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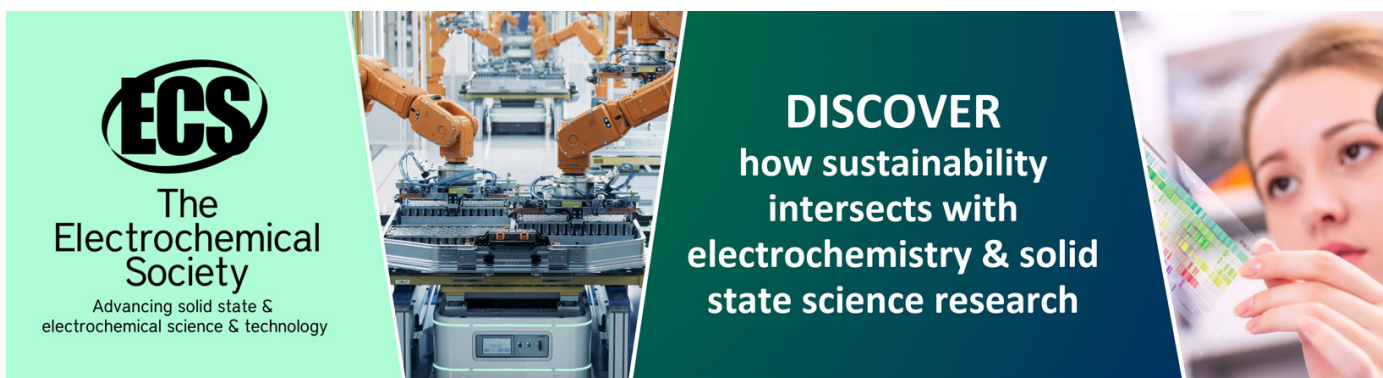
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Antibiotic resistance of *Lactobacillus fermentum* isolates of dadiah Lintau

R Amelia^{1*}, H Harun², Y E Pratama³ and I Suryanis⁴

¹ Faculty of Medicine, Baiturrhamah University, Padang, Indonesia

² Faculty of Medicine, Andalas University University, Padang, Indonesia

³ Faculty of Animal Science, Andalas University, Padang, Indonesia

⁴ Faculty of Health Science, Baiturrhamah University, Padang, Indonesia

Corresponding author : rinitaamelia@gmail.com

Abstract. Antibiotic resistance is one of the tests that Lactic Acid Bacteria must pass in order to boost their potential as probiotics. This study aims to determine the resistance of lactic acid bacteria (LAB) isolate *Lactobacillus fermentum* to tetracycline, chloramphenicol, ampicillin, kanamycin, and penicillin and to measure levels of resistance. The disc diffusion method was used to assess the zone of inhibition of LAB growth against antibiotics, and a quantitative descriptive method was used to analyze the results. According to the study's findings, *L. fermentum* had an overall mass of 86×10^8 CFU/ml and was strongly resistant to the medicines penicillin, tetracycline, and chloramphenicol, with only modest resistance to amoxicillin and kanamycin.

Keywords: Antibiotic, Dadiah, *Lactobacillus fermentum*, Resistance

1. Introduction

Lactic acid bacteria (LAB) are single-celled organisms and include gram-positive bacteria and facultative anaerobes, which means they do not form spores, form rods, and cocci and are capable of producing lactic acid as a result of their cell metabolism [1]. Probiotic microorganisms such as *Bifidobacterium* and lactic acid bacteria (LAB) can colonize the intestine, maintain the balance of the intestinal microflora, as well as produce antimicrobial compounds (bacteriocins). In that case, they will have a positive impact on health. [2]. Before becoming a probiotic bacteria, LAB will undergo several tests in vitro to increase its potential as a probiotic, including the resistance test to gastric acid [3], the bile salt resistance test [4], the antimicrobial activity test against pathogenic microorganisms [5], and also its resistance to antibiotics. One of the important tests in increasing the potency of BAL is resistance to antibiotics. Probiotic bacterial strains resistant to antibiotics are very useful for restoring the gut microbiota after using antibiotics [6]. *Lactobacillus fermentum* isolated from dadiah has gone through a series of characteristic tests as a probiotic candidate [7], so it is necessary to carry out further analysis regarding the resistance to the isolated antibiotic.



Antibiotic resistance is a normal adaptation mechanism due to antibiotic exposure, which causes a bacterium to become resistant to one or more antibiotics [8]. Antibiotic resistance increased significantly due to errors or overuse of antibiotics. It was further explained that errors and excess use of antibiotics could lead to 2 (two) things: selection pressure on antibiotic-receiving bacteria to produce compounds that can be used to survive against antibiotics [9].

