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Antibiotic-Resistant Lactic Acid Bacteria *Pediococcus acidilactici* Isolated from Buffalo's Milk Fermentation (Dadiah) Bukittinggi, West Sumatera

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Abstract

The use of antibiotics is still carried out in the health world so that it will cause a big problem, namely antibiotic resistance. The use of antibiotics has also been limited but cannot be stopped. The use of probiotics as therapy in the world of health is increasing every year, so it is necessary to study the combination of antibiotics and probiotics so as not to reduce the effectiveness of probiotics later. This study aims to determine the resistance ability of lactic acid bacteria (LAB) isolates from Buffalos Milk Fermentation (called as dadiah) Bukittinggi against antibiotics. This research uses a descriptive, experimental method where the observations made are related to the total mass of isolates per ml and the clear zone of LAB isolates from dadiah Bukittinggi against antibiotics. In this study, five types of LAB isolates from dadiah Bukittinggi were used, and it was found that the total mass of isolates ranged from 4-194 x10^{8} CFU/ml. In addition to antibiotic resistance, it was known that dadiah LAB isolates resisted Kanamycin and Penicillin by 75%. While on the antibiotic chloramphenicol, ampicillin and tetracyclin, the four isolates are suspect to the antibiotic. The five LAB isolates of dadiah used in the study, DS1 isolate had the best total mass and was resistant to kanamycin and penicillin

Keywords: *Antibiotic; Dadiah; Lactic acid bacteria; Probiotic; Pediococcus acidilactici*

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INTRODUCTION

In this modern era, antibiotic resistance has become a severe challenge in global health. This phenomenon is exacerbated by the presence of antibiotic resistance in various pathogenic bacteria. Research related to the use of probiotics continues to grow from characterization to potential applications and applications of probiotics in improving nutrition and health (Nasab et al., 2021). Probiotics are one of the properties of Lactic Acid Bacteria (LAB), where LAB is one of the recommended food additives because it is included in GRAS (generally recognized as safe) products and with QPS (qualified presumption of safety) status (Duche et al., 2023). Not only added to processed food, LAB has also been continued as an additive in cosmetics (skincare) to improve aging and antibacterial in skin bacteria (Amelia et al., 2021). Besides that, LAB probiotics have also been used as food supplements in the form of tablets and capsules so that they can be consumed as therapy for the prevention and treatment of diseases.

Antimicrobial resistance (AMR) is a serious economic and public health hazard. The increasing prevalence of multidrug-resistant (MDR) pathogen strains makes antibiotics less effective. Although AMR microbial infections have received much attention from the scientific community and medical organizations (with a global annual mortality rate of >700,000-1 million and expected to reach 10 million by 2050) (Tang et al., 2023), there is ample evidence of ongoing gene exchange between pathogenic strains and apparently, benign or even mutually beneficial species. Therefore, these species are now considered "reservoirs" of antibiotic resistance genes (ARGs), which have the potential to spread and eventually share with pathogens or pathobionts through various pathways (Daniali et al., 2020.; Wong et al., 2022).

Isolating probiotic bacteria from traditional fermented foods and dairy products is a routine practice. Research developments related to LAB isolation continue to grow, and various types and strains have been discovered that have their advantages depending on the source of isolation; human-derived LAB strains from milk and newborn fecal samples may be more resistant to intestinal and gastric stress factors, making them better candidates for probiotic use (Panwar et al., 2021; Thakur et al., 2016). One of the foodstuffs that can be used as a source of probiotics is curd or "native community called as Dadiah", dadiah is a traditional food from West Sumatra made from buffalo milk fermented in bamboo tubes for 2-3 days at room temperature. The results of this spontaneous fermentation certainly have a variety of LAB microbiota bioversity derived from buffalo milk (Melia et al., 2018) and from the bamboo tube itself.

LAB isolation from dadiah of Bukittinggi City obtained *Pediococcus acidilactici* DS1, which has been characterized and has potential as a probiotic because it has resistance to gastric pH and is resistant to bile salts and also has good antimicrobial activity against pathogenic bacteria (Dewi et al., 2023). Research on antibiotic resistance in *P. acidilactici* from buffalo milk dadiah is essential because of its public health implications and the sustainability of the food industry. Understanding the mechanism of antibiotic resistance in this microorganism will provide better insights into managing health risks related to the use of dadiah as a food product, as well as making an important contribution to efforts to control antibiotic resistance more broadly. So, this research aims

to determine the antibiotic resistance of *P. acidilactici* BK01 bacteria isolated from Bukittinggi City to be applied in the world of health in the future.

