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ANALYSIS OF LACTIC ACID BACTERIA *WEISSELLA PARAMESENTEROIDES* LM-21 AS PROBIOTIC CANDIDATE

Analisis Bakteri Asam Laktat *Weissella Paramesenteroides* LM-21 sebagai Kandidat Probiotik

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ABSTRACT

Probiotics are live microorganisms that provide many health benefits. Weissella genus have been much research over this decade because probiotic potential. Weissella Paramesenteroides LM-21 isolated from Kimchi kiciwis leaf have health potential as glutathione antioxidant production. This research aims to analyze the ability of W. paramesenteroides LM-21 as a probiotic candidate. The methodes are cell viability test at low pH (pH setting of 2.0; 3.0; 4.0; 7.0), antibacterial activity against E.coli and S.aureus, analysis production of exopolysaccharides (EPS) and qualitative analysis of short chain fatty acid (SCFA) by HPLC. The results are W.paramesentreroides LM-21 could survive at low pH (viability at pH 2 is 6.2 log CFU/mL); showed inhibition zone against E. coli was 0.8 mm and S. aureus was 0.5 mm; ability producing EPS with ropy phenotype and crude extract of 30 mg/mL; and SCFA (acetic acid based on a retention time of 10.688 minutes and propionic acid of 26.787 minutes). Based on the results of this research, can be concluded that W. paramesenteroides LM-21 has excellent potential as a probiotic candidate

Keywords: *Lactic Acid Bacteria, Probiotic, Short Chain Fatty Acid, Weissella paramesenteroides*

ABSTRAK

Probiotik merupakan mikroorganisme hidup yang memberikan banyak manfaat kesehatan. Strain Weissella telah banyak diteliti pada dekade ini karena potensinya sebagai probiotik. Weissella parameseneteroides LM-21 diisolasi dari produk fermentasi Kimchi daun kiciwis memberikan potensi kesehatan yang mampu memproduksi antioksidan glutation. Penelitian ini bertujuan untuk menganalisis kemampuan bakteri W. Parameseneteroides LM-21. Metode penelitian ini adalah menguji kemampuan hidup sel pada pH rendah (pada pengaturan pH 2,0; 3,0; 4,0; 7,0), analisis produksi eksopolisakarida (EPS), dan analisis kualitatif short chain fatty acid (SCFA) menggunakan HPLC. Hasil dari penelitian ini adalah, strain W.parameseneteroides LM-21 mampu hidup di pH rendah (viabilitas sel pada pH 2 yaitu 6.2 log CFU/mL); menunjukkan zona hambat untuk E. coli 0.8 mm dan S. aureus 0.5 mm; mampu memproduksi EPS dengan fenotip kental (ropy) dan ekstrak kasar sebanyak 30 mg/mL; serta SCFA (berupa asam asetat berdasarkan waktu retensi 10.688 menit dan asam propionat pada 26.787 menit). Berdasarkan hasil penelitian ini dapat disimpulkan bahwa W.parameseneteroides LM-21 memiliki potensi yang sangat baik sebagai kandidat probiotik.

Kata kunci: *Bakteri Asam Laktat, Probiotik, Short Chain Fatty Acid, Weissella*

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INTRODUCTION

Probiotic terms is meaning “for life” and currently used to name bacteria associated with beneficial effects for humans and animals (FAO/WHO, 2002). Probiotic have many health benefits as improving nutritional value of foods, preventing cardiovascular and urinary diseases, lowering levels of cholesterol, reducing the risk of colon cancer, maintaining immunity system by gut mucosal health, and combating pathogenic bacteria (Zendeboodi *et al*., 2020). Probiotic candidates should have antimicrobial activity, fight various pathogenic bacteria, be able to survive at low pH. Lactic acid bacteria (LAB) occupy the highest percentage in producing probiotics. The effectiveness of LAB as probiotics is reinforced by bioactive bacteriocin compounds, most of which are considered safe or Generally Regarded as Safe (GRAS) by the US Food and Drug Administration (FDA). The European Food Safety Authority (EFSA) also grants Qualified Presumption of Safety (QPS) status to some LAB genera (Reuben *et al*., 2019).

