ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at www.jgpt.co.in

RESEARCH ARTICLE

Determination of Lactic Acid Bacteria Isolated from the Gastrointestinal Tract of Bali Cattle with Potential Probiotic Role

I Dewa Made Sukrama[1*], I Wayan Suardana[2], Christopher Ryalino[3]

1. *Department of Clinical Microbiology, Faculty of Medicine.*
2. *Department of Veterinary Public Health, Faculty of Veterinary Medicine.*
3. *Department of Anaesthesiology, Pain Medicine, and Intensive Care, Faculty of Medicine Udayana University, Bali, Indonesia.*

***Corresponding Author Email:** *dewa_sukrama@yahoo.co.id*

Abstract

Bali cattle is known as pioneer' cattle resulted by their high ability to adapt in poor quality of feed so that was assumed for finding the specific species of lactic acid bacteria. The study aimed to investigate the potency of lactic acid bacteria isolated from the gastrointestinal tract of Bali' cattle as a candidate for new probiotic. Fifteen isolates of lactic acid bacteria isolated from 15 intestinal tracts of Bali cattle were grown on the specific medium deMan Rogosa Sharpe (MRS) broth followed by Gram staining, catalase test, the formation of CO2 gas, and antimicrobial activities against Bacillus cereus. The result of this study confirmed 15 isolates were Gram-positive bacteria, and 14 out of 15 isolates were categorized as homofermentative. Probiotic potency test showed 11 out of 15 isolates were tolerant against lower pH (pH 2, 4 and 6), and also resistant to bile acid with NaDC concentration 0.2, 0.4, and 0.6 mM. Identification of isolate using API 50CH test kit found 4 out of 5 isolates as Lactobacillus sp. and the remainder as Lactococcus sp. The study concluded isolates of lactic acid bacteria isolated from the gastrointestinal tract of Bali cattle showed potential as a probiotic candidate.

Keywords: *Bali cattle, Gastrointestinal tract, Lactic acid bacteria, Probiotic.*

Introduction

Lactic acid bacteria (LAB) are Gram-positive bacteria, non-sporing, catalase negative, acid-tolerant and strictly fermentation[1]. These bacteria are non-pathogenic and save to use with the status of Generally Recognize as Safe (GRAS) [2]. Several strains of LAB normally found as normal flora in the intestinal tract of humans and animals [3] including the intestinal tract of Bali cattle. Bali cattle is known as pioneer cattle resulted by their high ability to adapt in poor quality of feed [4].

Furthermore, it is known that dietary as a factor contributes to the variety of normal flora in the intestinal tract of human and animal. Flora in the intestinal tract are increasing in number and complexity along the canal and is estimated to be abundance up to 10^{12} bacteria per gram of content of the gastrointestinal tract with an estimate not less than 500 species including LAB as dominant species [5].

Based on these facts it is possible to find out the specific LAB in the intestinal tract of Bali cattle. On the other hands, lactic acid bacteria have been receiving considerable attention as probiotics because of their innate ability to exert antagonistic activity against non-pathogenic and spoilage organisms. Probiotics are live microorganisms which when they are administered inadequate amounts, provide a benefit to the health of consumers [1,6]. Probiotic may be one of the most effective therapies for the prevention of several diseases [7].

Appreciable numbers of research have been devoted to isolating novel probiotic LAB with emphasis on their health-promoting properties and mode of antimicrobial action [8,9] including the future generation of probiotics based on genetically modified strains that will deliver therapeutic molecules to the host [10]. General aspects for the probiotic candidate that must be

fulfilled including biosafety (selected strains should be non-pathogenic and non-toxic), probiotic bacteria must be able to survive in the gastrointestinal tract, probiotics organisms must be resistant to bile acids, the ability to attach and colonize in the intestinal tissues, antimicrobial activity against potentially pathogenic bacteria, modulation of immune system, health aspects, production aspects, and quality control aspects.

Furthermore, probiotic strains must be characterized at a minimum with the following tests: (1) Assessment of the side effects of previous human studies; (2) Assessment of certain metabolic activities (e.g. D-lactase production, bile salt deconjugation; (3) Determination of antibiotic resistance pattern; and (4) Post-market surveillance of adverse incidents on consumer [12]. The requirements have been established in order to ensure efficiency, effectiveness, and benefit to the host from those microorganisms [5, 13].

