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Probiotic Characteristics of Lactic Acid Bacteria Isolated From *Sui Wu'u*: A Traditional Food From Bajawa, West Flores, Indonesia

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Abstract

The probiotic potential of lactic acid bacteria originating from traditional food will always be a concern for researchers, along with increasing public awareness of health. One source of LAB isolates with probiotic potential is traditional food. This study aims to investigate the probiotic potential of lactic acid bacteria from *Sui Wu'u*, a traditional food-based pork originating from Bajawa, East Nusa Tenggara, Indonesia. *Sui Wu'u* was prepared based on method of the Bajawa community and was analyzed after being stored for six months. Total LAB, morphological, and phenotypic identification was carried out as well as testing the ability of isolates to ferment sugar. Probiotics potency was tested on resistance to low pH and gastric acid, resistance to pathogenic bacteria, and exposure to antibiotics. The results indicated that LAB isolated from *Sui Wu'u* were dominated by the coci form. The four selected isolates had resistance with a survival rate of 88.3%–96.88% when exposed to pH 2.5 and a survival rate of 98.69%–99.03% when exposed to bile salts at 0.3%. Moderate resistance was exhibited by all isolates against *E. coli*, S. typhimurium, and S. aureus, while no resistance was observed against B. cereus. All strains demonstrated sensitivity to tetracycline, ampicillin, and chloramphenicol but exhibited resistant to streptomycin. All four isolates were identified as *Lactococcus Lactis*. These results show that four strains of *Sui Wu'u* have demonstrated their potential as probiotics.

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Introduction

Traditional food has received attention from researchers in various countries; apart from being rich in unique flavors, traditional food has also been shown to provide a number of benefits for maintaining and improving human health status. Further investigation has shown that these diverse health benefits are linked to the presence of microorganisms in traditional foods. The diversity and abundance of these microorganisms are predominantly influenced by raw materials, additives, processing, packaging, storage time, and storage conditions. In various traditional foods, these microorganisms contribute significantly and play a central role in the fermentation process.[1] Lactic acid bacteria (LAB) are categorized as microorganisms that are safe for humans with Generally Recognized as Safe (GRAS) status. The presence of LAB contributes to the abundance of bioactive peptides in traditional foods due to their primary and secondary metabolites. This bioactive peptide has biological functions including lowering cholesterol, antihypertensive, antidiabetic, antiaging, anticancer, immunomodulatory, and antimicrobial.[2,3]

The potential of lactic acid bacteria from traditional foods as probiotics has also been demonstrated. Probiotics, according to the World Health Organization (WHO), are live microorganisms that offer health benefits when administered adequately. Probiotics have a beneficial impacts on health, including increasing the body's resistance to intestinal infections and diarrhea, increasing the immune system response, acting as an antidiabetic, lowering blood pressure, reducing the risk of lactose intolerance and cardiovascular disease.[4] Several tests are necessary to identify potential probiotic LAB, including resistance to gastric acid and bile salts, intestinal tract adherence, non-induction of antibiotic resistance, non-pathogenic probiotic species, and aggregation capability.[5]

The probiotic properties of LAB are strain-dependent, and traditional foods could be a good source for investigating local probiotics. Several LAB isolates from indigenous fermented foods have been reported as potential probiotics.[6,7] *Sui Wu'u* is an indigenous food of East Nusa Tenggara, Indonesia, made from pork. The method involves using salt and corn flour, placing them in a bamboo container (*tuku*), and storing for months or years. The probiotic potential of LAB in this traditional food remains unexplored; this study aims to investigate LAB's probiotic potential from *Sui Wu'u*.

Materials and Methods

Production of *Sui Wu'u*

Sui Wu'u is is prepared following the traditional Bajawa community recipe. Pork, corn kernels, and salt are purchased from the local market. The dry corn kernels are pounded in a mortar and sifted to obtain corn flour, which is then dried again. Meanwhile, Meanwhile, the pork is cut into medium-sized portions, then evenly sprinkled with salt and left to rest for 5 min. After marination, the pork is coated with dry corn flour, covering the entire surface, and alternately placed in a bamboo container (*tuku*) with layers of corn flour. The container is tightly sealed and stored according to the predetermined time. The *Sui Wu'u* products were then immediately analyzed.

