Journal of Dry Zone Agriculture, 2021, 7(1):81-96

Biochemical characterization and antibacterial potential of lactic acid bacteria from *Idli* batter and their influences on properties of batter

D. Bernard[1]*, N. Jeyagowri[2] and T. Madhujith[3]

[1]*Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka*
[2]*Department of Bioscience, Faculty of Applied Science, University of Vavuniya, Vavuniya, Sri Lanka*
[3]*Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka*

Received 15th April 2021, Accepted 10th November 2021

Abstract

Idli is one of the flour-based foods fermented by lactic acid bacteria. This study was designed to characterize the lactic acid bacteria isolated from *Idli* batter and to determine their antibacterial activity. Physicochemical changes of batter during fermentation and sensory qualities of the final product (*Idli*) also were determined. Lactic acid bacteria were isolated from *Idli* batter and characterized up to genus level. Agar well diffusion assay was used to determine the antibacterial activity of isolates against foodborne pathogens (*Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus)*. Changes of pH, lactic acid bacterial count, titratable acidity of batter during fermentation and sensory properties of the final product were measured. Ten isolates (I-1 to I-10) were isolated from *Idli*

* Corresponding author
Postal address: Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka
Email: diluckmini@gmail.com
ORCID ID: https://orcid.org/0000-0002-8137-472X

batter, of which, six were rod and other four were cocci shaped. All ten isolates were Gram positive, non-motile, non-spore formers and catalase activity negative. It was confirmed that all isolates belong to lactic acid bacteria up to genus level. Based on diameter of the inhibition zone, among ten isolates, I-6 was considered as the highest potential bacteriocinogenic isolate. Inhibition zone diameter of isolates ranged from 7.3±1.53 to 16.3±0.58 mm. The pH dropped steadily from 6.28 to 3.72, while titratable acidity increased from 0.24 to 0.92% throughout the fermentation period (0-32 h). With fermentation, the lactic acid bacterial count was increased, and highest lactic acid bacterial count of 9.88 $\log_{10}$cfu/g was observed after 12 h of fermentation. *Idli* prepared from *Idli* batter after 8-12 h of fermentation scored maximum for the sensory quality. From this study it is concluded that all lactic acid bacteria isolates possess antimicrobial activity and physicochemical and sensory qualities of *Idli* batter changed with duration of fermentation.

Keywords: antimicrobial activity, *idli* batter, lactic acid bacteria, physicochemical, sensory quality

INTRODUCTION

Fermented foods have acquired an ever-increasing trend due to their health benefits and fermentation is one of the oldest food preservation methods. Fermented foods are popular nowadays across the world as they are considered as functional foods. The Lactic Acid Bacteria (LAB) are the dominant organisms involved in fermentation of foods and produce acids in optimum amount. The amount of acid production varies with type of food and it changes the sensory quality of food.

LABs are important group of probiotic bacteria. They have become a major focus in food industry as they are improving human health in natural way (Fernandez *et al*., 2003; Balasubramanian and Viswanathan, 2007). These organisms have been widely reported to exert many beneficial health effects, such as activation of immune system, prevention of cancer cell growth, maintenance of mucosal integrity and presentation of an antagonistic environment for pathogens (Rashmi and Gayathri, 2014).

It is important to isolate and screen the new probiotic LAB strains from fermented foods as they own strong probiotic functionalities. Antimicrobial activity against foodborne pathogens is one of such

probiotic functionalities. They have ability to produce organic acids, exopolysaccharides and proteolytic products and through these by products they show antimicrobial activity against many foodborne pathogens (Abd El Gawad *et al*., 2010; Ayad *et al*., 2004; Ayad and Shokery, 2011; Chelliah *et al*., 2016; El-Soda *et al*., 2003).

Fermented foods from Asian recipe are non-dairy products, prepared from different raw materials, such as vegetables, grains, fruits, etc. Relatively, rapid growth is initiated by LAB over a wide range of salt concentrations and temperature (25 to 37 ^{o}C) in numerous plant-based flour materials. "*Dosa*", yoghurt rice, "*Idli*" and hoppers are considered as some of traditional fermented products consumed by vast majority of the people in Sri Lanka and India for their breakfast and dinner (Wickramanayake, 2002).

