

Antimicrobial Activity of Lactic Acid Bacteria Isolated from Dangke Traditional Food, Enrekang Regency, South Sulawesi

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ABSTRACT

Introduction: Lactic acid bacteria (LAB) produce lactic acid as the end product of carbohydrate metabolism. LAB is known to have benefits, including balancing the microflora in the gut, is bacteriostatic, and is also reported to be able to stimulate the immune system. LAB can be isolated and characterized from various food products spread throughout Indonesia. Dangke is a food product in the form of processed cow's milk which is traditionally produced by the people of Enrekang Regency, South Sulawesi. **Aims:** To isolate and explore the characteristics of LAB isolated from dangke as well as in vitro studies on the potential and mechanisms of their inhibition of the growth of pathogenic bacteria. **Method:** Research started with the process of collecting samples of dangke in Enrekang Regency, isolation and identification of indigenous LAB in dangke samples, characterization and continued with in vitro studies on the potential of LAB from dangke to inhibit bacterial growth. **Results:** LAB isolated from dangke macroscopic characteristics in the form of round colonies, mucoid texture, entire edge, elevation convex and translucent with a size of 2.2 mm. Microscopic characteristics in the form of rod-shaped gram-positive bacteria. Isolated LAB has the ability to grow in extreme conditions approaching intestinal conditions, namely growing in an environment with an acidic pH range of 3-5 and tolerant to bile salts. Secondary metabolites produced can inhibit the growth of *S. aureus* bacteria in vitro. **Conclusion:** LAB isolated from dangke have demonstrated significant potential for probiotic and antibacterial applications.

KEYWORDS: LAB, antimicrobial, probiotic, dangke.

INTRODUCTION

Lactic acid bacteria (LAB) produce lactic acid as the end product of carbohydrate metabolism. LAB plays an important role in fermentation and improving food quality. LAB is widely used in food biotechnology, among others, to improve the preservation, taste and texture of fermented foods and feed products. (Kleerebezem et al., 2017) (Li & Han, 2017)

(Teusink & Molenaar, 2017). Gut health research indicates that LAB strains can improve gut health by balancing gut bacteria, inhibiting harmful bacteria growth, and producing beneficial compounds like lactic acid. These compounds can help fight infections and aid in digestion (Lynch et al., 2018) (Farida, 2019) (Zhao et al., 2021) (Vareille-Delarbre et al., 2019). Furthermore,

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several metabolites of lactic acid bacteria are also reported to stimulate the immune system (Zhao et al., 2021).

LAB is generally not pathogenic for humans and can grow in environments with low pH and high salt concentrations so that various LAB strains that have been isolated and characterized from various food sources have been designated as LAB and have potential as probiotics (Mora-Villalobos et al., 2020) (Mathur et al., 2020).

Several studies have succeeded in isolating LAB from various sources, including vaginal secretions from women of childbearing age (Putri et al., 2018), bilih fish (Prima & Rusfidra, 2021), windu shirmp (Sidabutar et al., 2015), sumba horse milk (Detha, 2019), Fermentation foods, (Priadi et al., 2020) The isolated LAB species were *Lactobacillus crispatus*, *Lactobacillus oris*, *Lactobacillus salivarius*, *Enterococcus faecalis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*. (Priadi et al., 2020) (Prima & Rusfidra, 2021) (Detha, 2019) (Sidabutar et al., 2015) (Putri et al., 2018) furthermore the benefits of the metabolites produced by LAB have been shown to inhibit bacterial growth (Sidabutar et al., 2015).

Dangke is a traditional Indonesian processed buffalo or cow milk product, primarily made by the people of Enrekang Regency, South Sulawesi. It is commonly consumed as a side dish to accompany rice in everyday meals (Nurhaedah et al., 2020). Many studies on the isolation of LAB from

traditional food from Enrekang District have been carried out and have succeeded in isolating LAB species including *Lactobacillus lactis*, *Lactobacillus bulgaricus*, *Leuconostoc dextranicum*, *Streptococcus thermophilus* dan *Lactobacillus plantarum* (Zakariah, Malaka, Laga, & Ako, 2019) (Kaswi et al., 2020) (Nur et al., 2015) (Vinet & Zhedanov, 2011) however, research on the characterization of phenotypes and genotypes as well as the potential of their metabolites as growth inhibitors in bacteria is still limited. The aim of this study was to characterize the phenotype of LAB isolated from dangke and to evaluate their inhibitory potential against pathogenic bacteria pathogenic bacteria.

MATERIAL AND METHODS

Material

Dangke samples were sourced from production house in Enrekang regency. MRS broth, MRS agar, Gram stain set, hydrogen peroxide (Himedia, India), antibiotic discs (Oxoid, UK), calcium carbonate, hydrochloric acid, alcohol, were obtained commercially in pro analysis grade with Certificate of analysis (CoA). Distilled water was sourced from laboratory stock. Vortex mixer, incubator, autoclave, micropipette, centrifuge, hot plate, and magnetic stirrer prepared in well calibrated equipment.