Isolation of Lactic Acid Bacteria Strains and Phenotypic characterization

The isolation of LAB follow the method of Garafalo *et al* [8] A 25 g *Sui Wu'u* sample was mixed with 225 mL of sterilized distilled water and homogenized for 10 min using a stomacher (Bag Mixer; Interscience, FRA). The enumeration of LAB was subsequently assessed using the serial dilution plating method on de Man, Rogosa, and Sharpe agar (MRSA, Oxoid, USA) followed by incubation at 37 °C for 48 h. Colonies were randomly selected based on consistent cell morphology observed under a microscope and purified through streaking. The purified isolates were subsequently cultured in MRS broth (Oxoid, USA). Furthermore, characterization was carried out using and catalase test and the Gram staining. Prior to utilization, all isolates were maintained and stored in MRS broth and glycerol (20%) at −20 °C.

Carbohydrate Fermentation

Carbohydrate fermentation profiles were analyzed utilizing the API50 CHL kit (Bio-Mérieux, FRA) as per the manufacturer's instructions.

Acid and Bile Salt Tolerance

The acid survival and bile tolerance of LAB isolates were assessed through incubation at pH 2.5 and exposure to 0.3% bile salt, respectively.[9] Cultures of LAB were grown in an MRSB medium and incubated for 24 h. Subsequently, a 1 mL inoculum

was prepared for centrifugation (10.000 ×g, 4 °C, 5 min). The acid tolerance procedures are as follows: Cell pellets of LAB were resuspended in phosphate-buffered saline (PBS; Oxoid), adjusted to pH 2.5 using 0.1 N HCl. Subsequently, an incubation was conducted at 37 °C for 1 h. In the bile tolerance assay, LAB cell pellets were resuspended in PBS enriched with 0.3% (w/v) bile salt and subsequently incubated at 37 °C for a duration of 3 h. The samples were collected before (0 h) and after incubation, cultured for MRSA at 37 °C for 48 h. The survival of LAB due to acid and bile salt exposure was determined by the difference in LAB count before and after incubation.

Antibacterial Activity

The antibacterial activity of the LAB isolates against *Escherichia coli*, *Salmonella typhimurium Bacillus cereus* and *Staphylococcus aureus* a was determined by the well diffusion method[10] in Mueller–Hinton agar (Difco, USA) followed by incubation at 30 °C for 48 h. Subsequently, inhibitory zones diameters were measured in millimetres.

Antibiotic Resistance

The susceptibility of the LAB isolates to antibiotic was assessed by employing chloramphenicol, ampicillin, streptomycin, and tetracycline[11] following EFSA[12] technical guidelines.

Identification of LAB Isolates Based on 16S rRNA Gene Sequence

Based on the rapid identification using the API 50 CH, the LAB isolates were identified as *Lactococcus lactis*. However, this traditional phenotypic method could result in inaccurate identification. Therefore, a genetic method is required to achieve an accurate and reliable identification. Since this genus is difficult to distinguish from other cocci bacteria such as *Enterococcus, Leuconostoc,* and *Streptococcus*, the identification of LAB in this study was further characterized by PCR amplification using genus-specific primers. The previously generated genus-specific lactococcal primers through the utilization of the 16S rRNA gene have been demonstrated to provide benefits in distinguishing between *Lactococcus* and *Enterococcus.*

The LAB isolates were grown in MRSB with a 10% glycine supplement, and the incubation was carried out at 37 °C for 24 h. DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA), following the manufacturer's instructions. The primers used were forward primer L1 (5'-AAC TCT GTT GTT AGA G-3') and reverse primer L2 (5'-ATC TCT AGG AAT AGC AC-3').[13] PCR amplifications were performed in 20 µL volumes with 10 µL Promega Go Taq Green Master Mix (Promega Corporation, USA), 1 µL primer (0.5 µM of each forward and reverse), 1 µL DNA template, and 7 µL nuclease-free water. The amplifications were performed using an Applied Biosystems 2720 Thermal Cycler (USA) with the following cycling conditions:

1 cycle of 95 °C for 3 min; 35 cycles of 94 °C for 30 s, 43 °C for 45 s, and 72 °C for 1 min; and 1 cycle of 72 °C for 5 min. The PCR products were then run on 2% agarose gel in 1× TAE buffer and gels stained in GelRed (Biotium, USA) staining solution.

The PCR products were then sequenced by 1st Base Laboratories (Malaysia) and analyzed further using MEGA software version 7.0. The homology searches were performed using the NCBI BLAST (the Basic Local Alignment Search Tool) algorithm. The MEGA7 software was also used to create phylogenetic trees.

Statistical Analyses

The statistical analyses of the data were performed using SPSS version 22 (IBM Corp. USA). The assessment of mean differences between was conducted using the Duncan test and were considered significant when $P \leq 0.05$. The data were expressed as mean ± standard deviations.