According to the WHO/FAO guidelines, probiotic must be identified at genus, species and strain level completely. It is recommended to employ a combination of phenotypic and genetic techniques to accomplish the identification, classification, and typing [14]. A lot of experimental procedures have been developed for the identification of LAB. One of them is the use of rapid identification through biochemical test miniature API50CH [15]. This study aimed to determine the LAB isolated from the intestinal tract of Bali cattle with the potential probiotic role.

Material and Methods

Cultivation of Lactic Acid Bacteria

Stock of lactic acid bacteria (LAB) isolates that were previously isolated from content of Bali cattle's gastric juice i.e. SR1, SR3, SR4, SR5, SR7, SR8, SR9 and SR10 as well as LAB isolates from content of Bali cattle's colon i.e. SK2, SK3, SK6, SK7, SK8, SK9, and SK13 were taken from 30% glycerol stock stored at -20 ˚C. Those isolates were then thawed at 4 ˚C for 15 minutes before planted on sterile MRS broth medium for subsequently incubated at 37 ˚C for 24 hours.

As a LAB, isolates were re-confirmed with Gram staining test, catalase test, and CO_2 production test employing methods proposed by Suardana et al. [16] and Sukrama et.al [17]. Rapid test to study antimicrobial activity of isolate was conducted by direct antagonism using cross streak method [18].

Probiotic Test of Lactic Acid Bacteria

Bile Tolerance

Bile tolerance as one of the probiotic-LAB criteria was analyzed according to the study proposed by Sujaya et al. [19]. Amounting 50 µL stock cultures were suspended into 5 ml MRS broth medium supplemented with different concentration of bile salt. Each isolate was inoculated into 4 different tubes i.e. the first tube as a control (MRS broth medium without the addition of sodium deoxycholate (NaDC), the second tube added 10 µL NaDC (0.2 mM), the third tube was added 20 µL NaDC (0.4 mM) and the fourth tube was added 30 µL NaDC (0.6 mM). The tubes were incubated at 37 ˚C for 24 hours anaerobically.

The LAB growth was measured by turbidity level (OD 660 nm) using spectrophotometer [20]. The strains were considered not resistant to NaDC if their absorbance value (AO) <0.1, and otherwise if (AO) >0.1 [19, 21]. In order to obtain representative data, the test was repeated 3 times.

Acid Tolerance

The acid tolerance of isolates was analyzed according to the method previously [19, 21]. Amounting 100 µL BAL cultures were grown into four Eppendorf tubes that each tube containing 900 µL MRS broth medium with pH 2, 3 and 4. The tubes were incubated for 3 hours in a water bath at 37 ˚C which followed by centrifugation at 7000 rpm for 5 minutes and the supernatant was discarded. The obtained bacteria pellets were washed twice using 300 µL saline solution by vortex and centrifuged for 5 min at 7000 rpm. The pellets were then suspended with 300 µL saline solution.

Totally 50 µL of these suspensions were suspended into 5 mL MRS broth with neutral pH for subsequently incubated for 24 h at 37^{O}C anaerobically. The acid tolerance of isolates was indicated by their growth on a medium that was measured using a spectrophotometer at 660 nm wavelength. The strains were considered not resistant to acid if their absorbance value (AO) <0.1, and otherwise if (AO) >0.1.

Representative data were obtained as an average of 3-time repetitions.

Identification of Lactic Acid Bacteria

Identification of LAB strains was performed using API 50 CHL Kit assays. Overnight cultures of LAB isolates were grown in 10 ml MRS broth at 30 ˚C and then washed twice with sterile physiological saline (0.9% sodium chloride). The pellets were suspended in API 50 CHL medium (API systems, Bio Me Âreux). The turbidity of the suspension was determined by the McFarland method according to the instructions provided by the manufacturer.

Using sterile Pasteur, the homogenized suspensions of the cells were suspended in the medium and then transferred into each of the 50 wells on the API 50 CH strips. This procedure was done for all isolates. All wells on the plate were overlaid with sterile paraffin oil (Merck) to create the anaerobic condition. The plates were then incubated at 30 ˚C anaerobically, and the results were read after 24h and verified after 48h. Fermentation of carbohydrates was identified by a yellow color except for masculine (dark brown). The results were analyzed using API WEB (Bio-Merieux) [15, 22].