Probiotics are able to produce metabolities that lower the pH of digestion, thus inhibiting and preventing the attachment of pathogenic bacteria such as *E. coli* and *S. aureus*s as the cause of various diseases in digestion. The human gut is an acidic pH environment inhabited by trillions of microbiomes including bacteria, archae, viruses and fungi (Hasibuan, 2017). The gut microbiome is essential for nutrient absorption, immune function, and overall metabolic health. Probiotics are able to decompose long chains of carbohydrates that are not digested by the body to produce Shorth Chain Fatty Acid (SCFA) end products. Deviation of SCFA in the form of acetic acid, propionic acid and butyric acid is correlated with inflammantory bowel disease, irritable bowel syndrome, type 2 diabetes, obesity, and autoimmune disorders (Sandhu & Radhakrishnan, 2025). Probiotic synthesized Exopolysaccharide (EPS) has been proven to be used as a food additive and is beneficial as an anticarcinogen, antitumor, cholesterol – lowering, anticancer, and immunomudulator (Mundiri *et al*., 2020). The increasing use of probiotic – based products is in line with increasing research on probiotics in the health sector. Massive exploration of probiotic candidate microorganisms continues.

Lactic acid bacteria is the most member of probiotic microorganisms, commonly used in dairy and fermented food products Probiotics can be obtained from plant foods as fermented vegetables. One of the LAB successfully isolated from keciwis kimchi was *Weissella paramesenteroides* LM-21 *(W. paramesenteroides* LM-21) (Muharram et al., 2025). The Weissella genus is gram-positive and facultatively anaerobic lactic acid bacteria, has emerged as a significant potency of human microbiota with diverse biotechnological and therapeutic applications (Ma et al., 2025). The probiotic properties of Weissella spp. strains have attracted attention due to their ability to produce EPS at higher levels than other BAL strains. Weissella isolated from wheat has been reported to produce the dextran aureus type of EPS (Han et al., 2024). Recent studies show that the isolate *Weissella paramesenteroides* LM-21, isolated from fermented kimchi, has been shown to produce the bioactive compound glutathione (Muharram *et al*., 2025). This study aims to analyze probiotic potential of *Weissella paramesenteroides* LM-21 on ability acid resistance, SCFA and EPS production, and antibacterial activity.

MATERIALS AND METHODS

Place and time of research

This research was conducted at the Biotechnology Laboratory and Microbiology Laboratory of Muhammadiyah University Bandung and the Central Laboratory Research of Padjajaran University. The research was conducted from May 2024 to June 2025.

Tools and materials

Equipment

Oose apparatus; petri dishes; spatula; analytical balance; centrifuge tubes; shaker incubator; oven; micropipettes; erlenmeyer flasks; pH meter; centrifuge; caliper (vernier caliper); and millipore filtration unit.

Materials

Weissella paramesenteroides LM-21 isolate; *Escherichia coli* isolate;

Staphylococcus aureus isolate; De Man, Rogosa and Sharpe Agar (MRSA); De Man, Rogosa and Sharpe Broth (MRSB); Nutrient Broth (NB); and Mueller–Hinton Agar (MHA; OXOID CM0337)

METHODOLOGY

Activation of Isolated

W. paramesenteroides LM-21 isolate were activated on MRS agar by oose and incubated for 24 – 48 hours. Single colony inoculated into 15 ml of liquid MRS in falcon tubes and incubated on a shaker incubator at 225 rpm at 30 degree celcius for 24 hours.

Viability test at low pH

The viability test of low pH was carried out by growing 1% of of fresh culture *W. paramesenteroides* LM-21 into MRS broth media which had previously been adjusted to pH 7.0 ; 4.0 ; 3.0 ; 2.0 using NaOH and HCl, respectively. Then incubated for 24 hours at 37 degree celcius. The viability of bacteria was counted using the total plate count method on MRSA media (Sunaryanto *et al*., 2014)

Antibacterial Test Against *E.coli* and *S.aureus*

Antibacterial testing against indicator bacteria was performed using six disc diffusion methods on agar media. A suspension of pathogenic bacteria was prepared using 5 ml of NB media. A total of 20 µl of antibacterial supernatant was dropped onto a 6 mm diameter sterile disc. The disc was placed on MHA media containing test bacteria (*E. coli* and *S. aureus*). Observations were made by measuring the diameter of the clear zone formed around the paper disc, which was then measured using a caliper after incubation for 24 hours at 37°C (Sidabutar et al, 2015).

Exopolysaccharide Production

EPS production refers to the research by Nudyanto and Zubaidah (2015). *W. paramesenteroides* LM-21 culture was incubated overnight then inoculating to produce EPS into medium containing 5% skim milk, 0.35% yeast exctract, 0.35% peptone, and 5% glucose and incubated at 30 degree celcius for 24 hours. EPS was extracted by centrifugation at 10,000 g for 20 minutes at 4°C, and precipitated with ethanol at 4°C. The final precipitate was collected after centrifugation at 10,000 g for 20 minutes at 4°C. The EPS extract (as crude EPS) was dried and weighed.