Results

Microbial Populations

The enumeration results showed that the total LAB was 3.7×10^3 CFU/g. A total of 26 LAB colonies were isolated from *Sui Wu'u* grown on MRSA. There were 24 cocci and two rods among the 26 LAB isolates. According to the result, all isolated were found to be Gram-positive and catalase-negative (Table 1), thereby aligning with the classification of LAB.

Carbohydrate Fermentation Analysis

The API 50CHL analysis showed selected LAB isolates from *Sui Wu'u* metabolized 22 (45%) of 49 carbohydrates (Table 2).

Table 1: Single colony morphology and microscopic characteristics of LAB isolates from *Sui Wu'u*

Isolate code	Colony characteristics				Catalase test	Gram stain	Cell shape
	shape	size	color	Colony edge			
SwR1	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR2	round	small	white	slippery /clear	-	+	cocci/forming clusters
SwR3	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR4	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR5	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR6	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR7	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR8	round	small	white	slippery /clear	-	+	cocci/ forming chains
SwR9	round	small	white	slippery /clear	-	+	rod /forming chains
SwR10	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR11	round	small	white	slippery /clear	-	+	cocci/forming clusters
SwR12	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR13	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR14	round	small	white	slippery /clear	-	+	cocci/forming clusters
SwR15	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR16	round	small	white	slippery /clear	-	+	rod/forming chains
SwR17	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR18	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR19	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR20	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR21	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR22	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR23	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR24	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR25	round	small	white	slippery /clear	-	+	cocci/forming chains
SwR26	round	small	white	slippery /clear	-	+	cocci/forming chains

The characteristic similarity (100%) between the LAB *Sui Wu'u* colonies became the basis for selecting six of the 26 LAB isolates to test their probiotic potential. The isolates were selected based on their clear appearance on MRSA media and good growth ability on the media. The six LAB isolates are SwR2, SwR9, SwR24, SwR23, SwR16, and SwR14.

Table 2: Carbohydrate fermentation of selected LAB isolates of Sui Wu'u. The ability of a selected LAB isolate to metabolize the corresponding carbohydrates is represented as positive (+) or lack of fermentation (−)

Carbo-hydrates	Isolate code						Carbo-hydrates	Isolate code					
	SwR							SwR					
	2	14	16	23	24	26		2	14	16	23	24	26
Control	-	-	-	-	-	-	Esculin	+	+	+	+	-	+
Glycerol	-	-	-	-	-	-	Salisin	+	+	+	+	-	+

Erythritol	-	-	-	-	-	-	Cellobiose	+	+	+	+	-	+
D-Arabinose	-	-	-	-	-	-	Maltose	+	+	+	+	-	+
L-Arabinose	+	+	+	-	-	+	Laktose	+	-	-	+	-	+
Ribose	+	+	+	+	+	+	Melibiose	+	+	+	+	-	+
D-Xilose	-	-	-	-	-	-	Sukrose	+	+	+	+	-	+
L-Xylose	-	-	-	-	-	-	Trehalose	+	+	+	-	-	+
Adonitol	-	-	-	-	-	-	Inulin	-	-	-	-	-	-
Methyl	-	-	-	-	-	-	Melezitose	-	-	-	-	-	-
Galaktose	+	+	+	+	+	+	Rafinose	-	-	+	-	-	+
Glukose	+	+	+	+	+	+	Starch	-	-	-	-	-	-
Fruktose	+	+	+	+	+	+	Glycogen	-	-	-	-	-	-
Mannose	+	+	+	+	+	+	Xylitol	-	-	-	-	-	-
Sorbose	-	-	-	-	-	-	Gentiobiose	+	+	+		-	+
Rhamnose	-	-	-	-	-	-	D-Turunose	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	D-Lykosa	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	D-Tagatose	-	-	-	-	-	-
Manitol	+	+	+	+	+	+	D-Fucose	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	L-Fucose	-	-	-	-	-	-
α-Met-D-Mannoside	-	-	+	-	+	+	D-Arabitol	-	-	-	-	-	-
α-Met-D-Glicoside	-	-	+	-	-	-	L-Arabitol	-	-	-	-	-	-
N-Ac-Glucosamin	+	+	+	+	+	+	Gluconate	-	-	-	-	-	-
Amygdalin	-	-	-	-	-	+	2-keto-Gluconate	-	-	-	-	-	-
Arbutin	+	+	-	+	+	+	5-Keto-gluconate	-	-	-	-	-	-

Isolate code: SwR (SwR2, SwR14,SwR16, SwR23, SwR24, SwR26)

Acid Tolerance

Six LAB isolates from *Sui Wu'u* showed varying tolerance after incubation at low acid (pH 2.5). The decrease in cell number ranged from 0.23–1.51 log CFU/mL with a survival rate of 79.65%–96.88% (Figure 1).

