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Isolation and Biochemical Characterization of Lactic Acid Bacteria from Fermented Foods

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A B S T R A C T

Probiotics are considered as successful major category of food supplements. Probiotics can be functional foods because their health benefits are essentially higher than traditional nutritional products. Probiotic bacteria was collected from home made and commercial fermented food samples. A total of 30 food samples were collected from local areas of Guntur in Andhra Pradesh. Bacteria were isolated on MRS agar medium after observation of growth and pure culture was obtained by sub-culturing on the same medium. Purity of each culture was confirmed by morphological investigation, Gram's staining and further identification by specific biochemical tests. The isolates from both dairy and non-dairy fermented foods were identified as rods, bacilli, cocci and chain shape. While some isolates showed positive results some showed negative results for catalase test, methyl red test, oxidase test, aescualin fermentation, starch hydrolysis, arginine hydrolysis, citrate utilization and voges prausker's test reaction. Based on morphological, cultural and biochemical characterization of 16 bacterial isolates out of 30 were identified as *Lactobacillus spp.*

Introduction

Microorganisms which have specific favorable effects on humans and animals are used as probiotics. Those organisms provide not only gut flora balance but also maintain good health condition (Schrezenmeir and Verse, 2001). The probiotic bacteria are *Bifidobacterium Enterococcus, Lactobacillus* and *Streptococcus* species. These bacteria play an extensive role in contrary to harmful microorganisms and strengthen the host's immune system (Soccol *et al.*, 2010). "Living microorganisms" are probiotics that provide

the host with health benefits when administered in sufficient quantities. Probiotics must meet certain key criteria such as tolerance to the intestine pH and bile salts, sensitivity to antibiotics and inhibition of growth in other harmful organisms. A huge number of microbes present in human intestine forms a wide range of health-active molecules. Diet and functional foods are essential modulators for gut microbiotas to improve the health of the hosts (Johan *et al.,* 2011).

In present conditions probiotics are being used worldwide as a major predominant category of food supplement. Probiotics can be functional food because their health benefits are significantly higher than the traditional food products. Several studies reported that probiotics can succeed in different types of infectious diseases, colon cancer, immune modulations and other chronic gastrointestinal inflammatory disorders as evidenced by high-quality scientifically based clinical information. Antimicrobial products of probiotic bacteria produce bacteriocins which are used against food spoiling organisms and to raise safety of foods (Nout,2014). During the process of fermentation, the microorganism's breakdown carbohydrates into carbon dioxide, organic acid and alcohol (Ansorena and Astisaran, 2016). Lactic acid bacteria (LAB) indicated as gram positive non-motile, non-sporulating bacteria produce more amount of lactic acid during fermentation process (Pelinescu *et al.,* 2009).

These are generally considered safe and suitable for medical applications (Hoque *et al.,* 2010).They have rod- or coccus-shaped cells (Zhang *et al.,* 2014) and are microaerophilic or anaerobic and acid-tolerant organisms (Shaukat, 2020).LAB initiates speedy and tolerable acidification of raw materials by producing different organic acids derived from carbohydrates. LAB is capable of producing lactic acid, bacteriocin, ethanol, exopolysaccharides, certain enzymes and flavouring compounds (De Vuyst & Leroy, 2007).

LAB has a prolonged history in cheese processing and consumption. Due to its organoleptic properties, the fermented food is more popular than unfermented foods among consumers. Due to the acid production, LAB reduces pH below 4°C and hinders pathogenic development. These pathogenic microbes spoil milk and cause diseases (Ananou *et al.,* 2007). *Lactobacilli* as beginners for fermented vegetables, milk products and sausages as well as inoculants are being used for food preservation. *Lactobacilli* are also proposed for the production of nutraceuticals (Widyastuti *et al.,* 2010).

More studies and considerable evidence that probiotics have an influence on several aspects of innate and acute immunity through IgA secretion and phagocytosis, which include alteration in T-cell responses, improvement in Th1 responses and attenuation of Th2 responses (Guarner *et al.,* 2003).

