Pro Food (Jurnal Ilmu dan Teknologi Pangan)
Volume 11, Nomor 2, November 2025, pp. 214 - 226
ISSN 2443-3446 (Online), ISSN 2443-1095 (Print)
doi: https://doi.org/10.29303/profood.v11i2.540

IDENTIFICATION OF LACTIC ACID BACTERIA POTENTIAL FROM WILD HORSE MILK *DANGKE* OF WEST NUSA TENGGARA AS INDIGENOUS PROBIOTIC CANDIDATES

[Identifikasi Potensi Bakteri Asam Laktat dari Dangke Susu Kuda Liar di Nusa Tenggara Barat Sebagai Kandidat Probiotik Lokal]

Husnita Komalasari[1]✉, Chairul Anam Afgani[2], Astri Melandani[1]

[1]Department of Food Technology, Faculty of Engineering, Bumigora University,West Nusa Tenggara, Indonesia

[2]Department of Technology of Agricultural Products, Faculty of Agricultural Sciences and Technology, Sumbawa University of Technology, West Nusa Tenggara, Indonesia

ABSTRACT

Dangke is a traditional food from Enrekang, South Sulawesi, and in the West Nusa Tenggara (NTB) region, *dangke* is also produced from local food which is wild horse milk. This study aimed to identify and characterize the microbiological profile of *dangke* of NTB to assess its potential as an indigenous probiotic candidate. The research used an experimental method with a Completely Randomized Design (CRD), considering the area of sample origin as the factor with Tukey's post-hoc test was applied at a 5% significance level. Multivariate Principal Component Analysis (PCA) was conducted using XLSTAT to map the microbiological characteristics. The results showed that sampling areas significantly affected microbiological properties such as total lactic acid bacteria (LAB), growth tolerance to bile salt, NaCl, and pH 2.5, as well as antimicrobial activity against E. *coli* and S. *aureus*. PCA revealed that *dangke* from Bima and Sumbawa had similar characteristics, which differed from those of Lombok and Dompu. These findings indicated that LAB from wild horse milk *dangke* had potential as indigenous probiotic candidates. In conclusion, this study identified and mapped the regional differences in *dangke* characteristics and highlighted their probiotic potential.

Keywords: *Dangke*, lactic acid bacteria, principal component analysis, probiotic indigenous

ABSTRAK

Dangke adalah makanan tradisional dari Enrekang, Sulawesi Selatan. Di wilayah Nusa Tenggara Barat (NTB), dangke juga diproduksi dari bahan makanan lokal berupa susu kuda liar. Penelitian ini bertujuan untuk mengidentifikasi dan mengkarakterisasi profil mikrobiologis dangke dari NTB guna menilai potensinya sebagai kandidat probiotik asli. Penelitian ini menggunakan metode eksperimental dengan Rancangan Acak Lengkap (RAL), dengan mempertimbangkan asal daerah sampel sebagai faktor dan uji post-hoc Tukey diterapkan pada tingkat signifikansi 5%. Multivariate Principal Component Analysis (PCA) dilakukan menggunakan XLSTAT untuk memetakan karakteristik mikrobiologis. Hasil menunjukkan bahwa area pengambilan sampel secara signifikan mempengaruhi sifat mikrobiologis seperti total bakteri asam laktat (BAL), toleransi pertumbuhan terhadap garam empedu, NaCl, dan pH 2,5, serta aktivitas antimikroba terhadap E. coli dan S. aureus. PCA menunjukkan bahwa dangke dari Bima dan Sumbawa memiliki karakteristik serupa, yang berbeda dari dangke Lombok dan Dompu. Temuan ini menunjukkan bahwa BAL dari dangke susu kuda liar memiliki potensi sebagai kandidat probiotik lokal. Kesimpulannya, penelitian ini mengidentifikasi dan memetakan perbedaan regional dalam karakteristik dangke serta menyoroti potensi probiotiknya.

Kata Kunci: *Bakteri asam laktat, dangke, principal component analysis, probiotik lokal*

✉ ***Corresponding Author:***
Husnita Komalasari
Bumigora University
Email: husnita@universitasbumigora.ac.id

INTRODUCTION

The word "probiotic" comes from the Greek word meaning "for life". This term was first used by Fuller (1992) to describe the secret of the substance with a microorganism that stimulates the growth of other beneficial microorganisms. Probiotics are living microorganisms that, if consumed in sufficient quantities, can provide health effects to their hosts (Komalasari et al., 2025). Several types of microorganisms have the potential as probiotics that have been successfully isolated from milk, namely *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Streptococcus thermophilus* (Nath et al., 2020; Taye et al., 2021; Yerlikaya, 2019).

Effective probiotics must have several criteria, such as providing beneficial effects on the host, being non-pathogenic and non-toxic, containing a large number of living cells, being able to survive and carry out metabolic activities in the intestine, remaining alive during storage and use, having good sensory properties, and being isolated from the host (Komalasari et al., 2025).

Probiotics are closely related to lactic acid bacteria (LAB), some types of LAB that are included in the probiotic group are *Lactobacillus* (*Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus casei), Bifidobacterium (Bifidobacterium bifidum, Bifidobacterium longum), Streptococcus thermophilus, Lactococcus lactis,* and *Enterococcus faecium* (Mulaw et al., 2019; Reuben et al., 2020; Silva et al., 2023; Tarique et al., 2022; Yang & Yoon, 2022; Zakariah, 2020). LAB is a group of microorganisms known to have the ability to improve digestive health and the immune system. These bacteria are widely found in fermented products such as yogurt, cheese, and other fermented drinks.

Based on research by Reuben et al., (2020) LAB is also found in many fresh products such as milk and dairy products. One of the dairy products is *dangke*, a traditional food from the Enrekang area, South Sulawesi, which is often referred to as local cheese. *Dangke* is processed enzymatically by adding papaya sap to milk and through a heating process. *Dangke* contains water, fat, protein, carbohydrates, ash, minerals, and vitamins (Arini et al., 2016; Komalasari et al., 2025).