METHOD

The research was conducted at the Livestock Product Biotechnology Laboratory, which is part of the Faculty of Animal Husbandry, Universitas Andalas. This study used *Pediococcus acidilactici* isolate (Dewi et al., 2023), which is now stored in the Laboratory of Biotechnology of Animal Products, Faculty of Animal Husbandry, Universitas Andalas. The materials used in this study include de Man, Rogosa, and Sharpe Agar (Merck), de Man, Rogosa, and Sharpe Broth (Merck), antibiotics (Kanamicyn, Ampicillin, penicillin, chloramphenicol, tetracycline), 70% and 96% alcohol, distilled water, aluminum foil, label paper, and plastic.

Preparation of LAB Cultures

Enrichment was carried out by taking 1 ml of storage culture stock and putting it into a test tube containing 9 ml of MRS broth. Then, the bacterial culture was incubated at 37^0 C for 24 hours under airtight conditions in an anaerobic jar. After that, the bacterial culture was ready for analysis.

Total Mass of Dadiah LAB

The total mass of LAB was determined using the method of Purwati et al., (2005). One ml of the isolate was dissolved in 9 mL of de Man, Rogosa and Sharpe (MRS) broth solution and vortexed until well mixed. The resulting dilutions were collected in 100µL Eppendorf tubes containing 900µL MRS Broth solution and vortexed until homogeneously mixed. Up to 10-7, all serial dilutions were performed with the same solution. On a petri dish containing MRS Agar, 100 µL of the 10-7 dilution was spread and leveled with a sterile hockey stick, then the inoculum was kept in an anaerobic container, which was incubated in an incubator for 48 hours at 37°C. Counting was done using a Colony Quebec Counter, which was used to count the number of colonies that developed. The results were given in log CFU/mL.

Antibiotic-Resistant

The study used a modified method by Ammor et al., (2007). The antibiotic susceptibility of the six isolated LAB strains was evaluated using the standard disc diffusion method. Briefly, 40 µl of each isolate was spread evenly on MRS agar using the pure plate method and left at room temperature for 15 minutes. A disc containing the antibiotic was placed on top of the MRS agar. The diameter of the inhibition zone was measured after 16 hours of incubation at 37 C. Resistance tests were conducted with five different antibiotics, including kanamicyn (30 µg), ampicillin (10 µg), penicillin (10 µg), chloramphenicol (30 µg), and tetracycline (30 µg).

Data analysis

The results of this study will be interpreted descriptively with a quantitative approach related to the total mass and clear zone area of antibiotic-resistant LAB.

RESULTS AND DISCUSSION

Total Mass of Lactic Acid Bacteria

Calculating the total mass of dadiah LAB was done to determine the amount of LAB per ml so that the LAB concentration to be applied in the product or as a therapy in experimental animals is known.

Table 1. The Total Mass of Dadiah LAB Isolate

Sample	Total LAB (x10^8 CFU/ml)
DS1	194
DS2	85
DS3	121
DS4	23
DS5	4

It can be seen that the total mass of LAB dadiah isolates ranged between 4 - 194 x10^8 CFU/ml; the results obtained from the study showed that 4 out of 5 isolates were following the standards set by FAO (2001) and WHO (2002) as a source of probiotics has established criteria that must be met by strains classified as probiotic organisms has a minimum number of live bacteria of at least 10^6-10^7 CFU/g. More precisely, such microorganisms must not cause disease, which means they must have a GRAS (generally regarded as safe) classification (Land et al., 2005). To achieve health benefits, it is essential to provide a minimum amount of 10^8 -10^{11} CFU (colony-forming units) of bacterial or yeast cells in a daily dose (Binda et al., 2020). Only one isolate (DS5) did not comply with standards, so only four isolates will be used for further analysis.

Nevertheless, as previously mentioned, the stability of probiotic preparations is often compromised by factors associated with the technological manufacturing process, ripening, and storage. Furthermore, it is crucial to ensure the viability of probiotics and preserve their functional qualities during the manufacturing process and storage period to achieve the desired probiotic impact in the gastrointestinal tract (Roos et al., 2002).