Prebiotics can be used to supplement probiotic products. It turns out that developing biological therapeutic formulas containing microbial and synergistic strains in both the colon and the intestine can lead to a more pronounced probiotic effect. These enhanced products may be more effective and have a greater protective and stimulating effect than their individual components (Bomba *et al.,* 2002).

Materials and Methods

Sample collection

Initially market survey was done for identification of commercially available foods in the local markets and also identified

homemade cultured or fermented products. 30 samples were collected for the study through random sampling method. Samples of commercially available probiotic foods taken from different local markets and some of cultured samples collected from households in Guntur.

Isolation of probiotic bacterial isolates

Serial dilution and pour plate technique were followed. Ten ml of the sample in 90 ml sterile water blank for liquid samples and ten-gram sample in case of solid samples in 100 ml sterile water blanks were mixed thoroughly and serially diluted up to 10^5 dilutions. From 10^2 and 10^3,10^4 one ml each diluent was transferred to sterile Petri dishes and the molten and cooled MRS medium was poured after solidification. The plates were incubated in an inverted position for 24-48hrs at 30℃. The characteristic LAB colonies growing over the incubated plates were picked up carefully and streaked on the MRS agar medium for further purification. The discrete single colonies were picked up and inoculated into MRS broth in the culture vials and the grown-out cultures were maintained at 4℃ in a refrigerator for further studies. The isolates were assigned the code numbers (Awan and Rahman, 2005).

Enumeration of isolates

10 g of each food sample was taken in 250 ml conical flask to which 90 ml of sterile saline was added aseptically. The sample was agitated for 15 minutes on cyclomixer and serial dilutions of samples were prepared. 1 ml of respective dilutions were poured in to the sterile petriplates followed by MRS Agar medium. Then the petri plates were incubated at room temperatures for 24-72 hrs in a bacteriological incubator. After incubation colonies formed and population was expressed as CFU x dilution factor g-1 of sample.

Colony characteristics

The morphological characteristics of the colony of each isolate were examined on MRS agar medium by incubating for specific period. Cultural characterization of isolates such as shape and stain reaction were recorded (Aneja, 2003).

Gram staining

A loop full of inoculum from young culture was taken and mixed with a drop of sterile distilled water placed in the center of the slide. The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension.

The smear was dried in air and fixed through mild heating by passing the lower side of the slide three to four times over the flame. Then each smear was covered with crystal violet for 30 seconds followed by washing each slide with distilled water for few seconds.

Then each slide was covered with Gram's iodine solution for 60 seconds, then washed with 95% ethyl alcohol followed with distilled water and drained. The bacteria that appeared purple were referred to as Gram-positive and those that were pink as Gram-negative (Aneja, 2003).

Bio chemical characterization of effective isolates

Catalase test

Catalase test was conducted by dripping two drops of hydrogen peroxide% at 24 hours aged cultures on an object glass slide. Catalase test positive reaction characterized by the formation of oxygen bubbles that indicate the bacteria produce the catalase enzyme which converts H_2O_2 to water (H_2O) and oxygen (O_2) (Rangaswamy and Bagyaraj,1993).

Oxidase test

The overnight cultures of the test isolate were spotted on plates poured with sterile trypticase soya agar (TSA) and the plates were incubated for 24 hours at 30℃.

After incubation, oxidase discs placed in the petri plates on the growth of culture within few minutes turns into blue colour indicates positive. No colour change indicates negative (Ifeanyi *et al.,* 2019).

Methyl red test

Sterilized glucose-phosphate broth tubes were inoculated with the test culture and incubated at 30℃ for 48 hours. After incubation five drops of methyl red indicator were added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test (Crown and Gen,1998).

Voges Proskauer's Test

To the pre-sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37℃ for 48 hrs.

After incubation ten drops of Barrit's reagent-A was added and gently shaken followed by the addition of Barrit's reagent-B. The development of pink colour in the broth was taken as positive for the test (Faddin, 2000).