Idli is a cereal and legume based fermented food. Raw materials used for the preparation of *Idli* batter are rice (*Oryza sativa*) and dehulled black gram (*Phaseolus mungo*). Following fermentation, the *Idli* batter is steamed and formed into round shaped, soft and porous textured product. Some studies have reported that LABs are involved in *Idli* batter fermentation and have probiotic potential. Those strains are *Lactobacillus collinoides* (Patel *et al*., 2012), *Leuconostoc mesenteroides, Streptococcus faecalis,* and *Pediococcus cerevisiae* (Mukherjee *et al*., 1965) (Balasubramanian and Viswanathan, 2007), *Lactobacillus delbrueckii* (Soni *et al*., 1986), *Lactococcus lactis* and *Lactobacillus plantarum* (Iyer *et al*., 2013). The LAB isolates from *Idli* batter also can be used as a starter culture during the fermented (functional) food production (Conter *et al*., 2005).

Even though, several studies have shown the biochemicals produced by LAB during fermentation of *Idli*, activity of LAB in the gut and their contribution for sensory quality of food, still, isolation and screening of new probiotic strains of LAB from fermented foods is a major research focus. *Idli* batter is fermented by LAB and the LAB isolated from this batter can be used for innovative industrial food applications. Considering the above facts, this study aimed to isolate and characterize the novel strains on LAB from *Idli* batter, determine the antibacterial activity of isolates and identify the acidity changes and sensory quality of *Idli* batter with fermentation.

MATERIALS AND METHODS

Materials

Black gram (*Phaseolus mungo*) and parboiled rice *(Oryza sativa)* were procured from local market in Vavuniya, Sri Lanka. All microbiological media and chemicals were obtained from HiMedia, India. The standard antibiotic discs were procured from Oxoid, UK. The pure cultures of standard food borne pathogens (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica*) were collected from Palmyrah Research Institute, Jaffna, where it was isolated and preserved. The culture was tested again for purity and species characteristics for confirmation.

Preparation of *Idli* batter

Idli batter was prepared by the procedure described by Wickramanayake (2002). The black gram and parboiled rice were soaked in water separately for four to six hours. The seed coat of black gram was removed, mixed in the proportion of 1 to 2, mashed and left overnight at ambient conditions.

Isolation of LAB

Agar microbial growth media, deMan Rogosa and Sharpe (MRS) was used for the isolation of LAB from the batter as reported previously by De Man *et al*. (1960). The pH of the MRS medium was maintained at 6.2. *Idli* batter (1 g) was suspended in 9 mL of 0.9% saline water and subjected to serial dilutions. Diluted mixture (0.1 mL) was pipetted out and spread out on the surface of an MRS medium agar plate. The plates were incubated in the inverted position for 48 h at 37 °C under anaerobic conditions using candle jar technique.

Ten well developed characteristic colonies growing on the MRS medium were picked up carefully and purified by streaking on MRS agar medium plates following four-way streaking technique and subsequently they were purified for 4 to 5 times to get pure cultures. The discrete single purified colonies were picked up and transferred into MRS broth in culture vials and the grown-out cultures were maintained at 4 °C in a refrigerator for further analysis (Pal *et al*., 2005). To prepare fresh pure cultures for further analysis, the preserved pure cultures were activated in MRS agar by streaking and incubated at 37 °C under anaerobic condition for 48 h before experiments.

Identification and biochemical characterization of pure culture

Gram staining as described by Rangaswami and Bagyaraj (1993) was carried out for morphological examination. Catalase activity (Murry, 1981), motility test (Barrow and Felthman, 1993) and spore forming ability (Barrow and Felthman, 1993) were used for biochemical identification of LAB from *Idli* batter.

Gram staining

The Gram characteristics of LAB were determined for the fresh isolates grown over 48 h using light microscope following staining. The gram-stained slides were observed under oil immersion (10 x 100) of light microscope (Olympus CH20i-India) and the cell morphology of isolated LAB were observed as described by Becking (1974).

Catalase test

A loopful of LAB culture grown over 48 h were transferred into a glass test tube containing 0.5 mL distilled water and mixed thoroughly with 0.5 mL of 3% hydrogen peroxide solution. Subsequently, the effervescence was observed.

Motility test

"Hanging drop method" was used to examine the motility of strains. At the centre of a coverslip, the bacterial culture was placed and each corner of the coverslip a drop of paraffin wax was placed. Over the coverslip a cavity slide was placed, and it was inverted to suspend the bacterial culture in the cavity slide's central depression. Finally, the motility was observed under high-power dry objective (x40) with reduced illumination of light microscope (Olympus CH20i-India).

