosidase enzyme functions to hydrolyze oligosaccharides and disaccharides in the small intestinal wall and α-1,4-D-glucose bonds (Sales et al., 2012). The results of the α-glucosidase inhibitory activity of the extract of LAB metabolite compounds in Sumbawa horse milk from five isolates were successfully identified as having inhibitory effects on the α-glucosidase enzyme. The inhibitory properties produced by the extract of LAB metabolite compounds can be seen in Table 3.

Table 3. The activity of LAB metabolite extracts against α-glucosidase enzyme

Sample	Condition	Consentration (µg/mL)	α-Glukosidase inhibitory (%)
Acarbose	Kontrol +	10	57.14
SK 1.5	*Enterococcus faecium*	49.5	78,57
SKP K.3	*Weissella confusa*	63.5	53.57
SKP K.5	*Lactococcus garvieae*	90	6,25
SKP K.9	*Enterococcus thailandicus*	71.5	39,28
SKL K.4	*Lactobacillus fermentum*	55.5	67,85

Note: SD (n = 3)

Based on Table 3 it can be seen that the extract of metabolite compounds using ethanol from the five isolates of LAB from Sumbawa horse milk can inhibit the α-glucosidase enzymes with varying abilities from 6.25 to 78.57%. Acarbose as a positive control has an inhibition of 57.14%. SK 1.5 (*E. faecium*) metabolite extract had the highest inhibitory activity against α-glucosidase (78.57%) compared to other LAB isolates, whereas SKL K.4 (*L. fermentum*) had an inhibitory activity of 67.85% which is at the second level. This is different from the research of Farida et al. (2019), in which extracts of the metabolite compound *L. fermentum* S21209 (LAB indigenous isolated from tempe) have the highest inhibitory activity against the α-glucosidase enzyme

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(75.22%) compared to other LAB bacteria. This shows that some LAB strains can inhibit the enzyme α-glucosidase (Chen et al., 2014). However, for the past three decades, *E. faecium* has been considered a bacterial species that includes the pathogen 'ESKAPE' (the bacterium that causes nosocomial infections, *E. faecium*, *S. aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacte*r spp.) (Lee et al., 2019).

Several previous studies discussing LAB can inhibit the activity of the α-glucosidase enzyme. Panwar et al. (2014) state that *Lactobacillus* strains in the human intestine have inhibitory activity of alpha and beta-glucosidase enzymes and can reduce blood glucose response in vivo. Some of these *Lactobacillus* strains are *L. fermentum*, *L. casei*, and *L. rhamnosus* which have broad-spectrum inhibitory activity (89%). Also, research by Nurhayati et al. (2017) states that LAB isolated from starch (*Canna edulis*) and taro (*Xanthosoma sagittifolium*) plants also has inhibitory activity against the α-glucosidase enzyme where one of the isolates of LAB GN 8 has an inhibitory activity of ± 93.75%.

The result of the antimicrobial activity and inhibitory activity test of the α-glucosidase enzyme showed that LAB from Sumbawa horse milk has the potential to be used as an antimicrobial and probiotic for type 2 diabetes mellitus patients. LAB has the potential as a probiotic for type 2 diabetes mellitus patients because of consumption probiotics can reduce glucose and diabetes in patients (Yun et al., 2009; Farida et al., 2019). LAB SKP K.9 (*E. thailandicus*) metabolite extract had the highest antimicrobial activity against the four pathogenic bacteria. LAB SK 1.5 (*E. faecium*) metabolite extract had the highest inhibitory activity against α-glucosidase (78.57%) compared to other LAB isolates. But from the current regulatory point of view, the genus *Enterococcus* is neither recommended for the QPS list nor has GRAS status. The development of highly adapted methods and legislation for *Enterococcus* strains are still required (Hanchi et al. 2018). The other LAB has the potential to be used probiotics for type 2 diabetes mellitus patients are LAB SKL K.4 (*L. fermentum*), LAB SKP K.3 (*W. confusa*), and LAB SKP K.5 (*L. garvieae*).

Conclusions

All the extracts of LAB metabolites from Sumbawa horse milk can inhibit the growth of pathogenic bacteria and inhibit the α-glucosidase enzymes with various abilities. The ability to extract LAB isolate metabolites for inhibiting the pathogenic bacteria with a variance between 2.23 cm to 2.7 cm. The extract of LAB SKP K.9 metabolic extract (*E. thailandicus*) has the highest antimicrobial activity against the four pathogenic bacteria. The ability to extract LAB isolate metabolites for inhibiting α-glucosidase enzymes with a range of 6.25% to 78.57%. Moreover, the inhibition of α-glucosidase enzyme obtained from LAB SKP K.5 isolate (*L. garvieae*) is 6.25%, 39.28% from LAB SKP K.9 (*E. thailandicus*), 53.57% from LAB SKP K.3 (*W. confusa*), 67.85% from LAB SKL K.4 (*L. fermentum*), and 78.57% from LAB SK 1.5 (*E. faecium*). The extract of LAB SK 1.5 metabolite compound (*E. faecium*) has the highest inhibitory activity against α-glucosidase enzyme (78.57%) compared to other LAB isolates. Therefore, the potency of LAB strains from Sumbawa horse milk can be an alternative to antimicrobial agents and probiotics for treating diabetes mellitus type 2 through α-glucosidase inhibition.

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