Gas from Glucose

Sterile test tubes of 10 ml glucose broth containing Durham's tube (inverted and dipped), were inoculated with Lactobacilli cultures at the 1% and incubated at 37°C for 24–48 h. Gas production that appeared in the form of a hollow space in Durham's tube was recorded as a positive result (Monica *et al.,* 2012).

Arginine Hydrolysis

Production of ammonia from arginine was tested by inoculation of the isolates in arginine broth. After 24 h of incubation, 100 µl of the sample was spotted on white tile and equal volume of Nessler's reagent was added.

Immediate appearance of dark orange color indicates the presence of ammonia due to hydrolysis of arginine (Ghosh *et al.,* 2011).

Aescualin fermentation

The isolates were also assessed for their ability to hydrolyze glycoside aesculin to aesculetin and glucose. For this, bile aesculin agar plates were streaked with the isolated cultures and incubated at 37 °C for 24–48 h. After incubation, the plates were examined for the presence of a dark brown to black halo around the bacterial growth, showing a positive result for aesculin hydrolysis (Monica *et al.,* 2012).

Nitrate Reduction Test

Nitrate reduction is an important criterion for differentiating and characterizing different types of bacteria. Therefore, the isolates were incubated at 37°C for 24 h in trypticase nitrate broth. After incubation, 0.5 mL each of sulphanilic acid (0.8%, in 5N Acetic acid) and α-naphthylamine (0.5%, in 5N Acetic acid) were added into the tubes. The appearance of red or pink color indicated the positive test for nitrate reduction (Monica *et al.,* 2012).

Citrate Utilization Test

The isolates were inoculated in Simmons citrate agar incubated at 37°C for 24 h. After incubation, the appearance of blue coloration indicated the positive test for citrate utilization and was recorded accordingly for the isolates tested (Faddin, 2000).

Starch Hydrolysis

Starch is a branched polysaccharide, used as a source of carbohydrate by certain bacteria. The starch containing medium is prepared by autoclaving at 121˚C for 15 min, poured onto the petri plates, allowed to dry and the isolates were streaked.

The plates were incubated for the period of 24 to 48 h at 37˚C. Iodine was added to the plate and incubated for 5 min to check for hydrolysis. Iodine forms complex with starch changing the background to blue – black. If starch is hydrolyzed, a clear zone was formed surrounding bacterial growth and if no hydrolysis the agar remained blue black with any clearance of the zone (Faddin, 2000).

Results and Discussion

Collection of fermented foods

The details regarding to collected samples from different local super markets, online sources and households in Guntur district of Andhra Pradesh.

Collection of fermented foods from commercial sources

The information related to collection of fermented foods from commercial sources is presented in Table 1

Collection of fermented foods from house holds

The information related to collection of fermented foods from households of Guntur is presented in Table 2.

Food samples like Home made curd (HCU), Dosa (DA), Dosa batter (DB), Idly (ID), Idly batter (IB), Punugu (PN) were the samples collected from households.

Isolation and population of LAB

The details of results pertaining to isolation and population of LAB from commercial and homemade fermented food samples are discussed here under Thirty fermented food samples were collected from various markets and households in Guntur and LAB were isolated on MRS (Mann, Rogosa, and Sharpe's agar) medium.

Sixteen of the thirty samples were tested positive for LAB population. The LAB population in fermented food products ranged from 1.5 to 10 x 10^4 (Table 4.5). Jersey Butter Milk had the maximum LAB population (10 x10^4 CFU/ml), followed by Amul Butter Milk (9.8 x10^4), Kombucha (8.8 x10^4 CFU/ml), Health Potion Drink (7.2 x10^4 CFU/ml), Mozzarella Cheese (6.0 x10^4 CFU/ml), Milky Mist Cheese (6.0 x10^4 CFU/ml) and Idly Batter (1.5 x10^4 CFU/ml).

