

**ORIGINAL ARTICLE****ARTICLE ACCEPTED DATE-Sep 22<sup>nd</sup> -2020****AUTHOR'S INTRODUCTION**

Rinita Amelia<sup>1</sup>, Dessy Abdullah<sup>2</sup>, Yudha E. Pratama<sup>3</sup>, Endang Purwati\*<sup>4</sup>

**Address(es):** Prof. drh. Hj. Endang Purwati MS, PhD

<sup>1,2</sup>Lincoln of University College, Faculty of Health and Science, Doctoral Program, Bharu City Malaysia

<sup>1,2</sup>Baiturrahmah University, Medical Faculty, Padang Indonesia

<sup>3</sup>Andalas University, Faculty of Animal Science, Doctoral Program, Padang, Indonesia

<sup>4</sup>Andalas University, Faculty of Animal Science, Professor of Biotechnology/Technology of Product Husbandry, Padang Indonesia.

\*Corresponding Author: [purwati17@yahoo.co.id](mailto:purwati17@yahoo.co.id) / [purwati17@ansci.unand](mailto:purwati17@ansci.unand)

**TITLE:****ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA FROM DADIAH ON BACTERIA THAT CAUSE SKIN INFECTIONS****ABSTRACT**

Dadiah is a fermented food made from buffalo milk containing lactic acid bacteria (LAB), the potential of LAB as an antimicrobial is expected to be able to inhibit or kill pathogens, especially those that cause inflammation in the skin. Dadiah used was taken in the Lintau area of Tanah Datar District, West Sumatra Indonesia. BAL isolation and identification used MRS Broth (MERCR) and MRS Agar (MERCK) media and pathogenic bacteria used by *Staphylococcus aureus*, *Propionibacterium acnes*, *Acinetobacter Baumannii*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. The results of this study obtained a total LAB of this study  $7 \times 10^{11}$  CFU / g and had the highest antimicrobial activity in the *Staphylococcus aureus* 14.53 mm 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 diseases in developing countries, especially in tropical climate countries. Humid and hot air throughout the year is very suitable in the development of infectious diseases of the skin. The prevalence of skin infections in developing countries can range from 20-80%. Indonesia is one of the developing countries whose inhabitants are prone to diseases of skin infections, both mainly caused by poor geographical and hygiene conditions to allow the opportunity of the rapid development of pathogenic bacteria. Therefore, it requires special attention from various parties in the prevention efforts of skin infections appropriately and optimally. The problem of antibiotic resistance has become a world issue that should be given with a quickly formulated solution as an alternative therapy (**Hafez *et al.*, 2013**). The problem of antibiotic resistance becomes an important issue in the handling of infections and needs to be solved through alternative therapy as candidates of synthetic antibiotics.

Probiotic, vitamins, and phenolics are just a few of the nutraceuticals. Nutraceuticals are important for healthy skin protection and potentially prevent and assist medical management of dermatologic problems (**Nwanodi, 2018**). This study discussed the role of probiotics of *Lactobacillus* as an effort to prevent and as a therapy for skin infections. Many studies have mentioned that lactic acid bacteria (LAB) may activate commensal bacteria and get rid of pathogenic bacteria through antimicrobial effects derived from bacteriocin contained in the LAB itself or the role of its peptides. Some studies also have mentioned the role of probiotics in nutrition as an attempt to cope with skin infections caused by various infectious agents such as bacteria of *Aeromonas bestiarum*, *Ichthyophthirius multifiliis*, *Staphylococcus aureus*, *Corynebacterium acnes*, *Streptococcus*, etc. (**Pieters *et al.*, 2008**).

Dadiah is a traditional food from West Sumatra made of buffalo milk that has a natural fermentation in a bamboo tube. According to the study by **Purwati *et al.* (2018)**, stating that especially Dadiah originally made from Lintau in Tanah Datar contains probiotics in a type of *L. Plantarum* has been demonstrated to have benefits in maintaining the balance of intestinal microflora and has a strong antimicrobial effect (**Purwati, Hellyward, and Purwanto, 2018**). Atopic dermatitis is also a skin inflammatory disease that often occurs both in children and elderly people (**Awasthi, Rothe, and Eichenfield, 2020**).