Endospore staining

On a microscopic slide, a thin smear of bacterial smear was prepared under aseptic conditions and heat fixed. Then the slide was placed over a steaming water bath and malachite green (primary stain) was applied for 5 min on the slide while covering the slide with blotting paper. Then the slide was removed from the water bath, the blotting paper was removed, and the slide was rinsed with water until water turned clear. Then the slide

was flooded with the counter stain safranin for 20 s and rinsed with water. Subsequently the slide was blot-dried and observed using oil immersion (10x100) of light microscope (Olympus CH20i-India).

Estimation of the growth of LAB count with fermentation

LAB counts were determined with fermentation time by using spread plate method (SLS: 516: Part 1: 1991). The freshly prepared *Idli* batter was allowed to ferment and during fermentation, samples of nearly 25 g were obtained from *Idli* batter for every 4 h interval up to 32 h and LAB counts were determined.

Serial dilutions were prepared up to 10^{-5}. Samples (0.1 mL) were taken from each dilution and poured on a MRS media plate. The sample was spread out evenly over the surface of agar using the sterile glass spreader, while the underneath of petridishes were rotated at the same time. Then, the plates were incubated in an inverted position for 48 h at 37 °C under anaerobic condition by candle jar technique. Three replicates were maintained. After the specified period of incubation, the colonies were counted in each dish containing not more than 300 colonies using the colony counter.

Evaluation of the biochemical changes during fermentation of batter

Idli batter was allowed to ferment, and pH (Sension+ PH 31-Spain) and titratable acidity were determined every 4 h interval up to 32 h of fermentation.

Evaluation of sensory quality of *Idli* with different fermentation time *Idli*

Idli prepared by fermenting the *Idli* batter for different time durations: 4, 8, 12 and 16 h were subjected for sensory attributes based on a 5-point hedonic scale using a panel of 30 semi-trained panellists.

Assessment of antimicrobial activity of isolated LAB

Antimicrobial activity of isolates was assessed against food borne pathogens, *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* by measuring the diameters of zone of inhibition (Chopra and Mehra, 2015) using agar well diffusion method.

The sample was centrifuged at 5000 rpm for 15 mins. The resulting cell debris that formed a pellet was discarded giving rise to a cell free supernatant. The pH of the Cell Free Supernatant (CFS) was adjusted to 7 with 1M NaOH (Sharpe *et al*., 1979). Sterile cotton swabs were dipped into the culture of test microorganisms; *E. coli*, *S. aureus* and *S. enterica* were inoculated by spread plate method over entire surface of Muller Hinton agar plates, which was preset. Agar well (9 mm) was created using sterile cork borer. CFS was poured into the wells and kept into the refrigerator at 4 °C for 2 h. Later, the plates were incubated anaerobically at 37 °C for 24 h. After 1 day of incubation at 37 °C, each plate was examined for zone of inhibition. The diameter of inhibitory zone was measured.

Statistical analysis

Three independent experiments for each isolate were carried out to determine the titratable acidity, pH, LAB count and antibacterial potential. The means of variable and standard deviations were calculated using Microsoft excel 2010. The data were analyzed using one way analysis of variance (ANOVA) using SPSS 16.0. The significant difference was compared using Turkey test.

RESULTS AND DISCUSSION

Ten well developed characteristic presumptive LAB were isolated from *Idli* batter. The characteristic colonies were identified on the surface of MRS agar petri plates. Different type of colonies: punctiform, small, moderate and large size colonies were observed on the surface. All colonies were non-pigmented in other words, creamy to white (Table 1).

Based on Gram staining, all isolates were Gram positive, 6 isolates were rod shaped while other 4 were cocci shaped. In case of endospore test, the isolates without green stained spores were termed as non-spore formers. All cultures were shown negative result thus they were identified as non-spore formers. The motility test has recognized that all isolates shown non-motile behaviour. Catalase test is used to determine if bacteria possess catalase enzyme which can detoxify H_2O_2. The LABs are negative to catalase test as they neither possess peroxidase enzyme nor produce bubbles when mixed with 3% hydrogen peroxide (Murry, 1981). Catalase test for all ten isolates were negative. indicating the absence of catalase enzyme and confirms that the isolates belong to LAB.