According to Shannon *et al.*, (2018), starter cultures were used in the production of cultured dairy products, cheese and fermented sausages to survive fermentation, the LAB and other microorganisms found in the finished product must deal with a variety of selective and competitive pressures such as salts, organic acids, ethanol, anaerobiosis and low pH. Furthermore, the incubation temperature during growth and nutrient content were usually well-suited to the needs of microorganisms.

Campo *et al.*, (2005) isolated *Lactobacillus delbrueckii* from yoghurt and found that the population count was 1.3 x10^5 CFU/g, The population count of *Lactobacillus bulgaricus* obtained from fermented beverages was 6.4 x 10^4 CFU/ml Thamer and Penna (2005) discovered a population of *L. bulgaricus* ranging from 7.3 to 11.4 x 10^4 CFU/mL in fermented milk beverages containing varying amounts of whey, sugar and fructo-

oligosaccharides. Sivieri *et al.,* (2011) stated that population of *L. bulgaricus* was slightly lower in the products with buttermilk addition, which could minimize the problem of excessive post-acidification during the product shelf life. Nivedita *et al.,* (2014) reported that *Lactobacillus fermentum* 49 × 10^5 CFU/g was obtained from dough and *Lactobacillus fermentum, Lactobacillus acidophilus* 34 × 10^5 CFU/g and 20 × 10^5 CFU/g respectively were obtained from Lassi.

Morphological characteristics of the isolates

Morphological characteristics were determined by culturing micro-organisms on MRS agar plates. The results pertaining to characteristics of colonies and gram's reaction discussed here under Table 4

Sixteen isolates were observed, isolated and characterized culturally and morphologically. The colour of the isolates differed. The LAB isolates took 48-72 hours to establish their growth on MRS agar medium. Of the 16 isolates, four isolates (SBM, SCU, JLS, SYD) were rod shaped, ten isolates (JBM, HCU, DB, ABM, IB, KM, MY, MZC, PY, SA) were oval shaped while one isolate (MMC) was chain shaped and one isolate (HP) was cocci shaped.

The colour of the isolates ranged from creamy white to white and thick white. Among sixteen isolates seven isolates (JBM, SBM, ABM, HP, KM, JLS, SA) were white in colour, 4 isolates (HCU, SCU, DB, IB) were thick white in colour, five isolates (MY, PY, MZC, SYD, MMC) were creamy white in colour. Twelve isolates (JBM, HCU, DB, ABM, HP, IB, KM, MY, MZC, PY, SA, SYD) showed gram positive reaction and four isolates (SBM, SCU, JLS, MMC) showed gram negative reaction. LAB belong to a gram-positive bacteria that produce lactic acid as their main fermentation product into the culture medium and generally recognized as safe (Konings *et al.,* 2000).

The present findings were in line with those of Kavitha and Jeevaratnam (2016) who reported that the isolates in the curd samples were both gram negative and positive and tentatively identified as *Lactobacillus acidophilus, Lactobacillus bulgaricus,* and *Lactobacillus lactis* like different species within the *Lactobacillus* genera.

Studies by Salvetti *et al.,* (2012), Vyas *et al.,* (2014) and Rao *et al.,* (2015) identified and confirmed that *Lactobacillus spp.* were gram-positive.

Misganaw and Teketay (2016) discovered that morphological LAB isolates from milk had chain, cocci, rod and ovoid shapes. Mannan *et al.,* (2017) reported that 25 *Lactobacillus* strains from local fermented foods such as yoghurt and cheese were gram-positive and rod shaped.

Biochemical characterization of Lactic Acid Bacteria isolates

Catalase test

The catalase test was found to be positive for isolates such as SA, KM, SBM, MY, JBM, JLS, MZC, HCU, IB and DB. All the isolates produced gas bubbles. Presence of catalase enzyme within them caused the formation of gas bubbles. Similarly, Maragkoudakis *et al.,* (2006), Kavitha *et al.,* (2013) and Timothy *et al.,* (2017) reported similar results for *L. casei* and *L. rhamnosus* obtained from traditionally fermented foods like curd and idly batter.