Results

Cultivation of Lactic Acid Bacteria

Cultivation of LAB isolates as a primary step in this study showed the contribution to the next step. The result of the cultivation of 15 LAB isolates based on morphological shape, catalase test, Gram staining, CO_2 formation, and antimicrobial activity is summarised in Table 1.

Table 1: Characteristic of LAB isolates isolated from intestinal tract of Bali cattle

Isolate codes	Source of isolates	Gram staining	Cell morphology	Anti-microbial activity	Catalase test	CO_2 formation	Category
SR-1	gastric juice	positive	rod	+	-	-	homofermentative
SR-3	gastric juice	positive	short rod	+	-	-	homofermentative
SR-4	gastric juice	positive	short rod	+	-	-	homofermentative
SR-5	gastric juice	positive	short rod	+	-	-	homofermentative
SR-7	gastric juice	positive	short rod	+	-	-	homofermentative
SR-8	gastric juice	positive	short rod	+	-	-	homofermentative
SR-9	gastric juice	positive	short rod	+	-	+	heterofermentative
SR-10	gastric juice	positive	short rod	+	-	-	homofermentative
SK-2	colon	positive	coccus	+	-	-	homofermentative
SK-3	colon	positive	short rod	+	-	-	homofermentative
SK-6	colon	positive	short rod	+	-	-	homofermentative
SK-7	colon	positive	short rod	+	-	-	homofermentative
SK-8	colon	positive	coccus	+	-	-	homofermentative
SK-9	colon	positive	short rod	+	-	-	homofermentative
SK-13	colon	positive	short rod	+	-	-	homofermentative

(+) positive; (-) negative reactions

The data in Table 1 shows that one out of 15 isolates tested i.e. SR-9 belonging as a heterofermentative group. Isolate SR-9 showed the presence of gas bubbles in the Durham tube when it was tested for CO_2 gas formation. On the contrary for the remainder isolates that are belonging to the homofermentative group [23]. The cell morphology of all isolates shows isolate SK8 as a short rod Gram-positive, and most of the isolates possess the ability to form killing zones around their growth. Clear zones around stab inoculant were showed for isolates number 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, and 14 after 24 hours of incubation. These results indicate that most of the isolates have good antimicrobial activity resulted by their ability to inhibit the growth of *Bacillus cereus* as a bacterial indicator.

Bile Tolerance

Bile tolerance as one of the general aspects for the probiotic candidate which must be fulfilled for the probiotic candidate. The result of the study is shown in Table 2.

Table 2: Growth of LAB strains in MRS broth with different concentration of NaDC

Isolate codes	OD 660 nm			
	control /0 NaDC	0.2 NaDC	0.4 NaDC	0.6 NaDC
SR-1	1.899+++	1.963+++	2.035+++	2.031+++
SR-3	1.979+++	1.775+++	2.007+++	1.980+++
SR-4	1.806+++	2.034+++	1.797+++	2.013+++
SR-5	1.686+++	2.004+++	1.995+++	1.818+++
SR-7	1.518+++	1.717+++	2.007+++	2.022+++
SR-8	1.754+++	1.814+++	1.754+++	2.003+++
SR-9	1.973+++	1.819+++	1.479+++	1.984+++
SR-10	2.005+++	2.047+++	1.622+++	2.035+++
SK-2	1.980+++	1.967+++	1.898+++	1.473+++
SK-3	1.427+++	1.321+++	.581++	-.002 -
SK-6	1.619+++	1.656+++	1.721+++	1.672+++
SK-7	1.372+++	.653++	.493+	.263+
SK-8	1.798+++	1.381+++	1.552+++	1.490++
SK-9	.828++	.814++	.700++	.337+
SK-13	1.275+++	1.249+++	.531++	.299+

Note: - = absorbance value <0.1 (no bile tolerance)
+= Absorbance value 0.1-0.5 (slightly bile tolerance)
++= absorbance value 0.5-1.0 (bile tolerance)
+++= absorbance value >1.0 (highly bile tolerance)

According to the data in Table 2, there are several isolates of LAB known to have highly bile tolerance that characterized by their grown on 0.6 NaDC except for isolates SK-3, SK-7, SK-9, and SK-13.