Isolation of LAB

Dangke samples (0.3 g) were aseptically inoculated into 3 mL of MRS broth, homoge-

nized using a vortex mixer, and incubated anaerobically at 37°C for 48 hours. Isolates from the MRS broth were streaked onto MRS agar plates supplemented with 1% CaCO₃ and incubated at 37°C for 48 hours. Colonies exhibiting clear zones were selected and further purified on MRS agar slants for subsequent identification (Suhaeni & Syakur, 2016).

Macroscopic and Microscopic Identification

Macroscopic characteristics, including colony morphology (shape, margin, color, and elevation), and the presence of a clear zone were observed on MRS agar plates supplemented with 1% CaCO₃. Microscopic examination was performed using Gram staining to determine the cell morphology and Gram reaction.

Biochemical characterization and probiotic potency for LAB isolates

Biochemical characterization of the isolates was conducted through catalase and motility tests. For the catalase test, a small amount of each isolate was applied to a glass slide, followed by the addition of 3% H₂O₂ solution. The presence of gas bubbles indicated a positive catalase test. To assess motility, isolates were inoculated into semi-solid media and incubated at 37°C for 48 hours. The absence of bacterial growth outside the inoculation line confirmed the non-motile nature of the LAB isolates (Detha, 2019).

Antimicrobial Activity of Lactic Acid Bacteri

Probiotic potency of the isolates was assessed by pH resistance parameter; Isolates were inoculated into MRS broth liquid media adjusted to pH 2, 3, 4, 5, 6, and 7. The inoculated media were incubated anaerobically at 37°C for 24-72 hours. Bacterial growth was observed in all media; growth indicating the ability of the isolates to survive and grow at each pH (Suhaeni & Syakur, 2016).

Inhibition test of bacterial growth

The inhibitory activity of LAB isolates from dangke against *Staphylococcus aureus* ATCC 6538 was determined using a modified well diffusion method according to (Suhaeni & Syakur, 2016) (Prima & Rusfidra, 2021) (Detha, 2019). LAB isolates were rejuvenated and enriched on De Man Rogosa and Sharpe agar (MRS agar) for 48 hours. A 1 mL aliquot of the culture was centrifuged at 10,000 rpm for 5 minutes, and the supernatant was collected. A single colony of overnight *S. aureus* ATCC 6538 was suspended into 0.9% NaCl solution and adjusted to a turbidity of 0.5 McFarland standard (amount of bacterial suspension approximately 1.5×10^8 CFU/mL). Adjusted turbidity of *S. aureus* ATCC 6538 suspensions than inoculated to MHA plates and wells were created on agar surface. Fifty microliters of the LAB supernatant were added to each well. Antibiotic discs containing 10 IU penicillin were used as positive controls while sterile filter paper discs served as negative controls

Table 1. Macroscopic & Microscopic Characteristics of LAB Isolates

Sample code	Whole colony						Gram Stain
	Whole	Texture	Entire	elevation	Optical density	Size (mm)	
ATD-2	Cyrcle	mucoid	Entire	Convex	Translucent	2.2	Bacillus Gram (+)



Figure 1. Whole colony (A) Macroscopic (B) & Microscopic (C) LAB Isolated from Dangke

followed by the placement of antibiotic discs the plates were incubated at 37°C for 24. The entire experiment was replicated three times. The inhibitory activity of the LAB supernatant was determined by measuring the diameter of the clear zones surrounding each well. The mean and standard deviation of the triplicate measurements were calculated and reported.

RESULTS AND DISCUSSION

Macroscopic and Microscopic Identification

Macroscopic characteristics were obtained by observing LAB isolate colonies on MRS agar medium while microscopic characteristics were obtained by gram staining to observe the morphology, arrangement and properties of gram cells under a microscope at 1000x microscope magnification. Table 1 & Figure 1 shows the shape of the bacterial colonies from dangke was round, mucoid in

texture, entire edge, elevation convex and translucent with a size of 2.2 mm while the results of gram staining showed that the isolate obtained was bacillus Gram (+). These results were consistent with previous studies which stated that the characteristics of the colonies in LAB were circular, mucoid texture, entire edges, while the results of gram staining showed isolate were gram positive (Zakariah, Malaka, Laga, Ako, et al., 2019). Additionally, LAB were gram-positive, non-motile, non-spore-forming bacteria. They are significant groups of probiotics, considered safe and recognized as Generally Recognized as Safe (GRAS). Moreover, they are considered most promising for developing antibiotic drugs with probiotic properties (Raman et al., 2022). Furthermore, this study clearly confirmed the absence of gram-negative bacteria, an early indication that dangke may free from pathogenic bacteria. As well as well-known

Table 2. Biochemical Characteristics and Probiotic Potential of LAB Isolates

Isolate Code	Biochemical Characteristics		Probiotic Potential Assay				
	Catalase	Motility	Ph Tolerant				Bile Salt Tolerant
			2	3	4	5	
ATD-2	-	-	-	+	+	+	+

that gram-negative bacteria represent a major class of pathogenic bacteria infecting all eukaryotes (Neyen & Lemaitre, 2016).