The dominant bacterial species found in this skin disease is *Staphylococcus aureus*. The colonization of these bacteria in the skin is closely related to the development of widespread infections. Thus it requires antimicrobial therapy to stop the growth of this bacteria (**Nakatsuji and Gallo, 2020**). Many broad-spectrum antibiotics can be used as an inhibitor of *Staphylococcus aureus* bacteria, such as penicillin, doxycycline, tetracycline, clindamycin, etc. Improper use of an antibiotic can cause drug resistance. It is also related to the emergence of increased risk of infection and bacterial colonization known as "Multidrug-Resistant Organisms (MDRO)" (**Weber, Mawdsley, and Kaye, 2020**). These Methicillin resistant *Staphylococcus aureus* (MRSA) and MDRO will be the main causes of nosocomial pathogenic infection in the world and require serious treatment since they can increase the comorbidity and death due to infection (**Sikorska & Smoragiewicz, 2020**). A major challenge posed by *S. aureus* is antimicrobial resistance. After the antibiotics penicillin and methicillin were introduced into clinical practice, strains of antibiotic-resistant *S. aureus* (MRSA) were identified (**Creech, Al-zubeidi, and Fritz, 2020**).

Therefore, this study was aimed to determine the role of Dadiah as a probiotic that can be used as a bacterial inhibitor of the cause of skin diseases especially in skin's infectious and inflammatory diseases, as a supporting or future therapy candidate against these skin diseases.

## MATERIALS AND METHODS

### Isolation Lactic Acid Bacteria

Isolation of BAL dadih ( **Purwati, Syukur and Hidayat, 2005**) used was 1 g, included in test tubes containing 9 ml of MRS broth (dilution 1:10), dilution of 10<sup>-1</sup>. 100 uL 10<sup>-1</sup> dilution, inserted in the tube contains 900 ul 1 MRS broth dilution 10<sup>-2</sup> - 10<sup>-7</sup>. Then, 100 ul of the serial dilution of 10<sup>-9</sup> inoculation on the media MRS Agar with the method of spread, put in an anaerobic jar, incubated for 48 hours with a temperature 37 0C. A single colony that characteristic LAB.

### Identification Lactic Acid Bacteria

Identification of LAB ( **Purwati, Syukur and Hidayat, 2005**) from dadiah samples done by 1 g put in a tube containing 9 ml MRS Broth (dilution 1:10), dilution of 10<sup>-1</sup>. Incubated in anaerobic conditions for 24 hours, After that, 100 uL 10<sup>-1</sup> dilution is inserted in the tube contains 900 uL 1 MRS broth dilution 10<sup>-2</sup>, done to a dilution of 10<sup>-8</sup>. Then, 100 uL of the serial dilution of 10<sup>-8</sup> inoculated on the media MRS



Agar with the method of spread, put in an anaerobic jar, incubated for 48 hours at 37 °C. A single (choice random) colony that characterize LAB (round slippery white yellowish) transferred the colony with the method of streak and incubated.

### **Antimicrobial Activity**

Antimicrobial resistance testing (**Patent IDS00002471**) was carried out with five bacterial test causes of the disease: LAB culture (1 mL) was centrifuged at 10,000 rpm for 5 minutes at 27° C and supernatant was used as much as 50 µl to test antimicrobial resistance. Added 0.2% of test bacteria (*Staphylococcus aureus*, *Propionibacterium acnes*, *Acinotobacter Baumannii*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*) on Nutrient Agar (NA) as a growth media for test bacteria, homogenized, and incubated. injected by micropipette and incubated at 37 °C. Clear and circular inhibition zones are measured after 12 hours.

## **RESULTS AND DISCUSSION**

### **Isolation Lactic Acid Bacteria**

Dadiah originating from the Lintau area of Tanah Datar District, West Sumatra Indonesia known to be total Lactic Acid Bacteria (LAB) isolated with MRS media as specific media for LAB growth was incubated for 48 hours. From this study, the amount of LAB was  $7 \times 10^{11}$  CFU / g. This result is higher compared to research **Syukur, Yolanda, and Fachrial, (2015)**, the total LAB obtained in prizes from Payakumbuh was  $8 \times 10^8$  CFU / g, research **Harun, Wirasti, Purwanto, and Purwati, (2020)** dadiah in Solok district LAB obtained as much as  $8 \times 10^8$  CFU / g as much. This is because the dadiah is a spontaneous fermentation product. After all, it does not use a starter in its fermentation. LAB was also found in buffalo milk taken in several areas in West Sumatra totaling  $3 \times 10^6$  -  $2 \times 10^8$  CFU / g (**Melia et al., 2017**). So that is what causes spontaneous fermentation in the production of dadiah. This is following the **FAO/WHO (2002)** because as BAL probiotic food produced must be in the amount of  $10^6$  -  $10^8$  CFU / Gram.