In the West Nusa Tenggara region, local people also produce *dangke* but with different raw materials, namely wild horse milk. Wild horse milk is one of the typical products of West Nusa Tenggara. Several studies have stated that consuming wild horse milk can cure various diseases (Prastyowati, 2021). The microorganisms found in NTB wild horse milk are *Lactobacillus, Ochrobacterium, Staphylococcus, Enterococcus sp., Lactococcus, and Dipodascaceae* family (Jatmiko et al., 2017; Kusdianawati et al., 2020; Mulyawati et al., 2019; Suryanti et al., 2019).

The composition of milk in mammal species is very diverse because it can be influenced by several factors (Malacarne et al., 2002; Minjigdorj et al., 2012; Wei et al., 2021). This is supported by the results of research stating that Sumbawa horse milk is dominated by *Lactobacilli* and *Weisella* or *Leuconostoc* bacteria (Suryani & Gaffar, 2024). Meanwhile, Bima wild horse milk contains *Lactobacillus acidophillus*, *Lactobacillus plantarum*, and *Lactococcus lactis* (Antara et al., 2009; Prastyowati, 2021). Based on this research, it is known that *dangke* and wild horse milk contain LAB which has the potential as a local probiotic candidate. Probiotics are live bacteria that can provide health effects to their hosts when consumed in sufficient quantities (Komalasari & Yoga, 2022). The high nutritional content in *dangke* and wild horse milk makes it a good growth medium for LAB. This study aims to investigate the potential of LAB isolated from wild horse milk *dangke* in West Nusa Tenggara as indigenous probiotic candidates. Therefore, it is important to identify and characterize LAB from wild horse milk *dangke* in NTB to evaluate their potential as indigenous probiotics.

MATERIAL AND METHOD

Sampling Method

The sample used in this study was wild horse milk *dangke* typical of West Nusa Tenggara (NTB) which was taken from 4 regions, namely Sumbawa, Bima, Dompu and Lombok which are part of the province of West Nusa Tenggara (NTB), Indonesia, using a random sampling method. The samples that had been obtained were stored at a temperature of 15°C up to 24 hours until the time of analysis was carried out. The analysis was carried out in the Food Microbiology laboratory of the University of Mataram from July to September 2024. The picture of the *dangke* sample used can be seen in Figure 1.

Figure 1. Wild Horse Milk Dangke

Isolation of Lactic Acid Bacteria (LAB)

The isolation of LAB which begins with 25 grams of sample ground with a mortar and suspended in 225 ml of buffer solution which is vortexed until homogeneous. Dilution is carried out up to 10^{-5}, tubes 10^{-3} to 10^{-5} are grown on de Mann Rogosa Sharpe Agar (MRSA) media using the streak plate and incubated at 37°C for 24 hours. Bacterial colonies that have different shapes are regrown on MRSA media using the streak plate technique to obtain pure isolates and are ready to be tested for probiotic potential (Hardiningsih et al., 1970).

Gram Staining

Gram staining begins with placing pure isolates on a glass slide containing a drop of physiological solution, followed by fixation over a flame. The preparation is dripped with crystal violet and left to stand, then washed with distilled water and given safranin dye. The preparation is rinsed again and air-dried. Cell morphology is observed using a microscope with a magnification of 1000 times. The results of gram-positive bacteria are marked with a purple color (Giyatno & Retnaningrum, 2021).

Bile Salt Tolerance Test

The bile salt tolerance test was performed by inoculating one loopful of bacterial culture into MRSB medium containing 0.5% bile salt, followed by incubating at 37°C for 48 hours. After incubation, bacterial growth was observed, and viable bacteria were quantified by colony counts (Mawardika et al., 2023).

NaCl Tolerance Test

The NaCl tolerance test was conducted by growing bacteria in MRSB medium containing 4% NaCl. Bacterial growth in the medium was observed after incubation at 37°C for 48 hours. After incubation, bacterial growth was observed, and viable bacteria were quantified by colony counts (Mawardika et al., 2023).

pH Tolerance Test

The pH tolerance test was carried out by inoculating the one loopful of MRSA bacterial culture into MRSB medium adjusted to pH 2,5 and 1. The test tubes was incubated at 37°C for 48 hours. Bacterial growth was indicated by turbidity (cloudiness) in the medium (Mawardika et al., 2023).

Antimicrobial Activity

The antimicrobial activity of the LAB isolates was tested using the well diffusion method. The cultures of pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* were compared with the Mc farland 0.5 standard and inoculated into NA (nutrient agar) plates containing wells with sterile cotton swabs. Furthermore, 100 µl of the LAB isolate was added to the wells. After incubation at 37°C for 24 hours, the diameter of the inhibition zone was calculated (Mawardika et al., 2023).

Data Analysis

The analysis method used in this study is a Completely Randomized Design (CRD), considering the area of sample origin as the factor with Tukey's post-hoc test was applied at a 5% significant level were analyzed using SPSS. In addition, to determine the profile characteristics and potential of LAB from wild horse milk *dangke*, the XLSTAT software was used with the Principal Component Analysis (PCA) method. PCA analysis is used to obtain a grouping of LAB potential from wild horse milk *dangke* samples as indigenous probiotic bacteria based on the dominant characteristics of each product.

Table 1. Average Observation Results and Significance Of Microbiological Analysis Results In Wild Horse Milk *Dangke* Samples

Sample Code	LAB (Log CFU/ml)	% Decreased Viability of Bile Salt Tolerance	% decreased Viability of NaCL Tolerance	% decreased Viability of Ph 2,5 Tolerance	% decreased Viability of pH 1 Tolerance	Antimicrobial Test of e. *coli* bacteria (cm)	Antimicrobial Test of S. *aureus* bacteria (cm)
A	$9{,}60^{C}$	$21{,}17^{A}$	$12{,}75^{A}$	$22{,}36^{C}$	100	$0{,}8^{B}$	$0{,}75^{C}$
B	$9{,}82^{B}$	$4{,}35^{D}$	$6{,}00^{D}$	$22{,}02^{D}$	100	$0{,}7^{D}$	$0{,}7^{D}$
C	$9{,}98^{A}$	$8{,}01^{B}$	$9{,}71^{B}$	$100{,}00^{A}$	100	$0{,}85^{A}$	$0{,}8^{B}$
D	$9{,}76^{B}$	$6{,}92^{C}$	$6{,}83^{C}$	$23{,}39^{B}$	100	$0{,}75^{C}$	$0{,}9^{A}$