The catalase test was negative for ABM, HP, SCU, SYD, MMC and PY isolates. Because the bacteria was not able to produce the catalase enzyme which converts hydrogen peroxide into water and oxygen, no bubble

was observed. Guessas *et al.,* (2004) stated that *Lactobacilli* species found in local raw milk were *Lb. curvatus, Lb. helveticus, Lb. plantarum, Lb. reuteri, Lb. casei, Lb. brevis, Lb. bulgaricus, Lb. paracasei* and *Lb. acidophilus* all of which showed catalase test to be negative.

Lactobacillus isolates from yoghurts, such as *Lactobacillus delbrueckii ssp. bulgaricus*, showed a catalase negative reaction in a study conducted by Mamata *et al.,* (2017) Catalase negative reactions have previously been reported by Djide *et al.,* (2008), Hawaz (2014), Ibrahim *et al.,* (2015), Sarangdhar *et al.,* (2015) and Saif *et al.,* (2016) in fermented products such as curd, yoghurt and cheese.

Oxidase test

The bacterial cultures were unable to oxidize the colorless reagent, tetramethyl p-phenylene diamine dihydrochloride to produce the purple compound in the oxidase test. The isolates lacked cytochrome C, which allows them to use free oxygen in their energy metabolism as stated by Mamata *et al.,* (2017).

Positive oxidase test was seen in isolates such as SA, KM, MY, SCU, JBM, JLS, MZC, HCU, DB and IB. Souid *et al.,* (2015) investigated that *Lactococcus lactis* from cheese that showed positive reaction in oxidase test. Ankur *et al.,* (2017) reported *Lactobacilli* isolates from fermented dairy products showed positive reaction. Lactic acid bacteria showed a positive reaction in fermented milk as observed by Sawadogo *et al.,* (2019).

Oxidase tests revealed negative results in isolates of SBM, HP, ABM, SYD, MMC, and PY. The present results were consistent with those of Ouoba *et al.,* (2009) who discovered that LAB isolated from African traditional alkaline fermented foods were oxidase negative. According to Mamata *et al.,* (2017) certain *Lactobacillus* isolates such as *Lactobacillus delbrueckii ssp. Bulgaricus* isolated from curd and probiotic beverages a negative oxidase reaction. *Lactobacillus* isolates demonstrated a negative reaction even in the studies conducted by Nazneen *et al.,* (2015) and Saif *et al.,* (2016).

Methyl red test

Methyl red test is based on the principle of fermentation of glucose to pyruvic acid and oxidation of pyruvic acid to other acids such as lactic, acetic and formic acids which results in decrease in pH. The methyl red then turns red indicating a positive reaction.

The isolates such as SBM, HP, JBM, MZC, SYD, IB and DB tested positive for methyl red test. These results were in line with Kamel *et al.,* (2012) who stated that lactic acid bacteria in fermented dairy products showed a positive reaction. Apoorva *et al.,* (2013) reported that *Lactobacillus. fermentum, L. brevis* from dairy products (curd, buttermilk, paneer whey) and beverages exhibited positive reactions.

The isolates such as SA, KM, ABM, MY, SCU, JLS, MZC, HCU, MMC and PY tested negative for the methyl red test. The current results are in line with Jagadeeswari *et al.,* (2010) who stated *Lactobacillus* isolated from traditional fermented foods like dosa batter, appam batter, buttermilk, yoghurt and cabbage exhibited negative reaction.

Kavitha *et al.,* (2016) reported that *Lactobacillus acidophilus* from dahi sample showed negative reaction. Ankur *et al.,* (2017) concluded that some isolates of LAB from fermented foods showed positive while some others showed negative reaction for methyl red test. *Lactobacillus bulgaricus* and *Lactobacillus fermentum* from curd and milk

respectively showed negative reactions according to Timothy (2017). Another study conducted by Ngene *et al.,* (2019) found that *Lactococcus lactis, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus casei, Lactobacillus plantarum* from yoghurt showed negative reaction.