Antibiotic Resistance

Figure 1 shows the results obtained in this study, with some isolates being resistant to several antibiotics. For the assessment of bacteria used as feed additives, strains can be categorized as susceptible or resistant to antimicrobials. Susceptible (S): A bacterial strain is defined as susceptible if it is inhibited at a concentration of the specific antimicrobial that is equal to or lower than the set cut-off value (S ≤ x mg/L). Resistant (R): A bacterial strain is defined as resistant if it is not inhibited at a concentration of the specific antimicrobial that is higher than the set cut-off value (R > x mg/L). Interpretation of antibiotic resistance results refers to the Guidelines for the assessment of bacterial

susceptibility to antimicrobials of importance to humans and animals (FEEDAP, 2012) and by considering the type of bacteria according to (Cavalieri et al., 2005).

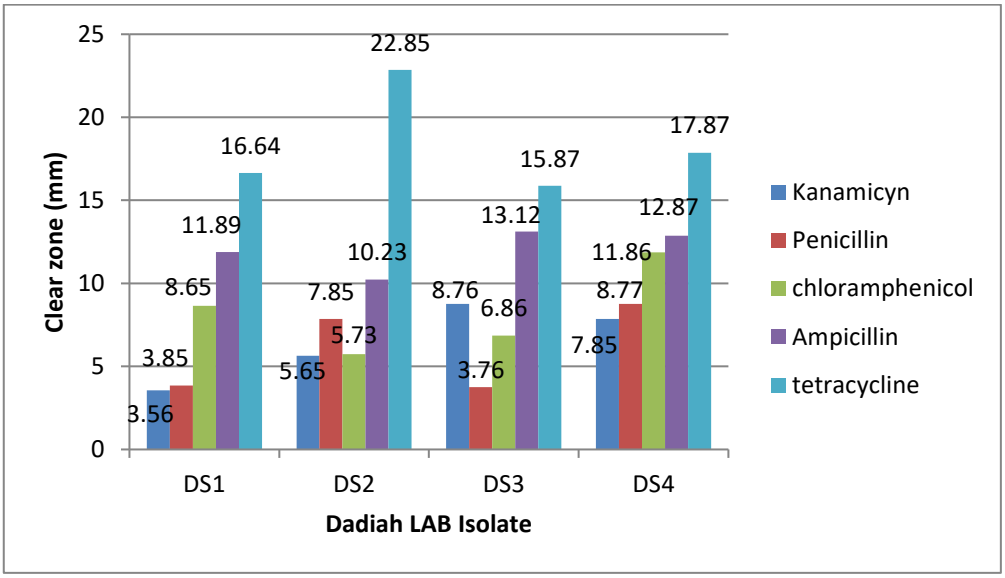

Figure 1. Inhibition Zone Graph of Dadiah LAB Isolates Against Antibiotics

The interpretation of figure 1 can be seen in table 2. The cut-off values identified should be seen as a pragmatic response intended to introduce consistency in the separation of resistant from susceptible strains. Limit values are not designed for any purpose other than the assessment of microbial products for the possible presence of antimicrobial resistance (FEEDAP, 2012).

Table 2. Antibiotic Resistance of Dadiah LAB

Isolate	Antibiotic disc				
	Kanamicyn	**Penicillin**	**Cloramfenikol**	**Ampicillin**	**Tetracyclin**
DS1	R	R	S	S	S
DS2	R	R	S	S	S
DS3	R	R	S	S	S
DS4	S	S	S	S	S

Desription: R= resistant ; S= susceptible

It can be seen from table 2 that the LAB isolate dadiah has resistance to the antibiotics Kanamycin and Penicillin 75%. While on the antibiotics chloramphenicol, ampicillin and tetracyclin, the four isolates are suspect to these antibiotics. This is because each isolate is strain dependent, or the ability of each strain to produce metabolism according to the strain and also the source of isolation. The occurrence of antibiotic resistance among LAB from food sources usually varies among different studies. Firstly, other studies applied various methods to evaluate antibiotic resistance, including E-test, agar dilution, micro broth culture, and disc diffusion, the results of which cannot be directly compared (Ammor et al., 2007). In addition, culture conditions such as culture medium or inoculum volume can also affect susceptibility testing and its results. In

addition, the location of specific resistance genes (in chromosomes on plasmids) Gevers et al., (2003) or the involvement of other non-specific mechanisms may also explain the different results.