### **Identification Lactic Acid Bacteria**

Incubation of prizes on MRS Broth media for 24 hours which were then grown on MRS media. For 48 hours in 37° C incubators, a single colony was obtained through macroscopic observations with round, cream-white and convex shapes. Microscopic observations obtained results by carrying dadiah bacteria are Gram-positive and rod-shaped. Lactic acid bacteria (LAB), which is a group of Gram-positive bacteria, negative catalase that can produce lactic acid by fermenting carbohydrates, the cells



are coccus or bacillus, arranged in pairs or chains, non porpora, facultative anaerobes, non-motile and mesophyllic (**Ray and bhunia, 2004**).

### Antimicrobial Activity

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

Tabel 1. Diameter Clear Zone

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

Testing antimicrobial activity using the well method is known that randomly selected 3A isolates from curd have the potential as probiotics because they can inhibit the growth of pathogenic bacteria that cause skin diseases. The clear zone produced by lactic acid bacteria in table 1 shows the existence of antimicrobial activity from BAL isolated from dadiah from Lintau, Tanah Datar District. The ability of BAL to inhibit pathogenic bacteria due to the presence of BAL metabolites in the form of lactic acid, peroxide, and bacteriocin.

Clear zones with the highest numbers were produced in the *Staphylococcus aureus* ATCC test bacteria (14.53 mm) produced by dadiah isolates. The clear zone in this study is smaller when compared to the study of **Juliyarsi et al., (2018)** by using LAB isolated from tempoyak which can inhibit the bacterium *Staphylococcus aureus* 19.3 mm.

In *Propionibacterium acnes* test bacteria obtained the clear zone with the smallest diameter compared to other test bacteria that is 7.6 mm, this result is smaller when compared with *Lactobacillus casei* strain Shirota bacteria of 11.2 mm and greater than the inhibitory power by bacteria *L. acidophilus* HM1 which is a 3.3 mm study (**Shokryazdan et al., 2014**). The clear zone of *Acinetobacter baumannii* (10.24 mm) produced from this study is greater than that of **Halder et al., (2017)** which obtained the clear zone produced *Lactobacillus acidophilus* LMEM8 against *Acinetobacter baumannii* which is 5.13 mm. The results of this study are better than the **Mandal and Halder, (2018)** testing the antimicrobial activity of *Lactobacillus fermentum*



MTCC 9748 in the bacteria test *Klebsiella pneumoniae* NTCC 703603 resulting in a 9.17 mm clear zone. Whereas the *Pseudomonas aeruginosa* test bacteria (13.18 mm) was not much different compared to the research (**Prabhurajeshwar and Chandrakanth, 2017**) testing of *Lactobacillus* spp against *Pseudomonas aeruginosa* bacteria with a range of clear zones 11-21 mm.

## CONCLUSION

Lactic acid bacteria isolated from dadiah from lintau are probiotic candidates and other further tests need to be carried out which are able to inhibit the growth of pathogenic bacteria that cause skin diseases caused by *Staphylococcus aureus*, *Propionibacterium acnes*, *Acinotobacter Baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which are caused by the bacteria *Staphylococcus aureus*, *Propionibacterium acnes*, *Acinotobacter Baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* the largest against *Staphylococcus aureus* (14.53 mm).

## REFERENCES

- Awasthi, S., Rothe, M. J., & Eichenfield, L. F. (2020). Atopic dermatitis : Kids are not just little people. *Clinics in Dermatology*, 33(6), 594–604. <https://doi.org/10.1016/j.clindermatol.2015.09.002>
- Creech, C. B., Al-zubeidi, D. N., & Fritz, S. A. (2020). Prevention of Recurrent Staphylococcal Skin Infections, 29(2015), 429–464. <https://doi.org/10.1016/j.idc.2015.05.007>
- FAO/WHO. 2001. Joint Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk With Live Lactic Acid Bacteria.
- Hafez, M. M., Maghrabi, I. A., & Zaki, N. M. (2013). Toward an Alternative Therapeutic Approach for Skin Infections : Antagonistic Activity of *Lactobacilli* Against Antibiotic-Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. <https://doi.org/10.1007/s12602-013-9137-z>
- Halder, D., Mandal, M., Chatterjee, Shiv, S., Pal, Nishith, K., & Mandal, S. (2017). Indigenous Probiotic *Lactobacillus* Isolates Presenting Antibiotic like Activity against Human. *Biomedicines Journal*, 5(31). <https://doi.org/10.3390/biomedicines5020031>