Gas formation from glucose

The isolates such as KM, SBM, MY, HCU, IB and DB showed positive reaction for gas formation from glucose. Isolates such as SA, ABM, HP, SCU, JBM, JLS, MZC, SYD, MMC and PY did not express formation of gas from glucose. They were negative.

The isolates could produce any gas as shown by a hollow space in the inverted Durham's tubes and therefore concluded as positive for gas production from glucose. Some of the isolates of LAB showed both positive and negative results for gas formation as per the study conducted by Monica *et al.,* (2012). *Lactobacillus brevis, Lactobacillus divergens, Lactobacillus fermentum, Lactobacillus buchneri, and Lactobacillus reuteri* were found in dairy products and produced gas from glucose reported by Sheela *et al.,* (2015).

Some LAB isolated from fermented products were shown to be positive and some were shown to be negative in gas formation from glucose in studies conducted by Kuikui (2015).

According to Bennani *et al.,* (2017) *Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus delbrueckii subsp. Lactis, Lactobacillus delbrueckii subsp. and Lactobacillus plantarum* were all homofermentative while *Lactobacillus brevis* was found to be heterofermentative for fermenting glucose.

Arginine hydrolysis

Isolates such as SA, KM, ABM, SBM, MY, SCU, JBM, MZC, MMC, IB, and DB were showed positive reactions. Isolates such as HP, JLS, SYD, HCU, and PY isolates were negative.

The isolates produced yellow color in the arginine hydrolysis test, indicating that they cannot produce ammonia from arginine. The organisms could not use the amino acid arginine as a source of carbon and energy (Chris *et al.,* 2006).

Tanasupawat *et al.,* (2006) stated that LAB isolates from fermented sausages showed both negative and positive results. Pooja *et al.,* (2015) reported that LAB isolate like *L. fermentum, L. paracasei, L. amylophilus* isolated from dairy and non-dairy fermented products like curd, dosa batter and fermented cabbage showed both positive and negative results.

Rhaiem *et al.,* (2016) reported that LAB isolates *Lactobacillus plantarum* and *Lactobacillus delbruecki* did not produce ammonia from arginine, whereas one isolate of *Lactobacillus fermentum* did produce ammonia from arginine. Another study conducted by Sneha *et al.,* (2017) reported that LAB strains from curd and fruit juice showed both positive and negative results.

Aescualin fermentation

The Aescualin fermentation test showed positive reaction in isolates such as SA, KM, ABM, SBM, MY, SCU, JBM and MZC. These isolates had ability to hydrolyze glycoside aesculin to aesculetin and glucose. The isolates such as MMC, IB, DB, HP, JLS, SYD, HCU and PY showed negative reaction.

Table.1 Collection of fermented food samples from commercial sources

S.No	Food sample	Code	Source
1.	Buttermilk (Amul)	ABM	Reliance super market, Guntur
2.	Buttermilk (Jersey)	JBM	Reliance super market, Guntur
3.	Butter milk (Sangam)	SBM	Reliance super market, Guntur
4.	Blue berry yoghurt	BBY	Vijetha super market, Guntur
5.	Cheese (Amul)	AMC	Spencer's super market, Guntur
6.	Cheese (Milky mist)	MMC	D- mart super market, Guntur
7.	Curd (Sangam)	SCU	Local super market, Guntur
8.	Greek Yoghurt	GY	Vijetha super market, Guntur
9.	Health potion drink	HP	Online stores
10.	Kombucha	KM	Online stores
11.	Lassi (Britania)	BLS	Reliance super market, Guntur
12.	Lassi (Jersey)	JLS	Reliance super market, Guntur
13.	Lassi (Amul)	ALS	Reliance super market, Guntur
14.	Mango yoghurt	MY	Super market, Guntur
15.	Mozzarella Cheese	MZC	Heritage, Guntur
16.	Mango yoghurt drink	MYD	Spencer's super market, Guntur
17.	Misti Doi	MD	Vijetha super market, Guntur
18.	Probiotic milk drink	PML	Vijetha super market, Guntur
19.	Peach yoghurt	PY	D-mart super market, Vijayawada
20.	Raspberry Yoghurt	RY	Local super market, Guntur
21.	Sauerkraut	SA	Online stores
22.	Straw berry yoghurt drink	SYD	Heritage, Vijayawada
23.	Straw berry yoghurt	SY	Heritage, Vijayawada