Acid Tolerance

Acid tolerance as one of the probiotic criteria among LAB isolates at low pH (pH 2, 3 and 4) showed a variation characterized by OD values that are showed in Table 3.

Table 3: Growth of LAB strains in MRS broth with different pH

Isolate codes	OD 660 nm			
	control (pH 6,5)	pH 2	pH 3	pH 4
SR-1	(2.035±0.002)	(1.977±0.007)+++	(1.995±0.006)+++	(2.071±0.011)+++
SR-3	(1.990±0.004)	(1.923±0.004)+++	(1.907±0.053)+++	(2.128±0.003)+++
SR-4	(2.055±0.008)	(2.032±0.027)+++	(2.057±0.010)+++	(2.018±0.003)+++
SR-5	(1.991±0.002)	(1.982±0.001)+++	(1.940±0.002)+++	(2.010±0.005)+++
SR-7	(2.118±0.002)	(1.931±0.008)+++	(1.958±0.010)+++	(2.117±0.012)+++
SR-8	(2.016±0.010)	(1.872±0.057)+++	(1.931±0.035)+++	(2.015±0.002)+++
SR-9	(1.991±0.003)	(1.892±0.010)+++	(1.899±0.006)+++	(2.092±0.006)+++
SR-10	(2.076±0.002)	(1.892±0.004)+++	(1.932±0.010)+++	(2.046±0.004)+++
SK-2	(1.855±0.002)	(1.724±0.039)+++	(1.220±0.034)+++	(1.577±0.020)+++
SK-3	(1.630±0.034)	(0.702±0.010)++	(1.366±0.029)+++	(1.225±0.016)+++
SK-6	(1.634±0.003)	(1.713±0.014)+++	(1.490±0.009)+++	(1.557±0.021)+++
SK-7	(0.850±0.050)	(0.650±0.017)++	(1.294±0.029)+++	(1.491±0.019)+++
SK-8	(1.558±0.048)	(1.522±0.064)+++	(1.577±0.031)+++	(1.552±0.051)+++
SK-9	(1.601±0.162)	(1.401±0.027)+++	(1.900±0.008)+++	(1.521±0.020)+++
SK-13	(1.728±0.041)	(1.567±0.026)+++	(1.519±0.034)+++	(1.315±0.021)+++

Note: - = absorbance value <0.1 (no acid tolerance)
+= Absorbance value 0.1-0.5 (slightly acid tolerance)
++= absorbance value 0.5-1.0 (acid tolerance)
+++= absorbance value > 1.0 (highly acid tolerance)

The data in Table 3 above shows that most of the isolates are categorized as high tolerance to acids at pH 2, 3 and 4, and only SK3 and SK7 are categorized as acid tolerance at pH 2.

Identification of Lactic Acid Bacteria

The results of API 50 CH test kits on 5 out of 15 isolates selected show variation in the fermentation of carbohydrates (Table 4). The results were analyzed using API WEB and that are summarised in Table 5.

Table 4: Carbohydrates fermentation by lactic acid bacteria isolates using API 50 CHL