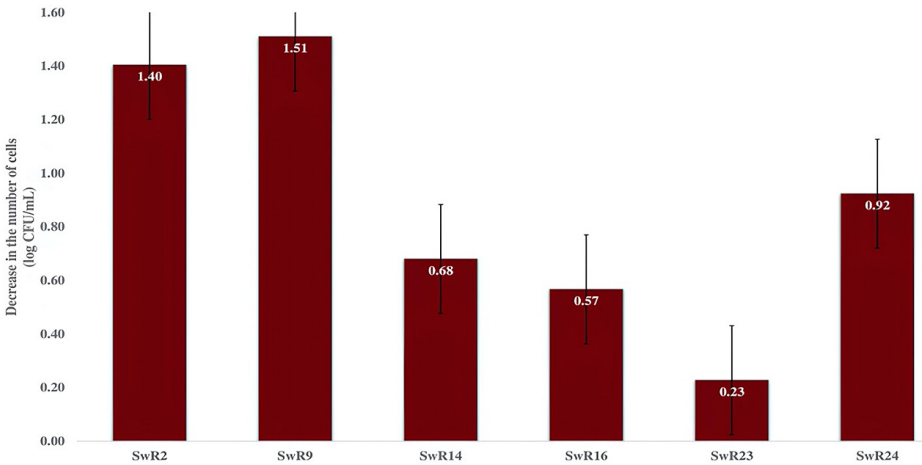

Fig. 1: Acid tolerance of LAB *Sui Wu'u*

The isolate SwR24 exhibited the lowest decline in LAB count after acid exposured (0.23 log CFU/mL). Based on our results, the four selected isolates demonstrated notable tolerance to low pH conditions (survival rate >85%); these isolates were SwR24, SwR23, SwR16, and SwR14.

Table 3: Tolerance of LAB isolated from *Sui Wu'u* to Acid and bile salt

Isolate code	Decrease of viable cells After 1 h incubation at pH 2.5 (log CFU/mL)	Decrease of viable cells After 3 h incubation at 0.3% bile salt (log CFU/mL)	Total decrease of viable cells (log CFU/mL)
SwR14	0.68 ± 0.25^{b}	0.10 ± 0.09^{a}	0.78 ± 0.33^{b}
SwR16	0.57 ± 0.36^{b}	0.09 ± 0.05^{a}	0.66 ± 0.42^{b}
SwR23	0.23 ± 0.04^{a}	0.08 ± 0.06^{a}	0.30 ± 0.05^{a}
SwR24	0.92 ± 0.06^{b}	0.10 ± 0.05^{a}	1.02 ± 0.10^{b}

Each value is presented as the mean ± SD of three independent experiments (n = 3). In instances where data within column share a common lowercase, no significant difference is observed ($p<0.05$).

Table 4: Antibacterial activity of LAB *Sui Wu'u* against selected indicator bacteria

Isolate code	Inhibition zones (mm)				Mean	Inhibition category
	B. cereus	*S. typhimurium*	*E. coli*	*S. aureus*		
SwR14	-	3.72 ± 0.20^{a}	3.80 ± 0.12^{a}	3.81 ± 0.09^{b}	3.78 ± 0.05^{a}	moderate
SwR16	-	4.34 ± 0.14^{b}	3.47 ± 0.23^{a}	3.45 ± 0.29^{a}	3.75 ± 0.51^{a}	moderate
SwR23	-	3.66 ± 0.23^{a}	3.39 ± 0.26^{a}	3.26 ± 0.27^{a}	3.44 ± 0.20^{a}	moderate
SwR24	-	3.44 ± 0.38^{a}	3.30 ± 0.31^{a}	3.29 ± 0.35^{ab}	3.34 ± 0.08^{a}	moderate
inhibition zones mean (mm)	-	3.79 ± 0.39	3.49 ± 0.22	3.45 ± 0.25	3.58 ± 0.22	

Inhibition categories: weak (0–3 mm), moderate (3–6 mm), strong (6–9 mm), and very strong (>9 mm) inhibition strength. Each value is presented as the mean ± SD of three independent experiments (n = 3). In instances where data within column share a common lowercase, no significant difference is observed ($p<0.05$).