- Harun, H., Wirasti, Y., Purwanto, B., & Purwati, E. (2020). Characterization of lactic acid bacteria and determination of antimicrobial activity in dadih from Air Dingin Alahan Panjang district, Solok regency-West Sumatera. *Systematic Reviews in Pharmacy*, 11(3), 583–586. <https://doi.org/10.31838/srp.2020.3.76>
- Juliyarsi, I., Hartini, P., . Y., Djamaan, A., . A., Purwanto, H., ... Purwati, E. (2018). Characterization of Lactic Acid Bacteria and Determination of Antimicrobial Activity in Tempoyak from Padang Pariaman District, West Sumatra, Indonesia. *Pakistan Journal of Nutrition*, 17(10), 506–511. <https://doi.org/10.3923/pjn.2018.506.511>
- Mandal, S., & Halder, D. (2018). Exploring Anti- *Klebsiella pneumoniae* Activity of Probiotic *Lactobacilli* of Curd Origin, 1(4), 49–53. <https://doi.org/10.31080/ASMI.2018.01.0038>
- Melia, S., Purwati, ., Yuherman, Jaswandi, Aritonang, S. N., Purwanto, H. 2019. Supernatan Bebas Sel *Lactobacillus fermentum* L23 yang berpotensi sebagai Antimikroba. No. Paten IDS000002471. Dirjen Kekayaan Intelektual, Kementerian Hukum dan HAM.
- Melia, S., Purwati, E., . Y., . J., Aritonang, S. N., & Silaen, M. (2017). Characterization of the Antimicrobial Activity of Lactic Acid Bacteria Isolated from Buffalo Milk in West Sumatera (Indonesia) Against *Listeria monocytogenes*. *Pakistan Journal of Nutrition*, 16(8), 645–650. <https://doi.org/10.3923/pjn.2017.645.650>
- Nakatsuji, T., & Gallo, R. L. (2020). The role of the skin microbiome in atopic dermatitis. *Annals of Allergy, Asthma and Immunology*, 122(3), 263–269. <https://doi.org/10.1016/j.anai.2018.12.003>
- Nwanodi, O. (2018). Skin Protective Nutraceuticals : The Current Evidence in Brief, 2. <https://doi.org/10.3390/healthcare6020040>
- Ray, B dan A. Bhunia. 2004. Fundamental Food Microbiology. 3rdEd. Florida. CRC Press. London. New York.
- Pieters, N., Brunt, J., Austin, B., & Lyndon, A. R. (2008). Efficacy of in-feed probiotics against *Aeromonas bestiarum* and *Ichthyophthirius multifiliis* skin infections in rainbow trout ( *Oncorhynchus mykiss*, Walbaum ), 105, 723–732. <https://doi.org/10.1111/j.1365-2672.2008.03817.x>
- Prabhurajeshwar, C., & Chandrakanth, R. K. (2017). ScienceDirect Probiotic



- potential of *Lactobacilli* with antagonistic activity against pathogenic strains : An in vitro validation for the production of inhibitory substances. *Biomedical Journal*, 40(5), 270–283. <https://doi.org/10.1016/j.bj.2017.06.008>
- Purwati, E., S. Syukur, dan Z. Hidayat. 2005. *Lactobacillus sp.* Isolasi dari Bivicophitomega sebagai Probiotik. Di dalam Proceeding Lembaga Ilmu Pengetahuan Indonesia, Jakarta.
- Purwati, E., Hellyward, J., & Purwanto, H. (2018). Isolation , Characterization And Identification Of Dna ' S Lactic Acid Bacteria ( Lab ) Of Dadih Lintau ( Tanah Datar District ) Which Is Antibiotic Resistance, (2), 28–31.
- Shokryazdan, P., Sieo, C. C., Kalavathy, R., Liang, J. B., Alitheen, N. B., Jahromi, M. F., & Ho, Y. W. (2014). Probiotic Potential of *Lactobacillus* Strains with Antimicrobial Activity against Some Human Pathogenic Strains, 2014.
- Sikorska, H., & Smoragiewicz, W. (2020). International Journal of Antimicrobial Agents Role of probiotics in the prevention and treatment of meticillin-resistant *Staphylococcus aureus* infections. *International Journal of Antimicrobial Agents*, 42(6), 475–481. <https://doi.org/10.1016/j.ijantimicag.2013.08.003>
- Syukur, S., Yolanda, S., & Fachrial, E. (2015). Isolation , antimicrobial activity and bioremediation of heavy metal Cadmium ( Cd ) by using lactic acid bacteria from Dadih Origin Lareh Sago Halaban , *Journal of Chemical and Pharmaceutical Research* , 7(9), 235–241.
- Weber, S., Mawdsley, E., & Kaye, D. (2020). Antibacterial Agents in the Elderly. <https://doi.org/10.1016/j.idc.2009.06.012>