Table 1: Morphological and Biochemical characteristics of LAB isolates from *Idli* batter

Isolates	Colony morphology	Gram reaction	Motility	Spore forming ability	Catalase activity	Genus Name
I-1	Circular, Moderate size, White creamy and Smooth	Positive, cocci	Non-motile	Non-spore formers	Negative	*Lactococcus* spp.
I-2	Circular, Large size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	*Lactobacillus* spp.
I-3	Circular, Punctiform, White creamy and Smooth	Positive, cocci	Non-motile	Non-spore formers	Negative	*Lactococcus* spp.
I-4	Irregular, Moderate size, White creamy and Smooth	Positive, cocci	Non-motile	Non-spore formers	Negative	*Lactococcus* spp.
I-5	Circular, Small size, White creamy and Smooth	Positive, cocci	Non-motile	Non-spore formers	Negative	*Lactococcus* spp.
I-6	Spindle, Large size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	*Lactobacillus* spp.
I-7	Irregular, Small size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	*Lactobacillus* spp.
I-8	Irregular, Large size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	*Lactobacillus* spp.
I-9	Spindle, Moderate size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	*Lactobacillus* spp.
I-10	Spindle, Small size, White creamy and Smooth	Positive, rod	Non-motile	Non-spore formers	Negative	*Lactobacillus* spp.

From 10 isolated colonies, 6 isolates were found to be Gram positive, rod shaped, non-motile, non-spore formers and negative to catalase activity, belonging to *Lactobacillus* spp. Other 4 isolates were Gram positive, cocci shape, non-motile, non-spore formers and negative to catalase activity, belonging to *Lactococcus* spp. In summary, according to the above tests, it was confirmed up to the genus level that all strains were LAB and among them 6 were *Lactobacillus* spp.

The antimicrobial activity as measured by the zone of inhibition is given in Table 2. Among ten isolates, isolate I-6 was considered as the highest potential bacteriocinogenic isolate against all test organisms. Smaller zone of inhibition was noted by all isolates against *E.coli*. In case antagonism activity of isolates, isolates I-2, I-3, I-5, I-6, I-7 and I-10 were shown highest antagonism activity against *S. enterica*, than other indicator organisms.

Table 2: Antimicrobial activity of LAB isolates obtained from *Idli* batter

Isolates	*Salmonella entrerica*	*Escherichia coli*	*Staphylococcus aureus*
1	12.3 ± 0.58^{bcd}	9.3 ± 1.53^{ab}	12.7 ± 0.57^{ab}
2	16.3 ± 0.58^{a}	8.3 ± 1.53^{ab}	9.6 ± 1.15^{b}
3	14.7 ± 1.15^{ab}	8.3 ± 1.53^{ab}	10.7 ± 0.58^{ab}
4	9.7 ± 0.58^{de}	8.7 ± 1.15^{ab}	11.3 ± 0.58^{ab}
5	13.3 ± 1.53^{bc}	7.3 ± 1.53^{b}	12.7 ± 1.15^{ab}
6	16.3 ± 0.58^{a}	11.7 ± 1.15^{a}	13.3 ± 1.53^{a}
7	14.3 ± 0.58^{abc}	11.7 ± 0.58^{a}	12.7 ± 1.15^{ab}
8	9.3 ± 1.53^{e}	10.3 ± 1.53^{ab}	12.7 ± 0.58^{ab}
9	10.3 ± 1.53^{de}	11.3 ± 0.58^{a}	12.3 ± 1.53^{ab}
10	11.7 ± 0.58^{cde}	10.7 ± 0.58^{ab}	11.7 ± 1.15^{ab}

Each value in the table was represented as mean ± SD (n = 3). Values in the same column followed by a different letter (a-e) are significantly different ($p < 0.05$).

The isolates I-1, I-4, I-8, I-9, and I-10 was shown higher zone of inhibition against *S. aureus*, than other indicator organisms. The overall zone of inhibition diameter of isolates were fallen within the range from 7.3±1.53 to 16.3±0.58 mm. Tejero-Sarinena *et al*. (2012) has stated that production of antimicrobial substances such as hydrogen peroxides, acetic acid, lactic acid and bacteriocins are the reason for showing antimicrobial properties by probiotics.