Bile Salt Tolerance

The tolerance to 0.3% bile salts of the selected isolates from *Sui Wu'u* varied. The cell count decrease ranged from 0.08 to 0.10 log CFU/mL and a survival rate of 98.69%–99.03% (Table 3). The total cell number decrease of the selected LAB after being exposed to low pH and bile salt ranged between 0.30–1.02 log CFU/mL. The most promising isolate was SwR23, exhibiting a total reduction of 0.30 log CFU/mL.

Antibacterial Activity

The antibacterial effectiveness of the selected LAB isolates is demonstrated in Table 4; Figure 2A. The greatest inhibition of *Sui Wu'u* LAB was observed against the indicator bacteria *S. typhimurium*.

Antibiotic Resistance

The resistance of selected LAB isolates to several types of antibiotics is presented in Table 5; Figure 2B.

LAB Isolates identification through 16S rRNA Gene Sequencing

The bands resulting from PCR amplification are illustrated in Figure 3, with an expected size of 450 bp. Therefore, the four isolates were identified as belonging to the *Lactococcus* genus. The BLAST analysis result supported a 100% nucleotide

sequence similarity between the four isolates and *Lactococcus lactis*, in agreement with API identification. More than 94% similarity in nucleotide sequences showed high homology and exhibited an identical species attribution.[14] Moreover, in the phylogenetic tree, the four isolates also demonstrated that those isolates were in the same clade as the species *Lactococcus lactis*, with strong bootstrap values (100) (Figure 4).

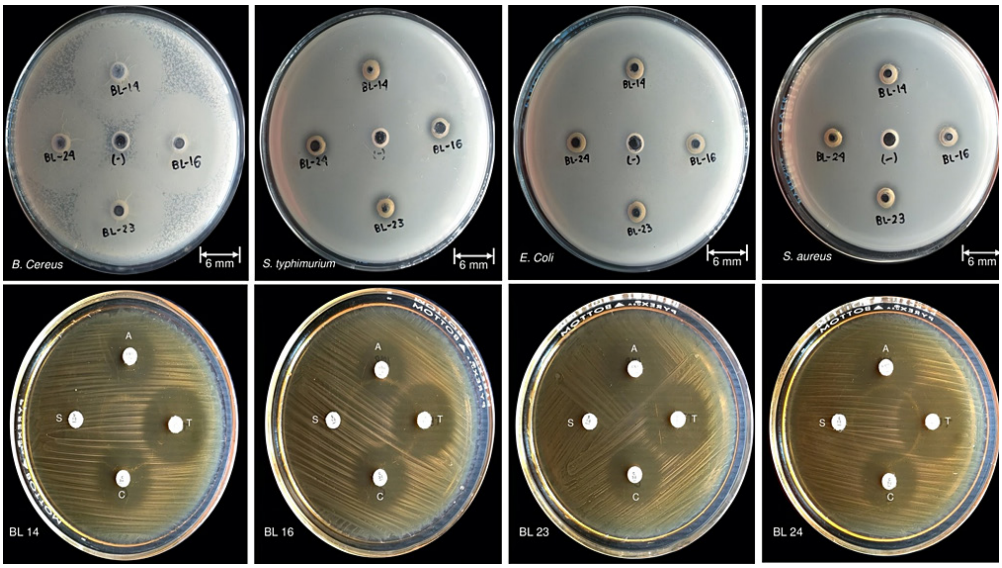

Fig. 2: Antibacterial Activity of LAB *Sui Wu'u* Against Selected Indicator Bacteria (2A); Antibiotic Resistance (2B; A: Ampicillin; S: Streptomycin; T: tetracycline; C: Chloramphenicol); BL14 = SwR14, BL16 = SwR16, BL23 = SwR23, BL24 = SwR24

Table 5: Antibiotic Resistance of selected LAB strains

Isolate code	Inhibition zones (mm)							
	Ampicillin	R	Streptomycin	R	Chloramphenicol	R	Tetracycline	R
SwR14	14.74 ± 0.03[a]	S	0.00	R	15.34 ± 0.04[a]	I	21.75 ± 0.58[a]	S
SwR16	14.68 ± 0.28[a]	S	0.00	R	16.02 ± 0.28[a]	I	21.22 ± 0.14[a]	S
SwR23	17.12 ± 1.20[a]	S	0.00	R	16.65 ± 0.26[a]	I	20.63 ± 1.22[a]	S
SwR24	18.00 ± 0.30[a]	S	0.00	R	17.10 ± 0.22[a]	I	21.72 ± 0.12[a]	S

Interpreting the sensitivity of LAB to antibiotics: S: sensitive; R: resistant; I: intermediate. Each value is presented as the mean ± SD of three independent experiments (n = 3). In instances where data within column share a common lowercase, no significant difference is observed ($p<0.05$).