Biochemical characterization and probiotic potency for LAB isolates

Biochemical characteristics were obtained by catalase test using the slide method and motility test using the upright method, while the probiotic potency test was obtained by testing the tolerance of LAB isolates to acidic pH and bile salt. Table 2 shows that the LAB isolate from dangke is catalase negative and non-motile, which means that the LAB isolate does not produce the catalase enzyme (non-pathogenic) and has no locomotion (non-immunogenic). In terms of probiotic potential, LAB isolates from dangke are tolerant to pH 3-7, tolerant to temperatures of 5°C and 36°C and tolerant to bile salt, this shows that LAB isolates from dangke can survive in an environment that resembles the intestinal environment. Lactic Acid Bacteria (LAB) do not form spores, are not motile, give a negative catalase reaction and can grow in aerobic or microaerophilic environments with an optimum temperature of 20 - 40°C (Marco et al., 2017).

Inhibitory Potent of LAB Isolates Against Pathogenic Bacteria

The inhibitory activity of LAB isolates against *S. aureus* ATCC 6538 was assessed using a modified well diffusion method, as described in previous studies (Suhaeni & Syakur, 2016) (Prima & Rusfidra, 2021) (Detha, 2019) with some modifications. As shown in Table 3; Supernatant of LAB isolate inhibited the growth of *S. aureus* ATCC 6538 forming an inhibition zone of 6.98 mm. This indicates that the supernatant possesses inhibitory activity, albeit weaker than the 10 IU penicillin as positive control which produced a 27.3 mm zone of inhibition. According to CLSI guidelines, an inhibition zone between 5-9 mm is categorized as weak. These findings diverge from those reported by Fatma et al. (2019) where an 11 mm inhibition zone (medium inhibition category) was observed (Maruddin et al., 2019). Several factors may account for this discrepancy as Ren et al. (2018) Noted that pH of the supernatant, the incubation period during LAB

Table 3 Inhibitory potent of LAB isolates against *Staphylococcus aureus* ATCC 6538

Sample code	Inhibitory zone (mm)
ATD-2	6.96±0.55
Penicillin	27.3±1.8
Blank paper disc	0

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subculturing, and kind of purification process of the antimicrobial substance were significant factors that led to the observed discrepancy in inhibitory activity (Ren et al., 2018). Furthermore, several advanced exploratory studies on LAB isolated from traditional food products reported that LAB can produce antimicrobial components such as organic acids, hydrogen peroxide and bacteriocins which can suppress the growth of pathogenic bacteria. (Lynch et al., 2018) (Farida, 2019) (Cabello-olmo, Miriam Oneca, Maria Torre, Paloma Sainz, Neira Moreno-aliaga, Mar J, 2019). According to Cirat et al. (2024), the accumulation of organic acids produced by LAB lowers the pH, making the environment unfavorable for pathogenic microorganisms like *E. coli*, *Pseudomonas*, *Salmonella*, and *Clostridium*, which cannot survive in acidic conditions (Cirat et al., 2024), while excessive hydrogen peroxide generated by LAB may cause oxidative damage, impairing essential cellular processes such as RNA, DNA, and protein synthesis pathways in pathogenic bacteria like *Acinetobacter baumannii* & *Streptococcus sp* (Milani et al., 2021) more over bacteriocin the most targeted substant produced by LAB isolate is an antimicrobial peptides or proteins produced by bacteria, inhibit the growth of other bacteria by various mechanisms, including pore formation in the target cell membrane, blocking cell wall synthesis, and degrading the target cell's DNA (Simons et al., 2020) (Acedo et al., 2018).

CONCLUSION

LAB isolated from traditional dangke food from Enrekang Regency, South Sulawesi has macroscopic characteristics in the form of round colonies, mucoid texture, entire edges, elevation convex and translucent with a size of 2.2 mm. microscopic characteristics in the form of rod-shaped gram-positive bacteria (Monobacillus gram-positive). Isolated LAB has the ability to grow in extreme conditions approaching intestinal conditions, namely growing in an environment with an acidic pH range of 3-5 and tolerant to bile salts. Furthermore, secondary metabolites in supernatant produced by the LAB isolates exhibited inhibitory activity against *S. aureus* in vitro.

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