Description: Numbers followed by different letters in the same column indicate a significant difference at the 5% level

Table 2. Results of Observation of Morphological Colony Identification Analysis

Sample	Macroscopic				Microscopic	
	Shape	Color	Edge	Elevation	Shape	Color
A	Round	White	Flat	Convex	Basil	Purple
B	Round	White	Flat	Convex	Basil	Purple
C	Round	White	Flat	Convex	Basil	Purple
D	Round	White	Flat	Convex	Basil/coccus	Purple

RESULT AND DISCUSSION

Results

The potential of LAB from wild horse milk *dangke* in NTB as indigenous probiotic candidates was identified based on bile salt tolerance, NaCL tolerance, acid resistance (pH 2.5 and 1), antimicrobial activity against E. coli and S. Aureus. *Dangke* samples were obtained from Lombok (A), Bima (B), Dompu (C), and Sumbawa (D). The mean values and significance of the microbiological analysis are presented in Table 1.

In addition, morphological identification of LAB isolated from all wild horse milk *dangke* samples was performed using gram staining. The morphology of LAB cell and colony are shown in Table 2 and Figure 2. Generally, the colonies were round, white, with flat edges and convex elevation macroscopically, while microscopically the bacterial cells showed two main shapes, that is bacilli and cocci, appearing purple as Gram-Positive.

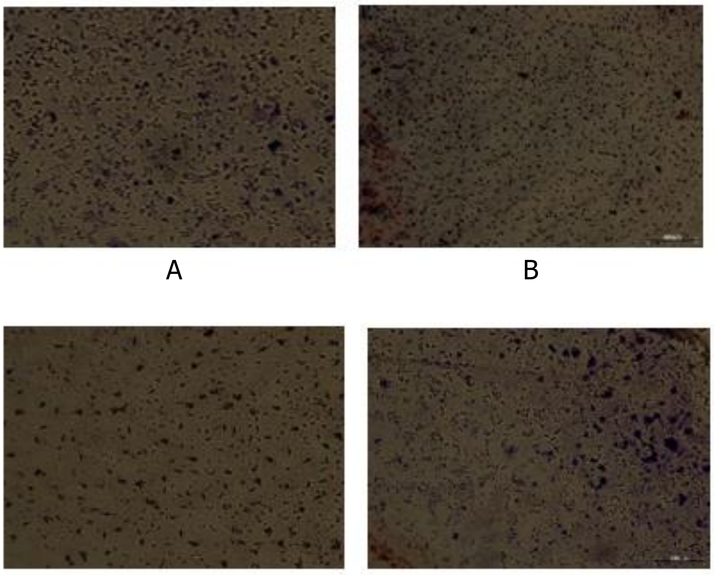

Figure 2. Gram Staining Results of LAB from Wild Horse Milk *Dangke*

PCA Results of Mapping The Characteristics of LAB Wild Horse Milk Dangke

PCA or Principal component analysis is a statistical technique used to identify data patterns by transforming original variables into linear combinations. In this study, PCA was applied to evaluate the microbiological parameters of *Dangke* samples from Lombok, Bima, Dompu, and Sumbawa. The dendogram of PCA results showing the relationship between samples and parameters is presented in Figure 3.

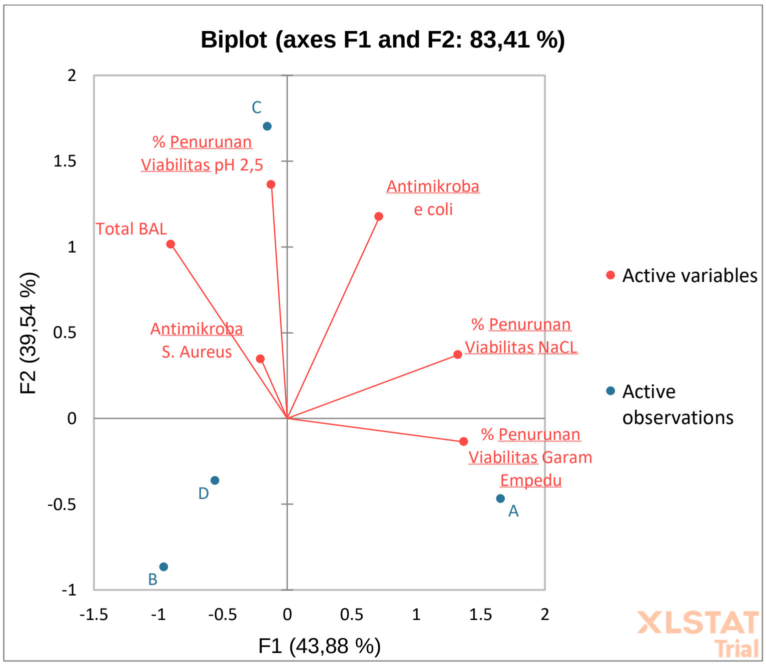

Figure 3. Dendrogram Results Graph of PCA Microbiological Properties Between Samples and Parameters

Figure 3 shows the PCA biplot between the parameters and the samples analyzed. It is known that the biplot axes F1 and F2 are 83.41%, this result indicates that the analyzed microbiological parameters influence the *dangke* sample of 83.41%. The use of F1 and F2 is based on eigenvalues that have a sufficient percentage to explain and represent the available data. The mapping results show that *dangke* samples from regions B and D have similar characteristics to each other but have different characteristics from samples A and C.