Discussion

Exploring LAB in traditional foods is still a researcher concern for various purposes, including obtaining LAB isolates with functional properties that are beneficial to human health, such as antihypertensive, antidiabetic, antioxidant, and anti-cholesterol[15] or obtaining LAB isolates as a starter culture to improve the physical, chemical and sensory attributes of the product.[16,17] The existence of LAB in various foods, especially fermented foods, can provide added value as functional foods.

This studi investigated the presence of LAB in *Sui Wu'u* after a storage period of three months. The viable cell count of LAB measured in this study was at 3.7 x 10^3 CFU/g, which was lower than

those of LAB measured in traditional Nham and Saigongyisan pork sausages, with a total LAB of 1.2 x 10^7–1.8 x 10^{10} CFU/g and 3.3 x107–2.5 x 1010 CFU/g, respectively, and those of LAB stored in bamboo.[18] The abundance of lactic acid bacteria in traditional foods is strongly influenced by the type of raw materials and additives, manufacturing processes, containers, and storage conditions. *Sui Wu'u* represents a pork product traditionally cured with salt and corn flour and stored for months in a bamboo container called "*tuku*". It is made mainly from pork back fat with quite high-fat content.[19] Several studies have shown that a high-fat content in a product can limit LAB growth.[20] However, the rate of LAB growth measured in this study indicated that the nutrients present in *Sui Wu'u* products are still sufficient to meet the requirement of LAB growth. Seventy-seven percent of 26 LAB isolates in this study showed the same morphological characteristics (Table 1). Matti *et al*.[21] have also characterized the LAB in traditional fermented fish products (Chao), in which cocci-shapes dominated 57% of LAB present. The probiotic potential has been attributed to certain LAB found in traditional foods. These live microorganisms, when present in adequate quantities can provide a positive effect or support the health organisms by improving the microflora in the digestive tract.

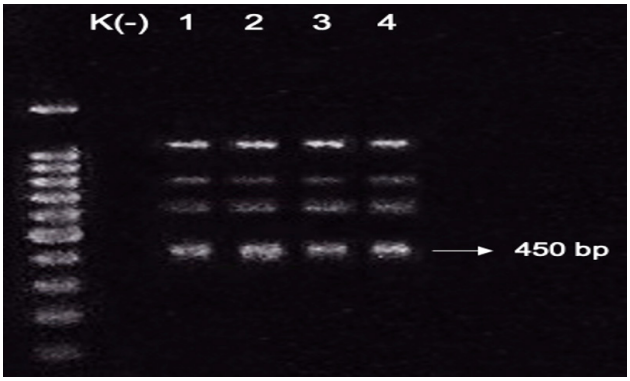

Fig. 3: Electrophoresis of PCR product from LAB isolates on a 2% agarose gel

K(-) = negative control; 1= SwR14; 2= SwR16; 3= SwR23; 4= SwR24.

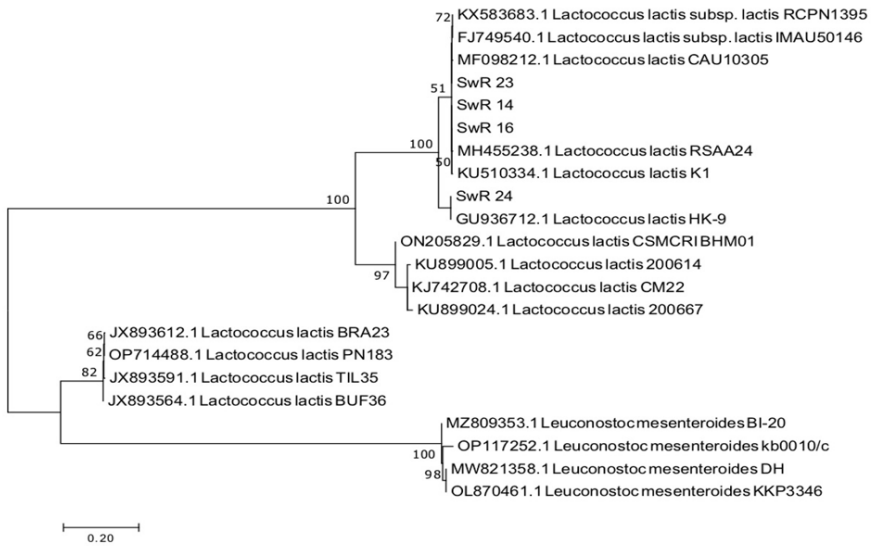

Fig. 4: Phylogenetic Analysis of 16S rRNA gene sequences of four LAB isolates from *Sui Wu'u*

The sugar fermentation ability of the selected *Sui Wu'u* isolate was assessed. SwR26 isolate was the most proficient, fermenting 21 out of 49 sugar types in 48 h, followed by SwR16, SwR2, SwR23, and SwR24 (Table 2). It is noted that LAB can ferment sugar into lactic acid. This ability is essential to getting an energy source and is strain-independent.