In addition, natural adaptations will occur in the recipient bacteria, eventually making the bacteria resistant and passing their resistance to certain antibiotics to their offspring. Scott [10] reported the emergence of antibiotic-resistance genes in bacteria isolated from various sources. This finding is very worrying because Schjorring and Krogfelt [11] found that these resistance genes can be transferred in vivo from antibiotic-resistant pathogenic bacteria to normal flora that live and develop in the digestive tract. If this happens, the emergence of antibiotic-resistant microbes will develop and expand so that they can become a serious threat to human health, especially when health conditions decline. The immune system does not function properly.

Bacterial resistance genes to antibiotics have been found in various sources. According to Imperial et al. [12], the spread of antibiotic-resistance genes can occur between probiotic, commensal, and probiotic bacteria pathogens in the digestive tract and can be a worrying problem. *Lactobacillus acidophilus* isolated from broiler feces is known to be resistant to the antibiotics nalidixic acid, gentamicin, ciprofloxacin, sulfamethoxazole, kanamycin, streptomycin, and vancomycin based on the inhibition zones they produce [13]. According to Sieo et al. [14], from several *Lactobacillus acidophilus* isolates taken from the digestive system of chickens, *Lactobacillus* sp. Resistant to 100% chloramphenicol, 58% erythromycin, and 17% tetracycline. *Lactobacillus* sp. Of those isolated from the cloaca of broiler chickens, 78% were resistant to macrolide antibiotics, and 87% were resistant to lincosamide. This study aims to determine the antibiotic resistance of *Lactobacillus fermentum* isolates isolated from Lintau dadiah

2. Material and Method

This research was conducted in the Animal Products Biotechnology Laboratory, Faculty of Animal Husbandry, Andalas University. The material used in this study was *Lactobacillus fermentum* (Amelia, 2021) isolate, which is stored at the Animal Products Biotechnology Laboratory, Faculty of Animal Husbandry, Andalas University. Material used in this research de Man, Rogosa, and Sharpe Agar (Merck), de Man, Rogosa, and Sharpe Broth (Merck), antibiotics (Kanamycin, Ampicillin, penicillin, chloramphenicol, tetracycline), alcohol 96%, alcohol 70%, H₂O₂ (3%), distilled water, aluminum foil, paper tag, and plastic.

2.1. Enrichment Lactic Acid Bacteria.

Enrichment of *L. fermentum* isolates was carried out by inoculating 1 ose of culture into the main culture into 5 ml of sterile MRSB media, then incubating at 37°C for 24-48 hours. The results are indicated by the presence of turbidity in the media [15].

2.2. Resistance Antibiotic Test

Resistance test of Lactic Acid Bacteria *Lactobacillus fermentum* from Dadiah Lintau used the well diffusion method modification Yang and Ranalla, [16]: As much as 20 ml of MRS Agar medium was added with 0.2% lactic acid bacteria, rejuvenated, and allowed to stand until it hardened in a petri dish. Then, the antibiotic disc was placed on the surface of the MRS agar. After that, it was incubated at 37°C. Observations were made on the clear zone, a circle, after 48 hours using a caliper. The results of bacteria sensitive to antibiotics were declared if the inhibition zone was formed of ≥ 21 mm with code S; intermediate (+++) with an inhibition zone of 16-20 mm (++) with code I; and resistant if the zone of inhibition is ≤ 15 mm (+) with code R [17].

3. Result and Discussion

In this study, it was found that the total mass of lactic acid bacteria from *L. fermentum* Dadiah Lintau was 86×10^8 CFU/ml. meaning that in 1 ml of *L. fermentum* there are up to $\times 10^8$ CFU/ml of bacterial mass present.

The most crucial aspect to consider when assessing the safety of probiotics is sensitivity to antibiotics. Application of probiotics could increase the risk of antibiotic resistance due to evidence of horizontal antibiotic resistance gene transfer between *Lactobacillus* and *Enterococcus faecalis* in vitro. [18]. The following table shows the antibiotic resistance of *Lactobacillus fermentum* isolated from dadiah Lintau.