The main physiochemical characteristic of *Idli* batter fermentation is lactic acid formation. It can be noted by changes in the pH and titratable acidity. Table 3 shows the changes of pH, titratable acidity, and LAB count during fermentation of *Idli* batter. The measurements were taken for every 4 h time interval up to 32 h of fermentation. During fermentation, the titratable acidity was increased slowly from 0.24 to 0.32 % at the 8 h of fermentation *Idli* and sudden increment has been noted at 8 - 12 h of fermentation from 0.32 % to 0.40 %. After 12 h, the titratable acidity remained steady up to 24 h and the titratable acidity unchanged in both 24 and 28 h of fermentation that was 0.52 %. At the period of 28 – 32 h the titratable acidity increased drastically from 0.52 % to 0.92 %. While titratable acidity increased from 0.24 to 0.92%, the pH dropped steadily from 6.28 to 3.72. Even though the fermentation temperature varied between 25 and 37 °C, the same observation has been recorded in several studies (Ghosh and Chattopadhyay, 2012; Rajalakshmi and Vanaja, 1967; Soni and Sandhu, 1989; Soni and Sandhu, 1990; Thakkar *et al*., 2015).

The increasing acidity in *Idli* batter mainly depends on S*treptococcus faecalis* as it produces lactic acid and reduces the pH, increases CO_2 concentration in the batter, which leavens batter property (Mukherjee *et al.*, 1965). With the growth of LAB, the pH of batter decreases. The reduction of batter pH helps the activity of yeast as the optimum pH for yeast ranges from 4.4 to 4.5 (Soni and Sadhu, 1990).

The LAB count increased from 7.55 to 9.88 $\log_{10}$cfu/ g during fermentation. High LAB count was observed after 12 h of fermentation *Idli* where the pH level was noted as 4.64. The count reduced with the increment of acidity. After 12 h of fermentation the LAB count dropped drastically from 9.88 to 5.91 $\log_{10}$cfu/g during 32 h of fermentation. It may be due to increment of acidity which may not be favourable to LAB population.

The sensory quality of *Idli* prepared from the batter with different fermentation durations was analysed (Figure 1). The colour, appearance,

taste, texture, mouth feel and the overall acceptability were taken into the analysis. The *Idli* prepared following 8 and 12 h of fermentation showed maximum desirable sensory quality where pH and acidity were shown within the range of 4.64 - 5.21 and 0.32 - 0.40%, respectively.

Table 3: Changes of pH, titratable acidity and LAB count with fermentation of *Idli* batter at 4 h time intervals from 0-32 h

Fermentation time (h)	pH	LAB (cfu/g)	Titratable acidity (%)
0	6.28 ± 0.02^{a}	7.55 ± 0.11^{d}	0.24 ± 0.02^{e}
4	5.86 ± 0.01^{b}	8.11 ± 0.1^{c}	0.28 ± 0.01^{de}
8	5.21 ± 0.06^{c}	8.89 ± 0.02^{b}	0.32 ± 0.07^{cde}
12	4.64 ± 0.04^{d}	9.88 ± 0.05^{a}	0.40 ± 0.06^{bcd}
16	4.58 ± 0.04^{d}	8.74 ± 0.09^{b}	0.44 ± 0.02^{bc}
20	4.39 ± 0.07^{e}	7.05 ± 0.07^{de}	0.48 ± 0.05^{b}
24	3.88 ± 0.03^{f}	6.84 ± 0.26^{e}	0.52 ± 0.11^{b}
28	3.78 ± 0.08^{fg}	6.97 ± 0.15^{e}	0.52 ± 0.04^{b}
32	3.72 ± 0.06^{g}	5.91 ± 0.46^{f}	0.92 ± 0.01^{a}

Each value in the table was represented as mean ± SD (n = 3). Values in the same column followed by a different letter (a-g) are significantly different ($p < 0.05$).

Idli prepared with the batter fermented for 4 and 16 h scored less for the overall acceptability. It may be due to the characteristic flavour and taste that may develop with prolonged fermentation. Four hours of fermentation might not be adequate to develop the desirable sensory attributes. Following 16 h of fermentation, higher acidity the sourness may be increased, and the activity of microorganisms may cause undesirable texture of *Idli*. It may be the reason for the lower scores in overall acceptability (Figure 1). The maximum score of sensory quality was obtained at the LAB count was noted high in number in the batter which was at 8 h and 12 h of fermentation. Therefore, it indicates that

LAB contribute to the textural and over all acceptability improvement of *Idli* batter. The same results were stated by different authors (Lee, 2001; Thakkar *et al*., 2015). They reported that the taste of *Idli* prepared from the *Idli* batter when the pH and acidity of *Idli* batter within the range of 4.0 - 4.5 and 0.5 - 0.6% at the time of fermentation has scored maximum.