As probiotic candidates, the LAB isolates must demonstrate upper gastrointestinal tract survival to reach the intestine and confer host benefits. Human stomach acid has a very low pH of 1.0–2.5; most microorganisms are killed in stomach acid. Six selected isolates from *Sui Wu'u* showed resistance above 50% to low pH (Figure 1). The survival rate of ≥ 50% at acidity pH 2.5 was declared high. The higher the survival rate of LAB after the acid exposure, the more resistant the LAB culture to low pH. Excessive acidity can induce cell death by compromising the integrity of the cytoplasmic membrane and releasing intracellular components. The ability of LAB isolates to survive in high acidity in the digestive tract is strain-independent. Certain strains demonstrated increased cytoplasmic membrane permeability and exhibit adaptability to acidity conditions. Their cellular pH homeostasis mechanism enables them to endure high acidity conditions by restricting protons diffusion across an extremely impermeable membrane, regulating the dimensions of channels, transporting protons through the ATPase enzyme to eliminate excess protons from the cell cytoplasm, and maintaining cell membrane fluidity through fatty acids modulation.[22]

The four selected isolates with the smallest decrease in cell number at low pH were also shown to have resistance to 0.3% bile salt (Table 3). The isolate SwR23 showed the lowest decrease in cell number. In the human intestinal environment, the averages concentration of bile salt is approximately 0.3% (w/v)23; hence, 0.3% bile salt is a critical concentration for selecting isolates that are tolerant and resistant to bile salt. The ability of bile acid tolerance, where it is suspected to be related to protein molecules or enzymes released by LAB, could potentially influence cell membranes, cellular homeostatic properties, or induce alterations in the structure of the surrounding bile salts. Yao *et al*.[24] stated that the enzyme Bile Salt Hydrolase (BSH) contributes to the tolerance of bile salts. The BSH can protect cells by catalyzing the deconjugation (breakdown) of bile salts by hydrolyzing amino amide bonds, taurine, or glycine. When exposed to bile salt stress, some probiotic LAB produce exopolysaccharide synthesis (EPS), such as glycosyltransferase.[25] In both tests (Table 3), the enumeration of LAB cells indicated a decrease of less than <1 log CFU/mL (75%). These results demonstrated that the four LAB isolates from *Sui Wu'u* were capable of surviving the passage through the stomach and small intestine and colonizing the large intestine. Thus, these four isolates have the potential to be probiotics. The additional tests involved assessing antibiotic resistance, pathogenic bacteria inhibition, and epithelial cells adhesion yielded significant result. *In vivo* efficacy tests and clinical trials are still required to declare LAB probiotic bacteria.

The ability to inhibit pathogenic bacteria is one of the properties of probiotics. The antibacterial activity of LAB isolates from *Sui Wu'u* was evaluated using the well diffusion method against the test bacteria *B. cereus* and *S. aureus* (Gram-positive), *S. typhimurium* and *E. coli* (Gram-negative). Four LAB isolates from *Sui Wu'u* had moderate inhibition against *S. aureus, E. coli*, and *S. typhimurium* but had no inhibition against B. cereus. The most significant inhibition of LAB isolates from *Sui Wu'u* against the tested bacteria was shown against *S. typhimurium* (Table 4; Figure 2A). These results differ slightly because LAB is known to to be more effective against Gram-positive bacteria compared to Gram-negative bacteria,[26] although the antibacterial effectiviness of LAB against Gram-negative bacteria has also been reported.[27] Gram-negative bacteria are known to have a polysaccharide coating on the lipopolysaccharide portion and divalent cations on the hydrophilic outer membrane, so the antimicrobial compounds from LAB are difficult to penetrate the membrane structure of Gram-negative bacteria. The inhibition of pathogenic bacteria by LAB depends on the strain and is influenced by factors such as the origin and type of organic acids produced.[28] Heterofermentative LAB generate lactic acid and other acids such as acetic acid, propionic, formic and acetic acid, while homofermentative LAB, in their metabolic process, release lactic acid as the primary metabolite from breakdown of sugars. Other metabolites, namely bacteriocins, have been reported in several studies to be produced by LAB.