Discussion

Total LAB

Wild horse milk *dangke* contains natural microorganisms, including LAB, which ferment sugars into lactic acid. LAB commonly found in milk play a role in fermentation and are widely used in producing fermented foods beverages such as yogurt, kefir, kimchi, and sauerkraut. Some examples of lactic acid bacteria include *Lactobacillus, Streptococcus, Leuconostoc,* and *Pediococcus* (Aini et al., 2022; Manalu et al., 2020; Pratama et al., 2020). *Dangke* naturally contains indigenous LAB (Syah et al., 2017). Previous studies reported that LAB isolated from cow's milk *Dangke* include *Lactobacillus fermentum*, L. *acidophilus* (Lutfiah, 2015), *Lactobacillus* spp. (Nur et al., 2017) and L. *fermentum* (Syah et al., 2017). While from buffalo milk *dangke* include L. *plantarum* and L. *fermentum* species (Kaswi et al., 2021; Lutfiah, 2015).

Based on the data in Table 1, namely on the total LAB parameter, it is known that the difference in sampling locations has a significantly different effect on the total LAB samples. The results showed that samples from Dompu (C) had the highest total LAB count, which was 9.98 log CFU/ml, followed by Bima (B) with 9.82 log CFU/ml, Sumbawa (D) with 9.76 log CFU/ml, and Lombok (A) which had the lowest total LAB, which was 9.60 log CFU/ml. These results are similar to the total LAB in similar products, namely LAB in yogurt products used as soft cheese starters by Adrianto (2020), which was 9.31 log CFU/ml. In addition, these results are higher compared to the total microbes in cow's milk *dangke* products, which are 7.0-7.5 log CFU/ml (Kesuma et al., 2013), the total bacteria in cow's milk *dangke* are 5.3-7.78 log CFU/ml (Al-Baarri et al., 2018) and 7.25-7.71 log cfu/ml (Zakariah, 2020).

The significant differences in total LAB in this study may be due to several factors that influence the sampling locations including differences in environmental conditions, types of feed consumed by wild horses, and different husbandry practices in each region. For example, differences in climate, temperature, and types of feed available in each region can support the growth of different LABs. In addition, the microflora in the surrounding environment, such as air, soil, and equipment used for milking, can also affect the presence of bacteria in the milk animal (Malacarne et al., 2002; Minjigdorj et al., 2012; Wei et al., 2021). Despite regional variations, all samples demonstrated high LAB levels, consistently within the log 9 CFU/ml range, indicating a generally strong presence of LAB in wild horse milk *dangke* across all sample areas.

LAB Morphology

The morphology of LAB refers to the characteristics of the shape and structure of bacterial cells that can be observed under a microscope. The results of research on the morphology of LAB isolated from wild horse milk *dangke* samples in several areas in West Nusa Tenggara show that they generally have similar characteristics. Macroscopically, lactic acid bacteria colonies from all wild horse milk *dangke* samples appear round, white, with flat edges and convex elevations. These characteristics indicate that these colonies grow well on the agar media used to form clear and easily recognizable colonies. While microscopically, most samples show bacterial cell morphology in the form of bacilli (rods) with a purple color after gram staining. These results indicate that these bacteria are gram-positive bacteria. Bacteria with this *bacillus* shape are typical of lactic acid bacteria groups such as *Lactobacillus* and *Leuconostoc*. The purple color seen under the microscope after gram staining indicates that these bacteria have thick cell walls and are rich in peptidoglycan which are typical of gram-positive bacteria.

However, there is a difference in the sample from Sumbawa (D), which shows a slightly different morphology compared to the other samples. In the Sumbawa sample, in addition to the bacillus shape, several coccus-shaped cells (round) were also found. This indicates that there is variation in the types of LAB present in wild horse milk *dangke* in the area, which may reflect the diversity of species or strains of LAB present in the environment. Some species of LAB, such as *Streptococcus* or *Enterococcus*, have a coccus shape, and this may explain the finding of coccus-shaped cells in samples from Sumbawa.

This diversity may be influenced by multiple factors, including enviromental conditions, feeding sources and local animal husbandry practices. Previous studies have identified several species of LAB found in wild horse milk in NTB, including *Lactobacillus, Ochrobacterium, Staphylococcus, Enterococcus sp., Lactococcus, and the Dipodascaceae* family (Jatmiko et al., 2017; Kusdianawati et al., 2020; Mulyawati et al., 2019; Suryanti et al., 2019). This diversity indicates that the microbiota in wild horse milk varies greatly, and certain factors can influence the types of bacteria that are dominant in each region or area.

The composition of milk in mammalian species, including wild horses, is very diverse and is influenced by various factors such as available feed, environment, and the physiological condition of the animal (Malacarne et al., 2002; Minjigdorj et al., 2012; Wei et al., 2021). Research shows that differences in milk composition can affect the types of bacteria that grow in the milk. For example, wild horse milk in Sumbawa is dominated by bacteria from the genus *Lactobacilli* and *Weisella* or *Leuconostoc* (Suryani & Gaffar, 2024), which are often found in fermented products and milk. On the other hand, wild horse milk in Bima contains slightly different types of bacteria, such as *Lactobacillus acidophilus, Lactobacillus plantarum, and Lactococcus lactis* (Antara et al., 2009; Prastyowati, 2021). The diversity of lactic acid bacteria species is also the cause of the morphological differences found in the study, where samples from Sumbawa showed a combination of *bacillus* and coccus-shaped bacteria. This can be caused by the dominance of the rod-shaped *Lactobacillus* and *Leuconostoc* bacteria, as well as the presence of coccus-shaped *Enterococcus* or *Streptococcus* bacteria (Suryani & Gaffar, 2024).

Previous studies, such as that conducted by Paramitasari et al., (2015), successfully isolated the bacteria *Lactobacillus fermentum* and *Lactobacillus acidophilus* from *dangke* made from cow's milk. These types of bacteria are also found in other dairy products, indicating that *Lactobacillus* is one of the dominant lactic acid bacteria genera in mammalian milk, including wild horses. In addition, research by Lutfiah (2015) identified *Lactobacillus fermentum* and *Lactobacillus plantarum* in *dangke* made from buffalo milk. These findings show that Lactobacillus species are commonly found in dairy products across different animal sources, including wild horses.

