Antimicrobial Activity of Lactic Acid Bacteria Found in Dadiah on Disease-Causing Skin Infections

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Abstract

Dadiah is a fermented food made from buffalo milk containing lactic acid bacteria (LAB). The potential of LAB as an antimicrobial organism is expected to inhibit or kill pathogens, especially those causing inflammations in the skin. *Dadiah* used in this study was obtained from the Lintau area of Tanah Datar District, West Sumatra, Indonesia. LAB isolation and identification involved the use of MRS Broth (MERCR) and MRS Agar (MERCK) media and the pathogenic bacteria used include *Staphylococcus aureus, Propionibacterium acnes, Acinetobacter Baumannii, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*. The results of this study obtained a total LAB of $7x10^{11}$ CFU / g and showed the highest antimicrobial activity in the *Staphylococcus aureus* with a14.53 mm clear zone and 13.18 mm bacteria in the *Pseudomonas aeruginosa* test bacteria.

Keywords: *Antimicrobial dadiah, lactic acid bacteria, skin diseases*

Introduction

Skin diseases are the most common types of diseases in developing countries, especially in those with tropical climate. Therefore, humid and hot air throughout the year is highly suitable for the development of infectious, disease-causing pathogens in the skin. The prevalence of such infections in developing countries ranges between 20-80%. As a developing country, Indonesia and its inhabitants are prone to skin diseases of which its main causes include poor geographical and hygiene conditions. Such conditions enhance the rapid development of pathogens and bacteria. Therefore, the attention and recommendations of appropriate health organizations in the preventive measures against skin infections is presently a necessity. The problem of antibiotic resistance has become a worldwide problem, especially during prevention and cure, that necessitates viable solutions in form of alternative therapy or treatments[1]. These forms of alternative therapy include synthetic antibiotics.

Probiotics, vitamins, and phenolics are a few examples of nutraceuticals. These substances are important for skin protection and health, and they are known to assist in medical treatment of dermatological problems[2]. This study aims to discover the role of probiotics (Lactobacillus) as a prevention and treatment for skin infections. Many studies have shown that lactic acid bacteria (LAB) helps to activate commensal bacteria due to its probiotic properties, and gets rid of pathogenic bacteria through the antimicrobial properties derived from either bacteriocin found in LAB itself, or the role of its peptides. Some studies also mention that the role of probiotics in nutrition involves boosting the immunity against skin infections caused by various pathogenic bacteria such as *Aeromonas bestiarum, Ichthyophthirius multifiliis, Staphylococcus aureus, Corynebacterium acnes, Streptococcus*, etc[3].

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Dadiah is a traditional food from West Sumatra made of buffalo milk that undergoes a natural fermentation process in a bamboo tube, *Dadiah* originally made from Lintau in Tanah Datar, contains probiotics such as *L. Plantarum* which has proven to be effective in maintaining the balance of intestinal microflora, and contains strong antimicrobial properties[4].

Atopic dermatitis is an inflammatory skin disease occurring often in children and the elderly [5]. The dominant bacterial species found in this skin disease is *Staphylococcus aureu*s. The colonies of this bacteria, when present in the skin, can result in a wide range of infections therein. Therefore, antimicrobial treatment is necessary to prevent such bacterial growth [6] . Many broad-spectrum antibiotics can be used as an inhibitor of *Staphylococcus aureus*, such as penicillin, doxycycline, tetracycline, clindamycin, etc. However, improper use of an antibiotic results in drug resistance. It is also associated with an increased risk of infection and emergence of bacterial colonies, known as "Multidrug-Resistant Organisms (MDRO)" [7]. These Methicillin resistant *Staphylococcus aureus* (MRSA) and MDRO are the main causes of nosocomial pathogenic infection worldwide, and require serious treatment, as they increase comorbidity and death due to the infection[8]. A major challenge posed by *S. aureus* is antimicrobial resistance. After the introduction of the antibiotics penicillin and methicillin into clinical practice, strains of antibiotic-resistant *S. aureus* (MRSA) were subsequently identified [9].

Therefore, this study was aimed to determine the role of *Dadiah* as a probiotic that can be used to inhibit bacteria-induced skin diseases, especially the inflammatory infections, as a supporting or future treatment alternative against skin diseases.

Materials and Methods

Isolation of Lactic Acid Bacteria

One gram of *Dadiah* sample was placed within test tubes containing 9 ml of MRS broth and diluted up to ten logarithmic (10- 10) fold. Furthermore, it was added to test tubes containing 900 ul of MRS broth variations of dilution ratio 10-1, 10-2 and 10-7. Furthermore, 100 ul of the serial dilution of 10-9 was inoculated into the MRS Agar media using the spread method, placed in an anaerobic jar and incubated for 48 hours with a temperature 37ºC. After incubation, a single colony of LAB was obtained and hence isolated [10].

Identification of Lactic Acid Bacteria

Identification of LAB from *Dadiah* samples was done by placing 1g of the sample in a test tube containing 9 ml MRS Broth diluted up to ten (10-1) logarithmic fold. It was further incubated in anaerobic conditions for 24 hours, after which a 100 uL 10-1 dilution was added to the tube containing 900 uL 1 MRS broth dilution 10-2, and a subsequent dilution of 10-8. Consecutively, 100 uL of the serial dilution of 10-8 was inoculated on the media MRS Agar with the method of spread, placed within an anaerobic jar, and incubated for 48 hours at 37 ^{0}C. A single (choice random) colony that is round, slippery, and either white or yellowish is characteristic of LAB. Finally, this bacterial colony was transferred via streak method and incubated [10].