Lactic acid bacteria are known as safe or GRAS. However, using LAB has health risks. LAB can transfer resistant genes to and from gut bacteria, potentially spreading antibiotic resistance.[29] An antibiotic susceptibility test of LAB is essential to ascertain the absence of antibiotic resistance properties in the LAB. Antibiotic resistance in bacteria is the ability of bacteria to survive the effects of antibiotics. Selected LAB isolates from *Sui Wu'u* showed different susceptibilities to antibiotics. Based on the results (Table 5; Figure 2B), all isolates were sensitive to ampicillin, tetracycline and chloramphenicol but resistant to streptomycin. Each antibiotic has a different mechanism against bacteria. Ampicillin works by inhibiting the biosynthesis of peptidoglycan in the bacterial cell wall; Chloramphenicol operates through the inhibition of protein synthesis by binding to the 50S ribosome, while tetracycline disrupts protein synthesis by binding to the 30S ribosome. Resistance to streptomycin was observed in four selected LAB isolates. LAB isolates can possess intrinsic antibiotic resistance from inherent traits or acquire resistance through genetic mutations or external DNA uptake.[30] Resistance of *Lactococcus lactis* isolated from traditional Iranian cheese to the antibiotics ampicillin and chloramphenicol was reported by Afshari *et al*.[31] The four isolates from *Sui Wu'u* were identified as *Lactococcus lactis*. A LAB species present in various traditional fermented foods such as fermented fish, dry sausages and pork products.[32,33,34] *Lactococcus* is a mesophilic (optimum growth is at 30 °C), Gram-positive bacterium within Firmicutes phylum exhibit characteristic of lack the catalase enzyme, facultative anaerobic, are non-motile, non-sporulating, spherical in shaped, and forms chains or pairs. This characteristic is in line with the morphological and phenotypic characteristics of LAB from *Lactococcus* described in this study. *Lactococcus* displays nutritional selectivity, relying on essential nutrients such as amino acids, vitamins, and fermentable carbohydrates to obtain energy.[35]

Lactococcus is is a homofermentative microorganism that converts sugars into lactic acid as the primary product during glucose fermentation, resulting in aromatic components that enhance the taste, aroma, and texture of various traditional foods like cheeses and sour milk through the process of fermentation. The contribution of this bacteria to the distinctive characteristics of fermented foods increases its use as a starter culture.[36] Exploration of the functional properties and probiotic potential of this isolate has also been investigated in several studies. Previous studies have shown that *Lactococcus lactis* effectively withstands low pH levels (pH 2 and 3) and 0.3% bile salt concentration. Furthermore, it displays inhibitory properties against various pathogenic bacteria.[37] This report has confirmed the results obtained in this study, particularly with regards to the susceptibility of *Lactococcus lactis* originating from *Sui Wu'u* towards S. aureus and *E. coli*. It is known that, apart from producing organic acids, *Lactococcus lactis* also produces bacteriocins and special antibacterial chemicals.[38] Another potential probiotic, namely susceptibility to antibiotics, was reported by Yerlikaya *et al*.[39] and is also in line with the results obtained in our study. Another advantage explored for *Lactococcus lactis* is its functional properties. Previous studies have shown that *Lactococcus lactis* has antioxidant, cholesterol-lowering, anti-diabetic, anti-depressant, and pro-inflammatory.[40,41]

Conclusion

All 26 LAB colonies found in *Sui Wu'u* products were mainly in the shape of cocci. The six selected isolates could metabolize carbohydrates (45%) and had low acid tolerance (pH 2.5), with a decrease in cell number ranging from 0.23–1.51 CFU/mL and a survival rate of 79.65%–96.88%. Four isolates were selected based on their resistance to low pH (survival rate >85%) and the isolate SwR23 was the most prospective isolate, with the lowest cell decrease (0.30 log CFU/mL) for both tests at low pH and bile salt. These four isolates had moderate inhibitory activity against *E. coli*, *S. typhimurium*, and S. aureus and did not show any activity against B. cereus. Selected LAB showed sensitivity to tetracycline, ampicillin, and chloramphenicol, but were resistant to streptomycin. All four isolates were identified as *Lactococcus lactis*. The results obtained in this study indicated the presence of LAB in *Sui Wu'u*, which had been stored for six months. Four selected LAB from *Sui Wu'u* based on tests conducted in this study have shown their potential as probiotics. In the future, animal studies will be needed to evaluate their probiotic potential.

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Conflict of Interest

There is no known conflict of interest.

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