LAB has the potential as a probiotic candidate with several requirements that must be met, namely being stable to acid (especially stomach acid), stable to bile salts and able to survive while in the upper part of the small intestine, producing antimicrobial compounds including organic acids, hydrogen peroxide, and bacteriocins, able to attach and colonize human intestinal cells, grow well and develop in the digestive tract, are safe for use by humans, and coaggregation forms a normal and balanced microflora environment. These findings highlight the potential of LAB isolated from wild horse milk *dangke* as promising candidates for developing local probiotic products, especially given their diversity and adaptation to the local environment.

Tolerance Against Bile Salt

Bile salt tolerance reflects the ability of LAB to survive and grow under digestive tract conditions, making i tan important indikator of their potential as probiotics. Since bile salts are a major digestive component, LAB that tolerate them are more likely to persist and function in food fermentation, whereas some strains show limited or No. growth at higher concentrations.

Based on the data in Table 1, namely the parameter of the percentage decrease in viability of bile salt tolerance, it is known that the difference in sampling locations has a significant effect on the parameter of the percentage decrease in viability of bile salt tolerance of wild horse milk *dangke*. The highest to lowest parameters of the percentage decrease in viability of bile salt tolerance are the Lombok (A), Dompu (C), Sumbawa (D), and Bima (B) samples, which are respectively 21.17%; 8.01%; 6.92%;

4.35%. These results are by the research of (Hafsan et. al., 2015) which states that L. *fermentum* and L. *acidophillus* bacterial cells isolated from *Dangke* cow's milk can grow and survive in bile salts with concentrations of 0.1%, 0.3%, and 0.5%. With the number of surviving LAB of 1.4 x 10^6, 1.7x10^6, 4x10^6 for L. fermentum bacteria and 0.7x10^6, 0.9x10^6 and 1.4x10^6 for L. *acidophillus* bacteria.

The results of the analysis showed that samples from Lombok (A) were more susceptible to bile salts, thus having lower resistance under these conditions. On the other hand, samples from Bima (B) showed the lowest percentage of viability decline, indicating that lactic acid bacteria in Bima wild horse milk were more resistant to bile salts, or in other words, were more able to survive under these stressful conditions. The degree of tolerance to bile salts is very important for LAB, because it affects their activity in the digestive tract, especially in the upper intestine, where bile is secreted. Bile, which acts as a surface-active compound, can damage the structure of bacterial cell membranes through lipolytic enzymes that react with fatty acids in the membrane. The diversity of fatty acid structures in bacterial membranes affects resistance to bile salts (Hafsan et. al., 2015).

A lower decrease in bile salt tolerance viability indicates a higher survivak ability of LAB in bile salt environments. This is very important in the context of the application of these bacteria as probiotics or in fermentation processes, because bacteria that are more resistant to bile salts tend to have a greater potential to survive in the digestive tract, where they can provide health benefits, such as improving the balance of intestinal microflora and aiding digestion. Differences in tolerance to bile salts can be influenced by several factors, including the types of lactic acid bacteria species present in each location, the composition of the microbiota in wild horse milk, and environmental factors where the wild horses live. For example, some species of *Lactobacillus* and *Lactococcus* can grow well at low to moderate bile salt concentrations (around 0.1%-0.3%), while other species, such as *Enterococcus faecium*, can survive at higher concentrations, even up to 0.5% or more. In addition, variations in diet, climatic conditions, and animal physiological factors can also play a role in the ability of bacteria to adapt and survive stressful conditions such as bile salts.

Tolerance against NaCl

The growth tolerance of LAB in NaCl media conditions refers to the ability of bacteria to survive and grow in an environment containing salt, especially sodium chloride (NaCl). NaCl salt is often used in tolerance tests to test the ability of bacteria to deal with high osmotic conditions, which can cause dehydration or osmotic imbalance in bacterial cells.

Based on the data in table 1, namely the parameter of the percentage decrease in viability of NaCl tolerance, it is known that the difference in sampling locations has a significant effect on the parameter of the percentage decrease in viability of *Dangke* bile salt tolerance of wild horse milk. The highest to the lowest parameter of the percentage decrease in viability of NaCl tolerance is the Lombok (A), Dompu (C), Sumbawa (D), and Bima (B) samples, which are respectively 12.75%; 9.71%; 6.83%; 6.00%.

Lactic acid bacteria (LAB), such as *Lactobacillus* and *Lactococcus*, generally have tolerance to NaCl at certain concentrations. Some LAB species can grow well at low to moderate NaCl concentrations (e.g. 1% to 4%), while others, such as *Enterococcus* or *Leuconostoc*, may be more tolerant to higher concentrations (up to 6-7% NaCl). The ability to grow at different NaCl concentrations can be used to distinguish the genera *Enterococcus* and *Lactococcus*, where *Lactococcus* bacteria are only tolerant at a concentration of 4% (Lutfiah, 2015; Mawardika et al., 2023).

The results showed that samples from Lombok (A) indicated that LAB in Lombok wild horse milk *dangke* were more susceptible to osmotic stress caused by salt (NaCl). On the other hand, samples from Bima (B) showed that LAB in Bima wild horse milk *dangke* were more resistant to the effects of NaCl. The lower the percentage of decrease in viability to NaCl, the higher the resistance of bacteria to high osmotic conditions. This indicates that lactic acid bacteria from Bima have a better ability to survive in higher salinity conditions, which may reflect the ability of these bacteria to survive in the digestive tract, where the concentration of body salts and other elements can affect the survival of microorganisms.

This difference in resistance can be influenced by environmental factors and different microflora compositions in each location. Variations in environmental factors, feed, and physiological adaptation of bacteria to salinity conditions in each region can affect the ability of bacteria to survive in media containing salt animal (Malacarne et al., 2002; Minjigdorj et al., 2012; Wei et al., 2021). This tolerance to NaCl is also very relevant in the context of probiotic applications, where LAB that are more resistant to high osmotic conditions will be better able to survive in the digestive tract and provide health benefits, such as maintaining the balance of the intestinal microflora. In addition, bacteria that are more tolerant to NaCl will also be more effective in fermenting food products containing salt. One of the important requirements for bacteria to be used as probiotics is their ability to survive and reproduce in the digestive tract, which often has varying salinity and osmotic conditions.