Table 1. Antibiotic resistance to *Lactobacillus fermentum*

Isolate	Kanamycin	Ampicilin	Penicilin	chloramphenicol	tetracycline
<i>L. fermentum</i>	R	R	R	R	R

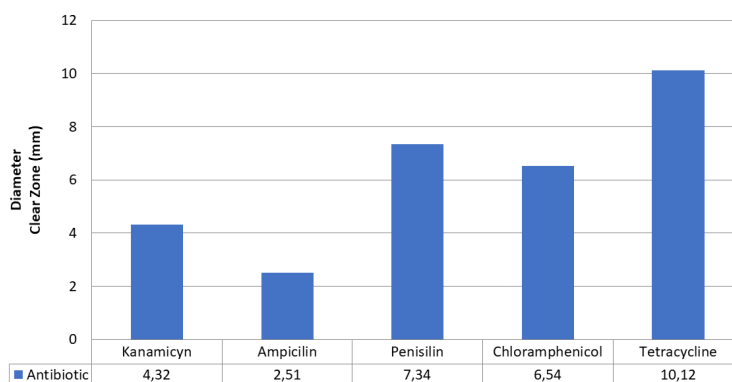


Figure 1. Graph of the clear zone area of *Lactobacillus fermentum* Dadiah Lintau against antibiotics

Antibiotics are a type of antibacterial compound, both natural and synthetic, that suppresses or stops a biochemical process in organisms [19]. Some examples of antibiotics often used are amoxicillin, tetracycline, and chloramphenicol. Antibiotics amoxicillin is a type of beta-lactam antibiotic with a broad spectrum because it can inhibit the growth of or kill Gram-positive and Gram-negative bacteria [19]. Antibiotic resistance in bacteria is the ability of bacteria to resist the effects of one or more antibiotics, which were previously sensitive to these antibiotics. The incidence of antibiotic resistance is said to be a widespread epidemic. This is because the incidence of antibiotic resistance has consequences in the form of increased morbidity and mortality, as well as increasing the duration and cost of the healing process due to infectious diseases [20].

In the process of becoming resistant to antibiotics, *Lactobacillus* sp. can do mechanisms intrinsically or naturally as well as acquired or secondary where *Lactobacillus* sp. mutation or receive resistance genes from other microorganisms. Intrinsically or naturally, *Lactobacillus* sp. already has resistance genes in their chromosomes, where these genes can be passed on to their off spring [21]. Meanwhile, the discovery of resistance genes makes it possible for *Lactobacillus* sp. to do several things; it first encodes an enzyme that can destroy the antibiotic agent before it can take effect. Second, efflux pumps remove the antibiotic agent from the cell before it can reach the target site and exert its effect. Third, the metabolic pathway that makes up the cell wall *Lactobacillus* sp., no longer has a place for agent-binding antibiotics or limiting antibiotic agent entry in intracellular targets through regulation of porin genes [22].

In Figure 1, it can be seen that *L. fermentum* is resistant to the antibiotic Canamicyn 4.3 mm according to Vlkova et al. [17], including resistance to Canamycin; this is because Canamycin is an

aminoglycoside class antibiotic; the mechanism of action is to bind to the 30S sub-unit of the ribosome so that the 70S sub-unit is not formed which causes inhibition of protein synthesis. In general, antibiotics of the aminoglycoside group have a broad spectrum of antimicrobial activity, and the aminoglycoside-inactivating enzymes are relatively active against most gram-negative aerobic bacilli. [23]. The resistance of gram-positive bacteria is due to gene modification carried out by the target bacteria (Ozteber, 2017) [24] and mutation of target genes in the bacterial cell membrane, which results in impaired inter-cell transport [25]. Also found gene *bifunctional aac(6')* *le-aph(2'')* He; the presence of this gene causes resistance to almost all aminoglycosides except streptomycin [26].

In Table 1, *L. fermentum* is resistant to ampicillin, with a clear zone (Figure 1) of up to 2.5 mm. The resistance of *L. fermentum* to ampicillin was the lowest compared to other antibiotics in this study. Antibiotic resistance of each genus and species of LAB has different abilities depending on the isolate's source and the environment in which the bacteria have adapted. Penicillin G and ampicillin resistance vary by species, with the *Lactocaseibacillus casei* strain (91.7%) having significantly greater resistance than the *L. brevis* strain (45.5%) and the *L. paracasei* (33, 3%). Amoxiclav showed a tendency that was comparable to this. Additionally, reports of significant levels of penicillin antibiotic resistance for *L. paracasei*, *L. casei*, *L. brevis*, and *L. plantarum* have been recorded. [27].

The research results found that *L. fermentum dadiah* Lintau was resistant to penicillin, as shown in Figure 1. It can be seen that it has a clear zone of 7.34 mm. *Lactobacillus* is generally susceptible to antibiotics, such as penicillin (ampicillin, oxacillin, and piperacillin), β -lactamase inhibitors, and cephalosporins (cephalothin and cefuroxime, ceftriaxone and cefoxitin), but in recent years several authors have reported resistance to penicillin G in several strains of *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, and *Lactobacillus plantarum* [28,29]. The results of research by Hummel [27] show that BAL starters or probiotic strains belonging to the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Streptococcus* are generally quite sensitive to clinically relevant antibiotics such as penicillin G, ampicillin, tetracycline, erythromycin, and chloramphenicol. This resistance was lower than 10% of the isolates studied [30], although Zarazaga et al. [31] reported a very high incidence of 26.2% of *Lactobacillus* isolates that were resistant to penicillin.