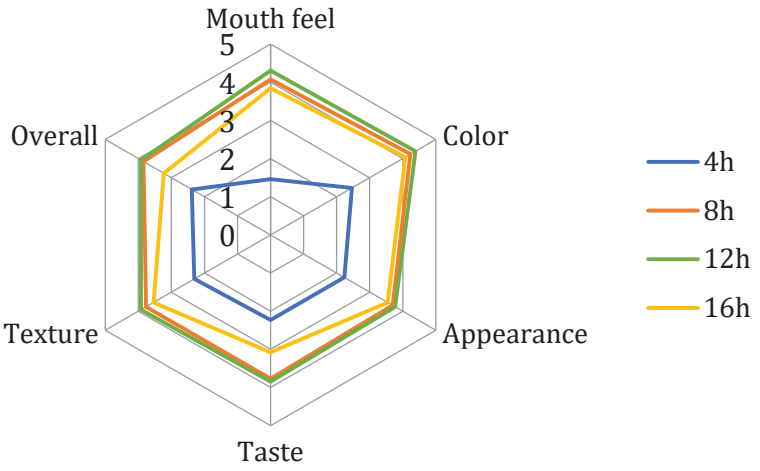

Figure 1: Web diagram representing sensory quality of *Idli* prepared using different fermentation durations

CONCLUSIONS

Lactobacilli spp are the predominant LAB microbial group involved in natural *Idli* batter fermentation which have antimicrobial activity against foodborne pathogens. It changes the biochemical characteristics of the *Idli* batter, subsequently it improves the texture and organoleptic characteristics of final steamed product. In future, species level identification of isolated LABs from *Idli* batter, assessment of exo-polysaccharide production ability of selected isolates, amount of bacteriocin production to control foodborne / spoilage bacteria and bacteriocin chemical characterization studies can be carried out to bring this study forward and more useful.

DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

REFERENCES

Abd El Gawad, I.A., Abd El Fatah, A.M. and Al Rubbayyi, K.A. 2010. Identification and characterization of dominant lactic acid bacteria isolated from traditional Rayeb milk in Egypt. Journal of American Science, 6(10):728-735.

Ayad, E.H.E., Nashat, S., El-Sedek, N., Metwally, H. and El-Soda, M. 2004. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food Microbiology, 21(6):715-725.

Ayad, E.H.E. and Shokery, E.S. 2011. Industrial important lactic acid bacteria isolated from Egyptian human milk and dairy products. Egyptian Journal Dairy Science, 39(2):197-207.

Balasubramanian, S. and Viswanathan, R. 2007. Properties of Idli batter during its fermentation time. Journal of Food Processing and Preservation, 31(1):32-40.

Barrow, G.I. and Felthman, R.K.A. 1993. Bacterial Characters and Charaterization. In: Barrow, G.I. and Felthman, R.K.A. (eds) Cowman and Steels manual for the identification of medical bacteria. 3rd edn. Cambridge University Press, UK, pp 21-45.

Becking, J.H. 1974. Family 2, Azotobacteriaceae. In: Buchanan, R.E. and Gibbons, N.E. (eds) *Bergey's Manual of Determinative Bacteriology*. 8th edn. Baltimore: The Williams and Wilkins Co, Baltimore, pp 253-261.

Chelliah, R., Ramakrishnan, S.R., Prabhu, P.R. and Antony, U. 2016. Evaluation of antimicrobial activity and probiotic properties of wild-strain *Pichia kudriavzevii* isolated from frozen *idli* batter. *Yeast*, 33:385-401.

Chopra, S. and Mehra, S. 2015. Identification of lactic acid bacteria from fermented Indian food and study its physical and cultural parameters on bacteriocins produced by them. International Journal for

Research in Applied Science & Engineering Technology (IJRASET), 3(7):339-343.

Conter, M., Muscariello, T., Zanardi, E., Ghidini, S., Vergara, A., Campanini, G. and Ianieri, A. 2005. Characterization of Lactic Acid Bacteria isolated from an Italian dry fermented sausage. Annali della Facoltà di Medicina Veterinaria - Università di Parma, 25(1):167-174.

De Man, J.C., Rogosa, M. and Sharpe, M.E. 1960. A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology, 23:130-135.