Carbon source	LAB isolates				
	SR10	SK6	SK7	SK8	SK13
Control	-	-	-	-	-
(1) Glycerol	+	-	+	-	-
(2) Erythritol	-	-	-	-	-
(3) D-Arabinose	-	-	-	-	-
(4) L-Arabinose	+	+	+	-	-
(5) Ribose	+	+	+	+	-
(6) D-Xylose	+	+	+	-	-
(7) L-Xylose	-	-	-	-	-
(8) Adonitol	-	-	-	-	-
(9) ß-Mathyl-xyloside	-	-	-	-	-
(10) Galactose	+	+	+	+	-
(11) D-Glucose	+	+	+	+	+
(12) D-Fructose	+	+	+	+	+
(13) D-Mannose	+	+	+	+	+
(14) L-Sorbose	-	-	-	-	-
(15) Rhamnose	-	+	+	-	-
(16) Dulcitol	-	-	-	-	-
(17) Inositol	-	-	-	-	-
(18) Mannitol	+	+	+	-	-
(19) Sorbitol	+	-	+	-	-
(20)α-Methyl-D-mannoside	-	-	-	-	-
(21) α-Mathyl-D-Glucoside	-	-	-	-	-
(22) N-Acetyl glucosamine	+	+	+	+	+
(23) Amygdaline	+	-	+	-	-
(24) Arbutine	+	+	+	+	-
(25) Esculine	+	+	+	+	+
(26) Salicine	+	+	+	+	-
(27) Cellobiose	+	+	+	+	+
(28) Maltose	+	+	+	+	+
(29) Lactose	+	+	+	+	-
(30) Melibiose	+	+	+	+	-
(31) Saccharose	+	+	+	+	+
(32) Trehalose	+	+	+	+	-
(33) Inulin	-	-	-	-	-
(34) Melezitose	+	+	+	-	-
(35) D-Raffinose	+	+	+	+	-
(36) Amidon	-	-	-	-	-
(37) Glycogene	-	-	-	-	-
(38) Xylitol	-	-	-	-	-
(39) ß-Gentiobiose	-	+	-	-	-
(40) D-Turanose	-	-	-	-	-
(41) D-Lyxose	-	-	-	-	-
(42) D-Tagatose	+	+	+	+	-
(43) D-Fucose	-	-	-	-	-
(44) L-Fucose	-	-	-	-	-
(45) D-Arabitol	-	-	-	-	-
(46) L-Arabitol	-	-	-	-	-
(47) Gluconate	-	-	-	-	-
(48) 2 Ceto-gluconate	-	-	-	-	-
(49) 5 Ceto-gluconate	-	-	-	-	-

Note: (+) fermented, (-) not fermented

The data in Table 4 show each isolate fermented carbon with a different number. Isolate SK7 is known as the highest with 25 fermentation, followed by SK10, SK6, and SK8 with 24, 23, and 17 fermentation, respectively. Table 4 also showed isolate SK13 as the lowest with 8 fermentations. The analyzed of the data in Table 4 using API WEB software show homology with several referent strains. Description of each isolate with its percentages of similarity is summarised in Table 5.

Table 5: Homology of LAB isolates against referent strain according to the API 50 CHL

LAB Isolates	Strains	Percentage of similarity
SR 10	*Lactobacillus pentosus*	99.9
SK6	*Lactobacillus plantarum 1*	93.7
SK7	*Lactobacillus pentosus*	99.9
SK8	*Lactococcus lactis ssp lactis 1*	61.3
SK13	*Lactobacillus acidophilus 3*	64.4

Data in Table 5 show isolates SR10, SK6, and SK7 have similarity more than 90% i.e. 99.9, 93.7, and 99.9% as strain Lactobacillus *pentosus*, *Lactobacillus plantarum 1,* and *Lactobacillus pentosus,* respectively. On the other hand, two isolates i.e. SK8 and SK13 show lower similarity i.e. 61.3 and 64.4% as *Lactococcus lactis ssp lactis 1,* and *Lactobacillus acidophilus 3,* respectively.

Discussion

Lactic acid bacteria are known as a source of probiotic because of their healthy effect on human consumption. As a probiotic, the LAB must have the ability to exert antagonistic activity against non-pathogenic and spoilage organisms. In this study the result indicated 15 isolates LAB originated from the digestive tract of Bali cattle are adequate as a candidate of probiotic. This statement is strengthened by the characteristic of isolates that showed criteria as a probiotic. All isolates were identified as Gram-positive, catalase negative, and have antimicrobial activity against *Bacillus cereus* as an indicator of pathogenic bacteria.

The ability of the LAB to inhibit pathogenic or spoilage bacteria is resulted in the production of organic acids, hydrogen peroxide, and bacteriocin which are known as an antimicrobial substance [24]. Bacteriocin is often defined as a protein with an intra-specific antagonist with its effect bactericidal or bacteriostatic [25]. This result is in accordance to the study previously which found *Lactobacillus brevis 1* and *Lactoccus lactis spp lactis 1* isolated from the gastric juice of Bali cattle that were known to have an antimicrobial activity to *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 [26].