It can be seen in Figure 1 that all four isolates are suspect to tetracycline; tetracycline has many properties considered ideal for antibiotic drugs, including activity against Gram-positive and harmful pathogens, proven clinical safety, acceptable tolerability, and availability of intravenous (IV) and oral formulations for most class members. Like all classes of antibiotics, the antimicrobial activity of tetracyclines is subject to class-specific and intrinsic antibiotic resistance mechanisms (Grossman, 2016). This is what makes the dadiah isolate suspect to this antibiotic. Flórez et al. (2005) also examined the antimicrobial resistance (AR) of lactic acid bacteria (LAB) in traditional Spanish blue vein Cabrales cheese. According to the findings, all *Lactobacilli* and *Leuconostoc* isolates were resistant to high concentrations of vancomycin. In addition, certain strains of *L. lactis, Enterococcus* spp, and *Lactobacillus* sp. They have shown resistance to antibiotics such as chloramphenicol, erythromycin, clindamycin, and tetracycline.

Investigations conducted by Herreros et al. (2005) identified AR LAB extracted from Armada cheese. According to their findings, none of the strains showed complete susceptibility to all the drugs tested, and they noted the presence of some resistance. In addition, most of the organisms tested showed resistance to cefotoxin, oxacillin, vancomycin, teicoplanin, nitrofurantoin and trimethoprim. This study showed significant similarities with our research in terms of multiple resistance (Erginkaya et al., 2018). In addition, the antibiotic resistance of probiotic bacteria (AR) was also examined, which showed that certain probiotic bacteria exhibited antibiotic resistance. This creates the potential for transfer of resistance from probiotics to human pathogenic bacteria, either through direct contact or indirectly through commensal flora (D'aimmo et al., 2007; Temmerman et al., 2003).

Based on the above investigations, it is evident that AR characteristics may vary depending on the product type or LAB species. Comprehensive investigations should be conducted to examine the characteristics of antibiotic resistance (AR) and identify the origin of bacteria exhibiting AR. In addition, these findings indicate the need for regular antibiotic susceptibility testing on food-associated microbes. The food chain is widely recognized as a major pathway through which antibiotic-resistant microorganisms are transmitted between animal populations and humans. More precisely, dairy products that undergo fermentation but do not undergo heat treatment before consumption serve as a means for antibiotic-resistant bacteria to be transmitted from the animals' natural microbial population to the human gastrointestinal tract.

The current analysis identified dairy products containing multi-resistant bacterial isolates. Findings from the current investigation may help reduce the spread of bacterial resistance. Natural fermentation makes these traditional dairy products very rich in lactic acid bacteria (LAB). The formation of these LABs is uncontrolled, which raises concerns about the spread of AR. Due to the unknown nature of LAB in these traditional dairy products, they could contain AR LAB. Therefore, precautions need to be taken for conventional milk production.

Antibiotic-resistant LAB may be beneficial for patients suffering from antibiotic-induced diarrhea as these strains can survive better under antibiotic stress and contribute to the maintenance of gastrointestinal stasis (Eaton & Gasson, 2001). However, from a food safety perspective, LAB used in food fermentation should not be resistant to antibiotics. When resistant LAB are used as probiotics or starter cultures, a large number of cells enter the human gut and interact with the native gut microbiota. Previous studies have shown that antibiotic-resistant genes show the potential to be transferred to commensal bacteria or enteric pathogenic bacteria through horizontal gene transfer (Mathur & Singh, 2005; Salyers et al., 2004) and may pose a serious threat to food safety and public health. To prevent the unwanted transfer of resistant genes, LAB used in the food industry should not carry resistances other than those specifically required. Therefore, regular examination of the antibiotic resistance profiles of commonly used LAB strains is essential (Wang et al., 2019). Based on research conducted by Dewi et al. (2023) through molecular analysis, it is known that isolate DS1 has a close kinship with *Pediococcus acidilactici* according to the phylogenetic analysis conducted.

CONCLUSION

The Isolate of lactic acid bacteria (LAB) from buffalos milk fermentation (dadiah) Bukittinggi had resistance to the antibiotics Kanamycin and Penicillin 75%. While on the antibiotic chloramphenicol, ampicillin and tetracyclin, the four isolates are suspect to the antibiotic. The five dadiah LAB isolates used in the study, DS1 isolate had the best total mass and was resistant to kanamycin and penicillin. Lactic acid bacteria isolates from dadiah Bukittinggi can be resistant to antibiotics, especially kanamycin and penicillin. so this dadiah isolate can be used as therapy for patients in conjunction with antibiotics.

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