Tolerance against Low pH

The growth tolerance of LAB in low pH media conditions indicates the extent to which these bacteria can survive and thrive in an acidic environment. This low pH condition is often used to test the resistance of bacteria to acid stress that occurs in various environments, such as the digestive tract. In this study, tests were carried out on pH 2.5 and pH 1 media conditions. This test is important to determine the ability of LAB as probiotic bacteria to survive in human stomach conditions which are known to have a very low pH, namely around pH 1-3, which can be a challenge for many microorganisms.

Based on the data in Table 1, namely the parameter of the percentage decrease in tolerance viability at pH 2.5, it is known that the difference in sampling locations has a significant effect on the parameter of the percentage decrease in tolerance viability at pH 2.5 wild horse milk *dangke*. The parameter of the percentage decrease in viability at pH 2.5 from highest to lowest is the Dompu (C), Sumbawa (D), Lombok (A), and Bima (B) samples, which are respectively 100%; 23.39%; 22.36%; 22.02%.

Samples from Dompu (C) showed the highest percentage decrease in viability, which was 100%, indicating that LAB isolated from wild horse milk *dangke* in Dompu were unable to survive at pH 2.5. This indicates the low tolerance of LAB to acidic conditions in these samples. Meanwhile, samples from Sumbawa (D), Lombok (A), and Bima (B) showed a lower decrease in viability indicating that LAB from these areas were more resistant to acidic pH conditions. According to (Vieco-Saiz et al., 2019), LAB can survive in acidic conditions, due to its ability to maintain pH conditions in cells, so it is resistant to membrane disruption. Meanwhile, the decrease in viability at pH 1 shows that the difference in sampling locations has an insignificant effect or based on the data there was a decrease of 100% or no LAB were able to survive. These results indicate that none of LAB can survive at such extreme pH conditions. At pH 1, most LAB may experience a significant decrease in viability, since this pH is very damaging to bacterial cell membranes. Only bacteria with very high acid tolerance, such as *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*, can survive under these conditions. This ability may be influenced by various factors, including differences in milk composition, local microflora, or specific adaptations of the bacteria to local environmental conditions.

Antimicrobial Resisstance

The antimicrobial ability of LAB refers to the ability of these bacteria to produce compounds that can inhibit or kill other pathogenic microorganisms. Antimicrobial tests were carried out on 2 types of bacteria, namely E. *coli* and S. *aureus*. Based on the data in Table 1, namely on the antimicrobial parameters of E. *coli*, it is known that the difference in sampling locations has a significantly different effect on the antimicrobial parameters of E. *coli* wild horse milk *dangke*. The highest to lowest inhibition of E. *coli* growth is the Dompu (C), Lombok (A), Sumbawa (D), and Bima (B) samples, which are respectively 0.85cm; 0.8cm; 0.75 cm; and 0.7 cm. While the highest to lowest inhibition of S. *aureus* growth is the Sumbawa (D), Dompu (C), Lombok (A), and Bima (B) samples, which are respectively 0.9cm; 0.8cm; 0.75 cm; 0.7 cm.

This shows that LAB isolated from wild horse milk *dangke* Dompu has stronger antimicrobial activity against E. *coli* compared to other areas. While LAB from wild horse milk *dangke* Sumbawa has more effective antimicrobial activity against S. *aureus* compared to other areas. The higher the inhibition of E. *coli* and S. *aureus* bacterial growth, the better the antimicrobial ability of the isolated LAB. These results indicate that LAB found in wild horse milk *dangke* has the potential to inhibit the growth of pathogenic microorganisms, although there is variation between locations. This variation could be caused by differences in the composition of local microflora or other environmental factors that affect the antimicrobial properties of LAB in each region animal (Malacarne et al., 2002; Minjigdorj et al., 2012; Wei et al., 2021). Overall, these findings indicate the good potential of LAB to be used in controlling pathogenic microbes, such as E. *coli* and S. *aureus*, which can contribute to improving food quality or health. The main mechanism of LAB is the production of lactic acid, which can lower the pH of the surrounding environment, creating unfavorable conditions for the growth of pathogenic microorganisms. In addition, LAB can also produce protein compounds called bacteriocins, which are antimicrobial and can inhibit or kill other bacteria, especially gram-positive bacteria. Some LAB species can also inhibit the activity of pathogenic enzymes required for their growth, such as protease and lipase enzymes. In addition, LAB also competes with pathogenic microbes for resources and growth sites, reducing the opportunity for pathogens to multiply. The antimicrobial ability of LAB can be influenced by environmental factors and the source of isolation of the bacteria. Overall, the antimicrobial ability of LAB plays an important role in controlling pathogenic microorganisms in various environments, both in the food industry and in health applications (Ayen et al., 2020; Yoo et al., 2023).

LAB are considered potential probiotics if they meet certain criteria, such as resistance to low pH, tolerance to bile salts and the ability to inhibit pathogenic microbes. These properties contribute to maintaining intestinal microflora balance by reducing the adhesion of pathogenic bacteria and thereby suppressing their growth in the digestive tract. In this study, LAB isolated from wild horse milk *dangke* demonstrated survival in bile salt, NaCl and acidic pH media, as well as antimicrobial activity against as E. *coli* and S. *Aureus*. These findings provide preliminary evidence of LAB's potential as probiotic candidate, although further studies are needed to confirm their full probiotic properties.

CONCLUSION

Dangke also called soft milk cheese is a typical food produced from fresh milk, one of which is wild horse milk typical of West Nusa Tenggara. Wild horse milk *dangke* has microbiological characteristics that vary depending on the region of origin. Analysis shows that differences in sample types affect parameters such as total LAB, percentage of growth tolerance in bile salt media, NaCL, pH 2.5, and antimicrobial properties for both pathogenic bacteria E. *coli* and S. *aureus*. These results indicate that LAB from wild horse milk *dangke* provides preliminary evidence of its potential as an indigenous probiotic candidate. PCA showed that *dangke* from Bima and Sumbawa had similar characteristics, which differed from those of Lombok and Dompu. Overall, this study succeeded in identifying and mapping differences in *dangke* characteristics based on their region of origin and their potential as probiotic candidates.