Exopolysaccharides Analysis

Weissella paramesenteroides LM-21 streaked using the four-way streak technique on MRSA medium with 10% sucrose and MRSA medium without added sucrose that had solidified incubated in an incubator oven for 24 hours at 37℃. The experiment was conducted in triplicate (Paiva et al., 2016).

Data analysis involved visual observation of EPS production in mucoid/ropy colonies. Mucoid colonies are those that produce mucus, while ropy colonies are those that, when picked up and pulled with an ose, appear as sticky threads longer than 5 mm (Lestari & Fibriarti, 2022).

SCFA Analysis

SCFA production refers to the research by Pabari et al, (2020) with adjustments. *W. paramesenteroides* LM-21 was grown in MRS Broth supplemented with 1% starch for 24 hours and then analyzed for SCFA production using HPLC. The SCFAs analyzed were propionic acid, butyric acid, and acetic acid, which were then compared with standard propionic acid, butyric acid, and acetic acid solutions. The supernatant of culture was transferred into vials using a syringe and a 0.45 µm Millipore filter, with a volume of 1.5 mL. Standard solutions of propionic acid, acetic acid, and butyric acid at 1000 ppm were prepared. These were then injected into the HPLC system under conditions of a flow rate of 0.6 mL/min.

RESULTS AND DISCUSSION

Activation of Bacterial Isolate

Isolates of *W. paramesenteroides* LM-21 on MRSA inoculated into 10ml of MRSB, after incubation on a shaker incubator for 24 hours showed, the media from the previously clear appeared to be cloudy and there was a ehite sediment indicating the growth of bacteria. Bacteria utilize nutrients in the media to compose their cell components so

that they can multiply. Liquid media is media that is not added to the solidifying material or agar so that the consistency is liquid. Liquid media is generally used to see the nature of bacterial growth such as uniform turbidity, forming sandy deposits or forming strands of hair or caput medusae (Suarjana *et al,* 2017). The activation results of the *W. paramesenteroides* LM-21 isolate are shown in Figure 1.

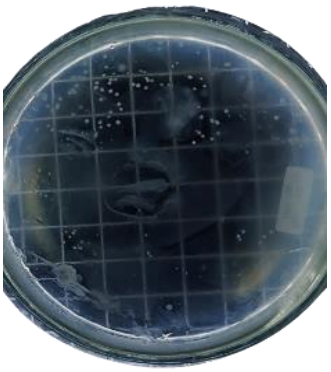
Figure 1. culture results of *Weissella paramesenteroides* LM21 bacteria

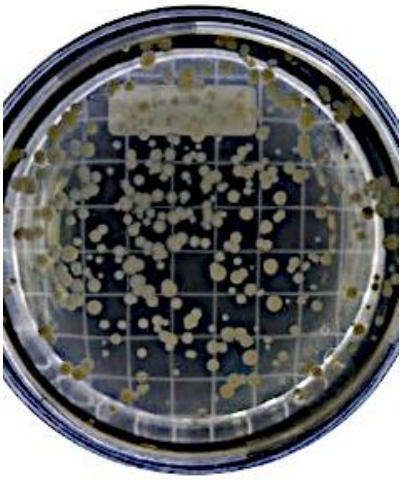
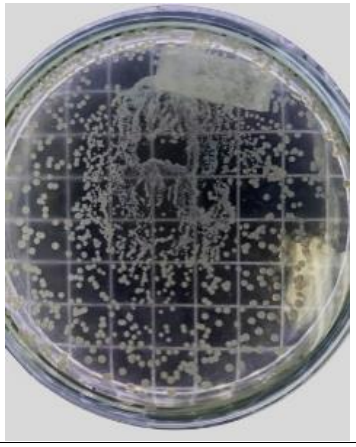
Viability test at Low pH

Viability test to low pH refers to research (Sunaryanto *et al*., 2014). *W. paramesenteroides* LM-21 activation results were inoculated as much as 1% of the total sterile MRS B containing stratch with pH variations of 7.0 ; 4.0 ; 3.0 and 2.0. After incubating for 24 hours at 37 degree celcius, the media from previously clear appeared to become cloudy indicating the presence of bacterial growth.