Antimicrobial Activity

The antimicrobial resistance testing was carried out using five bacteria samples that cause skin disease. LAB culture (1 mL) was centrifuged at 10,000 rpm for 5 minutes at 27^0 C, and supernatant volume of 50 uL was used to test for antimicrobial resistance. 0.2% of test bacteria (*Staphylococcus aureus, Propionibacterium acnes, Acinetobacter Baumannii, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*) was placed on Nutrient Agar (NA) as a growth media for test bacteria, homogenized, injected by micropipette, and incubated at 37 ^{0}C. Clear and circular inhibition zones are measured after 12 hours [11].

Results and Discussion

Isolation Lactic Acid Bacteria

Dadiah originating from the Lintau area of Tanah Datar District, West Sumatra, Indonesia is known to contain Lactic Acid Bacteria (LAB). It was isolated with MRS as a specific media for LAB growth and incubated for 48 hours. In this study, the amount of LAB used was 7x10^{11} CFU / g. This result is higher compared to those obtained, while the total LAB obtained from Payakumbuh Dadiah was 8x10^8 CFU/g [12], in a research at Solok district, the total LAB obtained was as much as 8x10^8 CFU/g [13]. This is because Dadiah kicks off

its fermentation spontaneously; without the need for a fermentation starter. LAB was also found in buffalo milk obtained from several areas in West Sumatra totaling $3x10^6$ - $2x10^8$ CFU/g [14]. This is consistent with the standards which state that LAB in probiotic food must be present within the range of 10^6 - 10^8 CFU / Gram [15].

Identification of Lactic Acid Bacteria

Incubation was done using MRS Broth and then cultured on MRS media. For 48 hours in 37^0 C incubators, a single colony was obtained through macroscopic observations with round, cream-white and convex shapes. Microscopic observations showed that the bacteria were Gram-positive and rod-shaped. Lactic acid bacteria (LAB), belongs to a group of gram-positive, catalase-negative bacteria, which synthesize lactic acid by the fermentation of carbohydrates. The cells are known as coccus or bacillus, usually arranged in pairs or chains. LAB are facultative anaerobes, non-motile and mesophyllic [16].

Antimicrobial Activity

Antimicrobial testing using *Staphylococcus aureus, Propionibacterium acnes, Acinetobacter Baumanni, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* commonly found in skin infections can be seen in Table 1.

Table 1. Diameter Clear Zone

Isolate	Bacteria test (mm)				
	Staphylococcus aureus ATCC	Propionibacterium acnes	Acinetobacter baumannii	Klebsiella pneumoniae	Pseudomonas aeruginosa
3A	14,53	7,6	10,24	10,15	13,18

Testing antimicrobial activity involved a well-known method of random selection of 3A isolates from curd which have the potential to function as probiotics, because they can inhibit the growth of pathogenic bacteria which cause skin diseases. The clear zone produced by lactic acid in table 1 shows the existence of antimicrobial activity from LAB isolated from Dadiah samples obtained from Lintau, Tanah Datar District. The ability of LAB to inhibit pathogenic bacteria is due to the presence of its metabolites such as lactic acid, peroxide, and bacteriocin.

Clear zones with the highest numbers were produced in the *Staphylococcus aureus* ATCC test bacteria (14.53 mm) from *Dadiah* isolates. This clear zone is smaller when compared to the study of which used LAB isolated from tempoyak which inhibits the bacterium *Staphylococcus aureus* which a diameter of 19.3 mm [17].

In *Propionibacterium acnes,* test bacteria obtained a clear zone of 7.6mm, the smallest diameter compared to other test bacteria. This result is smaller when compared with *Lactobacillus casei* strain of Shirota bacteria of 11.2 mm and therefore higher in inhibitory power than the *L. acidophilus* HM1 bacteria of 3.3 mm [18]. The clear zone of *Acinetobacter baumannii (*10.24 mm) obtained in this study is greater than that of which used the *Lactobacillus acidophilus* LMEM8 against *Acinetobacter baumannii* and obtained a diameter of 5.13 mm [19]. The results of this study are more productive than that of in testing the antimicrobial activity of *Lactobacillus fermentum* MTCC 9748 against *Klebsiella pneumoniae* NTCC 703603 which resulted in a 9.17 mm clear zone [20]. Moreover, the *Pseudomonas aeruhginosa* test bacteria (13.18 mm clear zone) was not significantly different compared to the research testing of *Lactobacillus* spp against *Pseudomonas aeruginosa* bacteria with a range of clear zones 11-21 mm [21].

Conclusion

Lactic acid bacteria isolated from *Dadiah* of Lintau origin is probiotic in nature, and it shows a highly significant inhibitory effect against *Staphylococcus*

aureus (of which the largest clear zone of 14.53mm was obtained), *Propionibacterium acnes, Acinetobacter baumannii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. However, further research is necessary in order to ascertain other probiotic, antimicrobial candidates which inhibit pathogenic bacteria which cause skin diseases and infections.

Conflict of Interest : None

Ethical Clearance: Not required in an in-vitro studys

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