Acknowledgments

Gratitude is conveyed to the Directorate General of Higher Education, Research, and Technology of the Republic of Indonesia for providing funding for activities in the Affirmative Beginner Lecturer Research Program for the 2024 Fiscal Year.

REFERENCES

Adrianto, L. (2020). Strategi sektor perikanan dalam pemulihan dan transformasi ekonomi nasional. *Prosiding Seminar Nasional Fakultas Pertanian UNS*.

Aini, A., Ustiawaty, J., Kurniawan, E., & Maulana, A. (2022). Isolate and characterization of lactic acid bacteria (lab) in local nira as probiotic starter candidates. *jurnal Biologi Tropis*, *22*(4). https://doi.org/10.29303/jbt.v22i4.4429

Al-Baarri, A. N. M., Legowo, A. M., Arum, S. K., & Hayakawa, S. (2018). Extending shelf life of Indonesian soft milk cheese (dangke) by lactoperoxidase system and lysozyme. *International Journal of Food Science*, *2018*. https://doi.org/10.1155/2018/4305395

Antara, N. S., Dibia, N., & Aryanta, W. R. (2009). Karakterisasi bakteri asam laktat yang diisolasi dari susu kuda Bima. *Agritech: Jurnal Fakultas Teknologi Pertanian UGM*, *29*(1).

Arini, N., Sudarwanto, M. B., Sudirman, I., & Indrawati, A. (2016). Improvement efforts of sensory quality and preservation cow's milk dangke with addition cow's milk fat and *Lactobacillus plantarum* supernatant. *International Journal of Dairy Science*, *11*(1). https://doi.org/10.3923/ijds.2016.20.27

Ayen, R. Y., Kusdiyantini, E., & Pujiyanto, S. (2020). Characterization of lactic acid bacteria and antimicrobial activity in Sui Wu'u from Bajawa District, Nusa Tenggara Timur, Indonesia. *Asian Journal of Pharmaceutical and Clinical Research*, 13(4). https://doi.org/10.22159/ajpcr.2020.v13i4.36760

Fuller, R. (1992). History and development of probiotics. In *Probiotics*. https://doi.org/10.1007/978-94-011-2364-8_1

Giyatno, D. C., & Retnaningrum, E. (2021). Isolasi dan karakterisasi bakteri asam laktat penghasil eksopolisakarida dari buah kersen (*Muntingia calabura* L.). *Jurnal Sains Dasar*, *9*(2). https://doi.org/10.21831/jsd.v9i2.34523

Hafsan et. al. (2015). Ketahanan bakteri asam laktat asal dangke terhadap garam empedu sebagai kandidat probiotik. *Prosiding Seminar Nasional Biologi*, *29*.

Hardiningsih, R., Napitupulu, R. N. R., & Yulinery, T. (1970). Isolation and resistance test of several isolates of *Lactobacillus* in low pH. *Biodiversitas Journal of Biological Diversity*, *7*(1). https://doi.org/10.13057/biodiv/d070105

Jatmiko, Y. D., Howarth, G. S., & Barton, M. D. (2017). Assessment of probiotic properties of lactic acid bacteria isolated from Indonesian naturally fermented milk. *AIP Conference Proceedings*, *1908*. https://doi.org/10.1063/1.5012732

Kaswi, K., Setiadi, G., & Su`ad, S. (2021). Pengaruh pendidikan dan pelatihan penguatan kepala sekolah dan iklim sekolah terhadap kinerja guru di sekolah dasar Kecamatan Winong. *Jurnal Pajar (Pendidikan dan Pengajaran)*, *5*(6). https://doi.org/10.33578/pjr.v5i6.8318

Kesuma, F. M. V., Sayuthi, S. M., Al-Baarri, A. N., & Legowo, A. M. (2013). Karakteristik dangke dari susu dengan waktu inkubasi berbeda pasca perendaman dalam larutan laktoferin ferdina. *Jurnal Aplikasi Teknologi Pangan*, *2*(3).

Komalasari, H., Afgani, C. A., & Melandani, A. (2025). Karakterisasi dan pemetaan profil fisikokimia dangke susu kuda liar khas Nusa Tenggara Barat. *Jurnal Teknologi Dan Mutu Pangan*, *3*(2), 80–87.

Komalasari, H., & Yoga, W. K. (2022). Potensi bakteri probiotik indigenous *Lactobacillus plantarum* dad-

13 sebagai starter pada pembuatan yoghurt fungsional: kajian pustaka. *Food Scientia : Journal of Food Science and Technology*, *2*(2), 199–217. https://doi.org/10.33830/fsj.v2i2.3694.2022

Kusdianawati, Mustopa, A. Z., Fatimah, & Budiarto, B. R. (2020). Genetic diversity of lactic acid bacteria isolated from Sumbawa Horse Milk, Indonesia. *Biodiversitas*, *21*(7). https://doi.org/10.13057/biodiv/d210744

Lutfiah, N. A. (2015). *Isolasi dan identifikasi bakteri asam laktat pada susu kambing saanen (Capra aegagrus* H.*).* [Skripsi]. Universitas Islam Negeri.

Malacarne, M., Martuzzi, F., Summer, A., & Mariani, P. (2002). Protein and fat composition of mare's milk: Some nutritional remarks with reference to human and cow's milk. *International Dairy Journal, 12*(11), 869-877. https://doi.org/10.1016/S0958-6946(02)00120-6

Manalu, R. T., Bahri, S., Melisa, M., & Sarah, S. (2020). Isolasi dan karakterisasi bakteri asam laktat asal feses manusia sebagai antibakteri *Escherichia coli* dan *Staphylococcus aureus*. *Sainstech Farma: Jurnal Ilmu Kefarmasian*, *13*(1).