Cell viability in LAB can be calculated using the total plate count method, based on the results of the resistance test of probiotic candidate bacteria to low pH or acidic conditions in (Table 1) shows that all bacterial isolates are able to survive in low pH, at pH 2, bacteria can survive of 6.2 log CFU / mL. This result similar by (Yadav et al., 2022), that *W. paramesenteroides* MYPS5.1 showed survival of 6.47 log CFU/mL at pH 2. The survival bacteria in low pH describe the ability of bacteria passage through the acidic environment of the stomach, which is extremely for living bacteria, and crucial for probiotic colonization in the intestine (Lachowicz-wi, 2022).

Tabel 1. Number of Bacteria at Various pH Level

pH	Number of Bacteria
pH 2 	6,62 log CFU/mL

pH	Number of Bacteria
pH 3	
	7,9 log CFU/mL
pH 4	
	8,2 log CFU/mL
pH 5	
	8,33 log CFU/mL

Antibacterial Test Against E.coli and S.aureus

The results obtained from the antibacterial activity test using the paper disc diffusion method showed that *Weissella paramesenteroides* LM-21 tested against *E. coli* and *S. aureus* had antibacterial activity, as indicated by the presence of a clear zone around the paper disc.

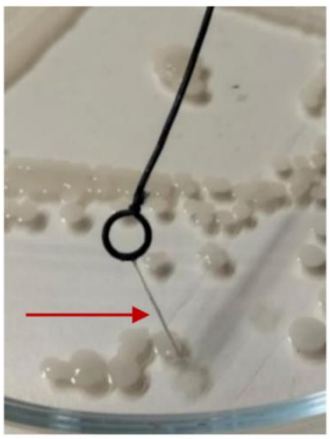
The diameter of the clear zone was measured using digital calipers, and the average diameter of the clear zone of *Weissella paramesenteroides* LM-21 against *E. coli* was 0.8 mm, while the average diameter of the clear zone against *S. aureus* was 0.5 mm (table 2). The results show that *Weissella paramesenteroides* LM-21 has weak antibacterial activity against *E. coli* and *S. aureus* bacteria. Based on the classification of David and Stout (1971), the antibacterial activity produced is classified as weak because it is below 5 mm.

Tabel 2. Inhibition zone against *E. coli* and *S. aureus*s bacteria

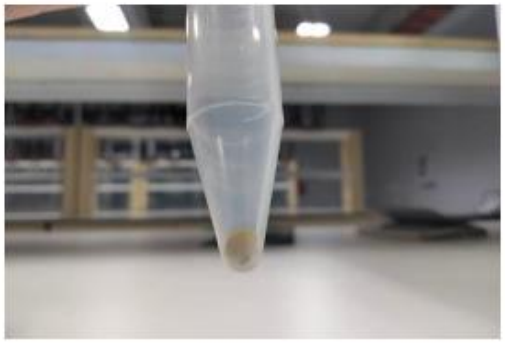
Sample	Inhibition zone (mm)	
	Escherichia coli	***Staphylococcus aureus***
K-	0,1	0,1
K+	10,47	10,17
F1–5	0,9	1,57
F21–25	0,1	1,57
F175	0,17	0,23

Analysis of EPS production

EPS production of LM-21 isolate obtained a dry weight of crude EPS of 1.2 grams from 40 mL of culture or 0,03 gram/mL. Based on figure 2, LM-21 strain have the ability to produce crude exopolysaccharide with ropy phenotypes. The ropy phenotype is visually of long filaments when a needle is lifted from the colony surfaces as well as from the cell pellet in fermented liquid (Prete et al., 2021), (Cirrincione et al., 2018). The ability of producing EPS is generally influenced by two factors, genetic factors and environmental factor (Tallon *et al*., 2003). Amount of EPS produced depends on the source of carbon and nitrogen, as well as the physicochemical conditions of bacterial growth such as temperature, pH, oxygen level, and others (Halim, 2013). Various sugars influences EPS production, however, sucrose enhances the EPS production. Weissella genus possesses probiotics properties and also produces many bioactive components such as bacteriocins, biogenic amines, enzymes, folate, etc (Kavitake et al., 2020). Weissella genus produces diversity of EPS with different chemical composition either homo or hetero polysaccharide (dextran, glucan, galactan, levan, and fructan type) using extracellular polysaccharide synthesis pathway (Zhou et al., 2018).



(a) Ropy texture of EPS



(b) crue extract of EPS

Figure 2. exopolysaccharides and crude EPS

SCFA Analysis

Analysis of SCFA content was carried out by HPLC method qualitatively. Based on the results of the study, HPLC peaks were obtained in standard solutions and samples of *Weissella paramesenteroides* bacteria in the following figure.3.