The ability of bacteria to be resistant to antibiotics chloramphenicol can be caused by the genes owned by the cell. This gene can cause bacteria to produce the enzyme chloramphenicol acetyltransferase, which can inactivate antibiotic compounds that enter cells before inhibition of protein synthesis occurs in bacterial cells [32]. This research shows that *L. fermentum* is resistant to chloramphenicol, whereas, in Figure 1, the resulting clear zone is 6.54 mm. LAB with the genus *Lactobacillus* generally has no innate resistance to antibiotics chloramphenicol [28]. However, studies have been carried out to determine the resistance of BAL to antibiotic chloramphenicol in several types of local Indonesian food such as bekasam, tempoyak, dadiah, and tape ketan which showed that of the 120 samples of LAB isolates tested, it was found that as many as 54 isolates had the gene *cat* which indicates that the BAL has a certain resistance to antibiotics chloramphenicol [33].

Hummel et al. [27] have found that gene *cat* can be owned by isolates of *Lactobacillus*, which are sensitive to chloramphenicol. However, gene *cat* properties possessed by bacteria are usually invisible in terms of RNA, both in conditions that can encourage the operation of this gene or not. In research by Dec et al. [34], 23% of 88 *Lactobacillus sp.* were resistant to the antibiotic chloramphenicol, with 37.5% of the 88 bacteria being detected to have the gene *cat*, which codes for the production of enzymes chloramphenicol acetyltransferase. Chloramphenicol acetyltransferase converts chloramphenicol to an inactive form, diacetyl chloramphenicol [34]. In Campedelli et al. (2018) [35], 49% of 182 *Lactobacillus sp.* Resistant against chloramphenicol with *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus manihotivorans*, and *Lactobacillus perolens* has the highest prevalence rate. Based on this research, *Lactobacillus sp.*, which is resistant to antibiotics, chloramphenicol was detected to have a resistance gene *cat* and *CmlA* located on the transposon and plasmid. In Souza et al. [36], 6 out of 37 (16.2%) *Lactobacillus sp.* were resistant to the antibiotic chloramphenicol. On Shao et al. [37], 12 of 32 *Lactobacillus sp.* resist the antibiotic chloramphenicol.

Teared *L. fermentum* on tetracycline, which is 10.12 mm (figure 1) is included in the classification of resistant to antibiotics [17]. The ability of BAL to be resistant to antibiotics is due to the presence of certain genes that increase the resistance of BAL to antibiotics. The existence of a genetic code that can increase the possibility of lactic acid bacteria cells to survive can be why lactic acid bacteria are resistant to this antibiotic, Tetracycline [38]. In Campedelli et al. [34], 50% of 182 *Lactobacillus sp.* are resistant to tetracycline antibiotics. Based on this research, *Lactobacillus sp.* resistance to tetracycline antibiotics detected resistance genes tet (M), tet (S), tet (Q), and tet (W) as ribosomal protection protein; also tet (L) and tet (P) play a role in efflux pump. In Souza et al. [35], 50% of 37 *Lactobacillus sp.* were resistant to tetracycline antibiotics with the detection of resistance genes tet (M) and tet (S) as as ribosomal protection proteins. In research by Dec et al. [33], 74% of 88 *Lactobacillus sp.* resistant to tetracycline antibiotics detected the tet resistance gene (L), which plays a role in efflux pump also tet (M) and tet (W) as ribosomal protection protein. On Preethi et al. [39], 14 With the discovery of the tet resistance gene (L), which functions in the efflux pump and tet (M) as ribosomal protective protein, fourteen different species of *Lactobacillus. sp* are resistant to tetracycline drugs. The resistance gene was found in mobile elements transposons and plasmids [34].

Knowledge about antibiotic resistance in LAB still needs to be improved, probably due to the many species found in this genus LAB and differences in the spectrum of resistance. It is necessary to carry out in-depth identification and research on BAL resistance to antibiotics so that BAL can be optimally used as a therapy for patients who use antibiotics in their treatment and therapy. *L. fermentum* is an established probiotic candidate that can be used in yogurt production. Aside from that, *L. fermentum* can support therapy in health by being resistant to an antibiotic, one of which is to prevent and treat childhood stunting.

4. Conclusion

Resistance of *Lactobacillus fermentum* isolated from *dadih* Lintau to several antibiotics. This study found that *L. fermentum* had strong resistance to the antibiotics chloramphenicol, tetracycline, and penicillin. As well as moderate resistance to the antibiotics kanamycin, ampicillin

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