These results also strengthening the study that found LAB isolate 18A isolated from Bali cattle's colon which was known to have inhibitory efficacy against gastrointestinal pathogens such as *Escherichia coli* KL 48 (2) and *Staphylococcus aureus* by 18.8% and 28.06% respectively [16]. According to the CO_2 formation, most of the isolates categorized as homofermentative except for isolate SR9 as heterofermentative. Homofermentative LAB includes some lactobacilli and most species of enterococci, lactococci, pediococci, streptococci, tetragenococci, and vagococci, which ferment hexoses by the Embden-Meyerhof (E-M) pathway. The second category heterofermentative LAB includes leuconostocs, some lactobacilli, oenococci, and weissella species. The apparent difference on the enzyme level between these two categories is the presence or absence of the key cleavage enzymes of the E-M pathway (fructose 1,6-diphosphate) and the PK pathway (phosphoketolase) [27]. The functional aspect of isolates as a probiotic candidate also be full filled by most of the isolates.

The bacteria must be able to survive in the gastrointestinal tract that means these probiotics organisms must be resistant to bile acids and tolerant to acidic pH. Data in Table 2 shows almost isolates survive on NaDC concentration 0.6 mM. Amounting 11 out of 15 isolates survive with a good condition characterized by optical value (OD) >1.0. As well as the data in Table 3 that also shows most of the isolates resistant to low pH (pH 2).

These results in accordance to previous study that found strains of *Sporolactobacillus*, *Bacillus laevolacticus*, *Bacillus racemilacticus* and *Bacillus coagulans* grown in MRS broth were tolerant to low pH conditions (2, 2.5, and 3) and also tolerant to bile concentrations over 0.3% (w/v) [20]. The external pH partly determines the cytoplasmic or intracellular pH, which affects enzyme activity and reaction rates, protein stability, the structure of nucleic acids, and many other biological molecules [28].

The declining growth of several isolates after an exposure to low pH refers to the theory stated by Yang et al. [29] which said that the effect of excessive acidification on the cell wall will cause the destruction of bacterial cell membranes. These phenomena resulted by several important components such as magnesium, potassium, and fat, go out of cells and they will cause the lysis of bacteria.

On the other hand, several isolates also appear highly resistance to acids based on the theory of Cotter and Hill [30] which suggested that in order to survive at low pH, acid-resistant bacteria will maintain their internal pH conditions relatively higher than their environment.

This mechanism is carried out by the activation of the ATP-ase enzyme, resulting in enough energy to move protons from inside to go out of the cell. The fulfilled of both criteria by LAB isolates indicated these isolates survive in the intestinal tract as a prerequisite of the probiotic candidate. The use of LAB as a probiotic culture or as food adjunct must be tolerant to acid and bile which enables a selected strain to survive, grow, and perform its therapeutic benefits in the intestinal tract [6].

Bacteria would contact at pH values ranging from 2.0 to 8.0 in the gastrointestinal tract if consumed [31]. Thus, probiotic cultures must survive in the environment with gastric and bile acids when viable cells go through to the gastrointestinal tract. Resisting at pH 3 for 2 h and growing in the medium containing 1,000 ppm of bile acids are considered as standards for acid and bile tolerance of probiotic culture [32].

The API 50 CH fermentation profiles may serve to characterize isolates to show their metabolic profiles. This method is considered as a well-established method for manual microorganism identification to the species level. Identification of 5 LAB species in this study show dominated by *Lactobacillus sp*. According to the previous study, several strains of Lactobacillus i.e. *Lactobacillus paracasei* and *Lactobacillus Plantarum* showed good probiotic potential and inhibited the growth of enteropathogenic bacteria including ETEC H10407, *Shigella flexneri* ATCC 12022, *Shigella sonnei* ATCC 9290, *Salmonella enteritidis* H7 and *Yersinia enterocolitica ATCC 23715* [33].

The result also confirmed the method as one of the strongly favoured phenotypic procedures in common use for identifying of Lactobacillus species, although API 50 CH profiles should be complemented with molecular genetic like 16S rRNA, random amplified polymorphic DNA (RAPD), PCR-DGGE, SDS-PAGE, and MALDI-TOP MS analysis to be effectiveness of identification [15, 16, 34, 35].