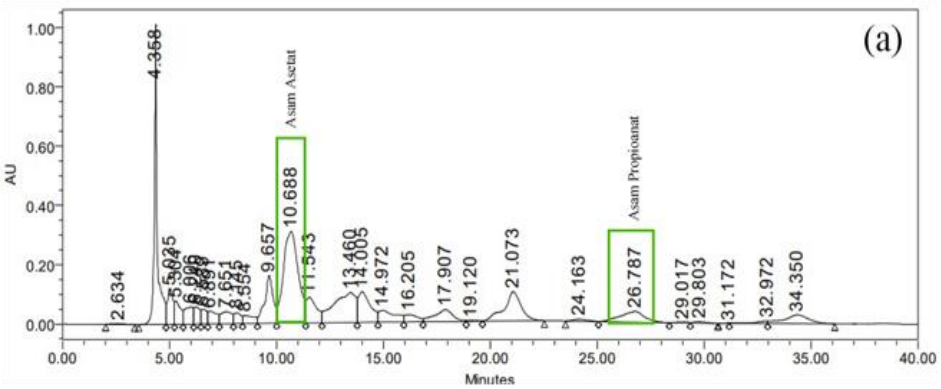


Figure 3. Standar SCFA peak content graph (Acetic Acid, Propionic Acid, and Butyrate Acid).

Based on figure 3, it is known that the standard chromatogram of acetic acid (a) appears at a retention time of 10.019 minutes, propinic acid (b) at 26.235 minutes, and butyric acid (c) at 1.658 minutes.

The results of HPLC testing showed that the *Weissella paramesenteroides* LM-21 found acetic acid and propionic acid compounds based on the same retention time, namely acetic acid at minute 10.688 and propionic acid at 26.787 minute. Meanwhile, butyric acid compounds cannot be detected in the sample. This is possible because optimization of methods such as substrate selection, fermentation conditions and optimization of appropriate HPLC conditions are still needed so that the compounds in the sample are detected properly and accurately during the HPLC process.

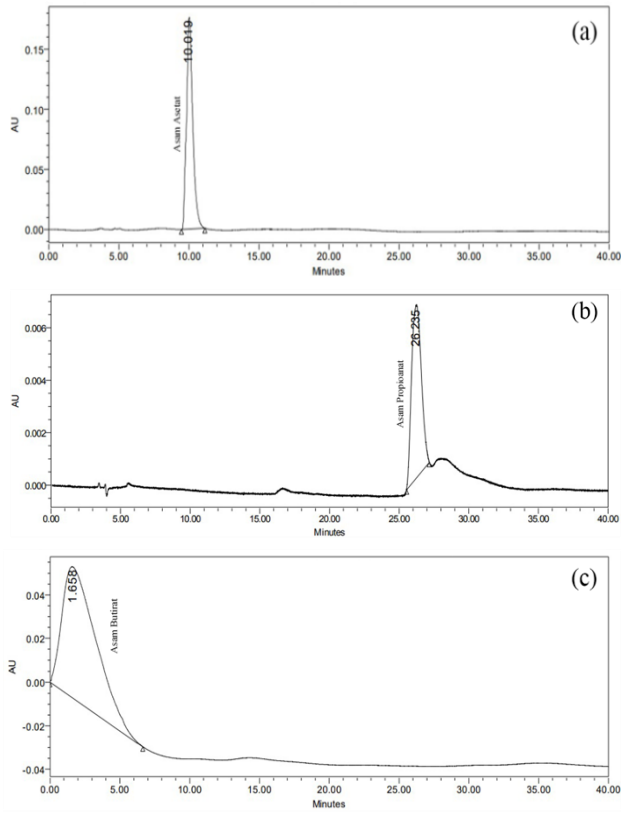


Figure 4. peak content graph of SCFA producing by *weissella paramesenteroides* LM21 (Acetic acid and Propionic acid).

CONCLUSION

New prospective probiotic widely exploring today owing their ability promoting health, therapeutic, and industrial application. Weissella genus has many recent study the strong potency of probiotic characteristics. This present study, new Weissella strain isolated from kimchi keciwis leaf, is *Weissella parameseneteroides* LM-21. Based on this studi, LM-21 strain have viability at low pH (6.2 log CFU / mL at pH 2), antibacterial activity against *E.coli* and *S.aureus* (weak activity), producing exopolysaccharide (a crude extract of 30 mg/mL), and short chain fatty acid (acetic acid and propionic acid by HPLC analysis). This research showed *W. parameseneteroides* LM-21 strain has strong potential of probiotic. Neverthless, further researches are required to confirmed the health benefit and assessment of safety aspect for therapeutic and industrial application.

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