Mawardika, H., Pertiwi, K. K., Wahyuni, D., & Aulia, Q. W. (2023). Karakterisasi dan uji aktivitas antimikroba bakteri kandidat probiotik dari terasi udang rebon. *Bioscientist : Jurnal Ilmiah Biologi*, *11*(2). https://doi.org/10.33394/bioscientist.v11i2.8981

Minjigdorj, N., Baldorj, O., & Austbø, D. (2012). Chemical composition of Mongolian mare milk. *Acta Agriculturae Scandinavica A: Animal Sciences*, *62*(2). https://doi.org/10.1080/09064702.2012.720999

Mulaw, G., Tessema, T. S., Muleta, D., & Tesfaye, A. (2019). In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented ethiopian food products. *International Journal of Microbiology*, *2019*. https://doi.org/10.1155/2019/7179514

Mulyawati, A. I., Jatmiko, Y. D., Mustafa, I., Ardyati, T., & Suharjono. (2019). Diversity of lactic acid bacteria isolated from fermented mare's milk products based on PCR-RFLP analysis. *IOP Conference Series: Earth and Environmental Science*, *230*(1). https://doi.org/10.1088/1755-1315/230/1/012104

Nath, S., Sikidar, J., Roy, M., & Deb, B. (2020). In vitro screening of probiotic properties of *Lactobacillus plantarum* isolated from fermented milk product. *Food Quality and Safety*, *4*(4). https://doi.org/10.1093/fqsafe/fyaa026

Nur, F., Hatta, M., Natzir, R., & Djide, M. N. (2017). Isolation of lactic acid bacteria as a potential probiotic in dangke, a traditional food from Enrekang, Indonesia. *International Journal of Sciences: Basic and Applied Research (IJSBAR)*, *35*(1).

Paramitasari, K. A., Sumantri, C., & Jakaria. (2015). The genetic variability of prolactin and signal transducers and activators of transcription 5A (STAT5A) genes in Bali cattle. *Media Peternakan*, *38*(1). https://doi.org/10.5398/medpet.2015.38.1.1

Prastyowati, A. (2021). Sumbawa wild horse milk: production, usage, chemical compound, microbial community, and probiotics potency. *Indonesian Bulletin of Animal and Veterinary Sciences*, *31*(3). https://doi.org/10.14334/wartazoa.v31i3.2850

Pratama, D. R., Melia, S., & Purwati, E. (2020). Perbedaan konsentrasi kombinasi starter tiga bakteri terhadap total bakteri asam laktat, nilai pH, dan total asam tertitrasi yogurt. *Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science)*, *22*(3). https://doi.org/10.25077/jpi.22.3.339-345.2020

Reuben, R. C., Roy, P. C., Sarkar, S. L., Alam, A. S. M. R. U., & Jahid, I. K. (2020). Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. *Journal of Dairy Science*, *103*(2). https://doi.org/10.3168/jds.2019-17092

Silva, S. P. M., Teixeira, J. A., & Silva, C. C. G. (2023). Recent advances in the use of edible films and coatings with probiotic and bacteriocin-producing lactic acid bacteria. *Food Bioscience, 56*. https://doi.org/10.1016/j.fbio.2023.103196

Suryani, E. M., & Gaffar, A. (2024). Isolasi dan karakterisasi bakteri asam laktat dari susu kuda Bima (*Equus* sp.) yang berpotensi sebagai probiotik. *Jurnal Ilmiah Biosaintropis (Bioscience-Tropic)*, *9*(2), 102–108.

Suryanti, S., Suryaningrum, T. D., & Utomo, B. S. B. (2019). The effect of concentration acetic acid in extraction of gelatin from nila fish (*Oreochromis niloticus*) to the physical characteristics. *IOP Conference Series: Materials Science and Engineering*, *546*(6). https://doi.org/10.1088/1757-899X/546/6/062032

Syah, S. P., Sumantri, C., Arief, I. I., & Taufik, E. (2017). Isolation and identification of indigenous lactic acid bacteria by sequencing the 16s rRNA from dangke, a traditional cheese from Enrekang, South Sulawesi. *Pakistan Journal of Nutrition*, *16*(5). https://doi.org/10.3923/pjn.2017.384.392

Tarique, M., Abdalla, A., Masad, R., Al-Sbiei, A., Kizhakkayil, J., Osaili, T., Olaimat, A., Liu, S. Q., Fernandez-Cabezudo, M., al-Ramadi, B., & Ayyash, M. (2022). Potential probiotics and postbiotic characteristics including immunomodulatory effects of lactic acid bacteria isolated from traditional yogurt-like products. *LWT*, *159*. https://doi.org/10.1016/j.lwt.2022.113207

Taye, Y., Degu, T., Fesseha, H., & Mathewos, M. (2021). Isolation and identification of lactic acid bacteria from cow milk and milk products. *Scientific World Journal*, *2021*. https://doi.org/10.1155/2021/4697445

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., & Drider, D. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontiers in Microbiology, 10*(57), 1-17. https://doi.org/10.3389/fmicb.2019.00057

Wei, W., Hu, X., Hou, Z., Wang, Y., & Zhu, L. (2021). Microbial community structure and diversity in different types of non-bovine milk. *Current Opinion in Food Science, 40,* 51-57. https://doi.org/10.1016/j.cofs.2021.01.008

Yang, S. Y., & Yoon, K. S. (2022). Effect of probiotic lactic acid bacteria (lab) on the quality and safety of greek yogurt. *Foods*, *11*(23). https://doi.org/10.3390/foods11233799

Yerlikaya, O. (2019). Probiotic potential and biochemical and technological properties of *Lactococcus lactis* ssp. lactis strains isolated from raw milk and kefir grains. *Journal of Dairy Science*, *102*(1). https://doi.org/10.3168/jds.2018-14983

Yoo, Y., Lee, J., Cho, J., & Yoon, Y. (2023). Antimicrobial properties of *Limosilactobacillus reuteri* strains for control of *Escherichia coli* and *Salmonella* strains, diarrhoea cause in weaning pigs. *Veterinarni Medicina*, *68*(5). https://doi.org/10.17221/112/2022-VETMED

Zakariah, M. A. (2020). *Eksplorasi Isolat Bakteri Asam Laktat dari Dangke untuk Peningkatan Kualitas dan Pengawetan Dangke*. [Skripsi]. Universitas Hasanuddin.