Conclusion

Our study showed that most of the LAB isolates isolated from the gastrointestinal tract of Bali cattle showed potential as a probiotic candidate. However, further study needs to be conducted in order to investigate the effectiveness of their potency.

Disclosure

The authors report no conflict of interests in this work.

References

1. Carr FJ, Chill D, Maida N (2002) The lactic acid bacteria: a literature survey. *Crit. Rev. Microbiol.*, 28: 281-370.
2. Aween MM, Hassan Z, Muhialdin BJ, Eljamel YA, Al-Mabrok AS, Lani MN (2012) Antibacterial activity of Lactobacillus acidophilus strains isolated from honey marketed in Malaysia against selected multiple antibiotic resistant (MAR) Gram-positive bacteria. *J. Food Sci.,* 77**:** M364-371.
3. Salminen S (1990) The role of intestinal microflora in preserving intestinal integrity and health with special reference to lactic acid bacteria. *Ann Med.*, 22**:**35.
4. Batan IW (2006) *Bali Cattle and Diseases*, Ed. Udayana Press, Denpasar.
5. Salminen S (2012) *Lactic acid bacteria : microbiological and functional aspects*, pp. 4th Ed. CRC Press, Taylor & Francis, Boca Raton.
6. Salminen S, Wright Av, Ouwehand A (2004) *Lactic acid bacteria : microbiology and functional aspects*, 3rd Ed. Marcel Dekker, New York.
7. Ji K, Jang NY, Kim YT (2015) Isolation of Lactic Acid Bacteria Showing Antioxidative and Probiotic Activities from Kimchi and Infant Feces. *J. Microbiol. Biotechnol.*, 25: 1568-1577.
8. Swain MR, Anandharaj M, Ray RC, Parveen Rani R (2014) Fermented fruits and vegetables of Asia: a potential source of probiotics. *Biotechnol Res Int.,* 250424.
9. Nermes M, Kantele JM, Atosuo TJ, Salminen S, Isolauri E (2011) Interaction of orally administered Lactobacillus rhamnosus GG with skin and gut microbiota and humoral immunity in infants with atopic dermatitis. *Clin. Exp. Allergy.*, 41:370-377.

10. Zoumpopouloua G, Potb B, Tsakalidoua E, Papadimitrioua K (2017) Dairy probiotics: Beyond the role of promoting gut and immune health. *International Dairy Journal,* 67: 46-60.

11. Shewale RN, Sawale PD, Khedar SD, Singh A (2014) Selection Criteria for Probiotic: A Review. *International Journal of Probiotics and Prebiotics,* 9:17-22.

12. Salminen S, Nybom S, Meriluoto J, Collado MC, Vesterlund S, El-Nezami H (2010) Interaction of probiotics and pathogens--benefits to human health? *Curr Opin Biotechnol.*, 21: 157-167.

13. Shen B, Brzezinski A, Fazio VW, Remzi FH, Achkar JP, Bennett AE*, et al* (2005) Maintenance therapy with a probiotic in antibiotic-dependent pouchitis: experience in clinical practice. *Aliment Pharmacol. Ther.*, 22:721-728.

14. FAO/WHO (2002) Guidelines for the Evaluation of Probiotics in Food. Report of a Joint FAO/WHO Working Group, London, Ontario, Canada.

15. Nigatu A (2000) Evaluation of numerical analyses of RAPD and API 50 CH patterns to differentiate Lactobacillus plantarum, Lact. fermentum, Lact. rhamnosus, Lact. sake, Lact. parabuchneri, Lact. gallinarum, Lact. casei, Weissella minor and related taxa isolated from kocho and tef. *J. Appl. Microbiol.,* 89: 969-978.

16. Suardana IW, Cahyani AP, Pinatih KJP (2016) Probiotic Potency and Molecular Identification of Lactic Acid Bacteria Isolated from Bali Cattle's Colon, Indonesia. *Global Advanced Research Journal of Medicine and Medical Sciences,* 5:143-149.

17. Sukrama IDM, Pinatih KJP, Suardana IW (2017) Molecular Analysis of Lactic Acid Bacteria Isolate Sr2 from Bali Cattle Rumen. *Journal of Global Pharma Technology,* 05: 44-49.

18. Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.*, 6:71-79.

19. Sujaya IN, Ramona Y, Widarini NP, Suariani NP, Dwipayanti NMU, Nocianitri KA, et al (2008) Probiotic potency of Lactobacillus spp. isolated from Sumbawa mare milk. *Jurnal Veteriner.*, 9:33-40.

20. Hyronimus B, Le Marrec C, Sassi AH, Deschamps A (2000) Acid and bile tolerance of spore-forming lactic acid bacteria. *Int. J. Food Microbiol.*, 61:193-197.

21. Kilic GB, Karahan AG (2010) Identification of lactic acid bacteria isolated from the fecal samples of healthy humans and patients with dyspepsia, and determination of their ph, bile, and antibiotic tolerance properties. *J. Mol. Microbiol. Biotechnol.,* 18: 220-229.

22. Brolazo EM, Leite DS, Tiba MR, Villarroel M, Marconi C, Simoes JA (2011) Correlation between api 50 ch and multiplex polymerase chain reaction for the identification of vaginal lactobacilli in isolates. *Braz. J. Microbiol.,* 42: 225-232.

23. McDonald LC, McFeeters RF, Daeschel MA, Fleming HP (1987) A differential medium for the enumeration of homofermentative and heterofermentative lactic Acid bacteria. *Appl. Environ. Microbiol.*, 53: 1382-1384.

24. Garzon K, Ortega C, Tenea GN (2017) Characterization of Bacteriocin-Producing Lactic Acid Bacteria Isolated from Native Fruits of Ecuadorian Amazon. *Pol. J. Microbiol.,* 66: 473-481.

25. Con AH, Gokalp HY, Kaya M (2001) Antagonistic effect on Listeria monocytogenes and L. innocua of a bacteriocin-like metabolite produced by lactic acid bacteria isolated from sucuk. *Meat Sci.,* 59: 437-441.

26. Suardana IW (2013) Study on Growth Patterns and Antimicrobial Activity of Isolate Lactococcus lactis spp lactis 1 Isolated from Gastric Juice of Bali Cattle, pp. *In* Satriawan IK, Mulyani PA, Gunam IBW (eds.), *The Book of Unud's Work for the Nation*, Ed. Universitas Udayana, Kampus Bukit Jimbaran

27. De Vos WM, Kleerebezem M, Kuipers OP (2013) xLactic acid bacteria - Genetics, metabolism and application. *FEMS Microbiol Rev.,* 29: 391.

28. Slonczewski JL, Fujisawa M, Dopson M, Krulwich TA (2009) Cytoplasmic pH measurement and homeostasis in bacteria and archaea. *Adv. Microb. Physiol.*, 55: 1-79, 317.

29. Yang YS, Chen MC, Liao CC (2001) *Mutant Bifidobacteria strains with acid,*

bile salt and oxygen tolerance. US patent application.

30. Cotter PD, Hill C (2003) Surviving the acid test: responses of gram-positive bacteria to low pH. *Microbiol Mol. Biol. Rev.,* 67: 429-453.
31. Hood VL, Schubert C, Keller U, Muller S (1988) Effect of systemic pH on pHi and lactic acid generation in exhaustive forearm exercise. *Am J. Physiol.*, 255: F479-485.
32. Itoh H (1992) Functional benefits from lactic acid bacteria used in cultured milk. *Anim. Sci. Technol.,* 63: 1276-1289.
33. Davoodabadi A, Soltan Dallal MM, Rahimi Foroushani A, Douraghi M, Sharifi Yazdi MK, Amin Harati F (2015) Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. *Anaerobe.,* 34: 53-58.
34. Gu RX, Yang ZQ, Li ZH, Chen SL, Luo ZL (2008) Probiotic properties of lactic acid bacteria isolated from stool samples of longevous people in regions of Hotan, Xinjiang and Bama, Guangxi, China. *Anaerobe.,* 14: 313-317.
35. Ahmadsah LSF, Kim E, Jung YS, Kim HY (2018) Identification of LAB and Fungi in Laru, a Fermentation Starter, by PCR-DGGE, SDS-PAGE, and MALDI-TOF MS. *J. Microbiol. Biotechnol.,* 28: 32-39.