El-Soda, M., El-Zeiney, M., Awad, S., Osman, G., Omarn, N., Gamal, G., Ezzat, N. and El Shafei, H. 2003. A culture collection of lactic acid bacteria isolated from raw milk and traditional Egyptian dairy products. Egyptian Journal Dairy Science, 31(1):23-41.

Fernandez, M.F., Boris, S. and Barbes, C. 2003. Probiotic properties of human Lactobacilli strains to be used in the gastrointestinal tract. Journal of Applied Microbiology, 94(3):449-455.

Ghosh, D. and Chattopadhyay, P. 2012. Application of principal component analysis (PCA) as a sensory assessment tool for fermented food products. Journal of Food Science and Technology, 49(3):328-334.

Iyer, B.K., Singhal, R.S. and Ananthanarayan, L. 2013. Characterization and in vitro probiotic evaluation of lactic acid bacteria isolated from Idli batter. Journal of Food Science and Technology, 50(6):1114-1121.

Lee, C. 2001. Fermentation technology in Korea. Seoul: Korea University Press, Korea.

Mukherjee, S.K., Albury, M.N., Pederson, C.S., Van Veen A.G. and Steinkraus, K.H. 1965. Role of *Leuconostoc mesenteroides* in leavening the batter of *Idli*, a fermented food of India. Applied Microbiology, 13(2):227-231.

Murry, R.G.E. 1981. Morphology. In: Gerhardt, P., Murry, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Kreig, N.R. and Phillips, G.B. (eds) Manual of Methods of General Bacteriology. 2nd edn. American Society for Microbiology, Washington DC, pp 5-64.

Pal, V., Jamuna M. and Jeevaratnam, K. 2005. Isolation and characterization of bacteriocin producing lactic acid bacteria from a south Indian special dosa (appam) batter. Journal of culture collections. 4:53-60.

Patel, A., Lindström, C., Patel, A., Prajapati, J.B. and Holst, O. 2012. Probiotic properties of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented foods. International Journal of Fermented Foods, 1(1):87-101.

Rajalakshmi, R. and Vanaja, K. 1967. Chemical and biological evaluation of the effects of fermentation on the nutritive value of foods prepared from rice and grams. British Journal of Nutrition, 21(2):467-473.

Rangaswami, G. and Bagyaraj, D.J. 1993. Microbial Biotechnology. In: Rangaswami, G. and Bagyaraj, D.J. (eds.) Agricultural Microbiology. 2nd edn. Prentice Hall of India Pvt. Ltd, New Delhi, pp 388-405.

Rashmi, B.S. and Gayathri, D. 2014. Partial purification, characterization of *Lactobacillus* sp G5 lipase and their probiotic potential. International Food Research Journal, 121(5):1737-1743.

Sharpe, M.E., Fryer, T.F. and Smith, D.G. 1979. Identification of Lactic Acid Bacteria. In: Gibbs, E.M. and Skinner, F.A. (eds.) Identification Methods for Microbiologists. Academic Press, London, ISBN-10: 0126477507, pp 233-259.

SLS 516. 1991. Microbiological analysis total plate count. Sri Lanka Standards Institute, Sri Lanka.

Soni, S.K, Sandhu, D.K, Vilkhu, K.S. and Kamra, N. 1986. Microbiological studies on Dosa fermentation. Food Microbiology, 3(1):45-53.

Soni, S.K., and Sandhu, D.K. 1989. Nutritional improvement of Indian dosa batters by yeast enrichment and black gram replacement. Journal of Fermentation and Bioengineering, 68(1):52-55.

Soni, S.K. and Sandhu D.K. 1990. Indian fermented foods: microbiological and biochemical aspects. Indian Journal of Microbiology, 30:135-157.

Tejero-Sarinena, S., Barlow, J., Costabile, A., Gibson, G.R. and Rowland, I. 2012. In vitro evaluation of the antimicrobial activity of a range of probiotics against pathogens: Evidence for the effects of organic acids. Anaerobes. 18(5):530-538.

Thakkar, P.N., Modi, H.A., Dabhi, B.K. and Prajapati, J.B. 2015. Evaluation of probiotic potential of Lactic acid bacteria isolated from South Indian special Dosa batter. International Journal of Pharmaceutical Science and Health Care, 5 (3):2249-5738.

Wickramanayake, T.W. 2002. Food and Nutrition. Reprinted, HARTI, Sri Lanka.