Table.2 Collection of fermented food samples from house holds

S. No.	Food sample	Code	Source
1.	Curd (Homemade)	HCU	House hold, Guntur
2.	Dosa	DA	House hold, Guntur
3.	Dosa batter	DB	House hold, Guntur
4.	Fermented grape juice	FGJ	House hold, Guntur
5.	Idly	ID	House hold, Guntur
6.	Idly batter	IB	House hold, Guntur
7.	Punugu	PN	House hold, Guntur

Table.3 Isolation and population count of LAB

S. No	Code	No. of isolates obtained	Lactic acid bacteria population (CFU/ml)
1.	JBM	1	10×10^4
2.	SBM	1	2.6×10^4
3.	HCU	1	4.0×10^4
4.	SCU	1	5.5×10^4
5.	DB	1	5.0×10^4
6.	ABM	1	9.8×10^4
7.	HP	1	7.2×10^4
8.	IB	1	1.5×10^4
9.	KM	1	8.8×10^4
10.	JLS	1	2.0×10^4
11.	MY	1	4.0×10^4
12.	MZC	1	6.0×10^4
13.	PY	1	1.6×10^4
14.	SA	1	2.5×10^4
15.	SYD	1	2.4×10^4
16.	MMC	1	6.0×10^4

Table.4 Characteristics of colonies and Gram's reaction

S. No.	Code	Colony characteristics		Gram's reaction
		Colour	Shape	+ve/-ve
1.	JBM	White	Oval	+ve
2.	SBM	White	Rod	-ve
3.	HCU	Thick white	Oval	+ve
4.	SCU	Thick white	Rod	-ve
5.	DB	Thick white	Oval	+ve
6.	ABM	White	Oval	+ve
7.	HP	White	Cocci	+ve
8.	IB	Thick white	Oval	+ve
9.	KM	White	Oval	+ve
10.	JLS	White	Rod	-ve
11.	MY	Creamy white	Oval	+ve
12.	MZC	Creamy white	Oval	+ve
13.	PY	Creamy white	Oval	+ve
14.	SA	White	Oval	+ve
15.	SYD	Creamy white	Rod	+ve
16.	MMC	Creamy white	Chain	-ve

Table.5 Bio chemical characterization of isolates

Isolate	Biochemical tests									
	Catalase test	Oxidase test	Methyl red test	Gas formation from Glucose	Arginine hydrolysis test	Aescualin fermentation test	Nitrate reductase test	Citrate utilization test	Starch hydrolysis test	Voges Prausker's test
JBM	+	+	+	-	+	+	+	-	-	-
SBM	+	-	+	+	+	+	+	-	-	+
HCU	+	+	-	+	-	-	-	-	-	-
SCU	-	+	-	-	+	+	-	-	-	+
DB	+	+	+	+	+	-	-	-	-	-
ABM	-	-	-	-	+	+	-	-	-	-
HP	-	-	+	-	-	-	+	+	-	-
IB	+	+	+	+	+	-	-	+	-	+
KM	+	+	-	+	+	+	+	-	-	-
JLS	+	+	-	-	-	-	+	-	+	+
MY	+	+	-	+	+	+	-	-	-	-
MZC	+	+	+	-	+	+	+	+	-	-
PY	-	-	-	-	-	-	+	+	-	-
SA	+	+	-	-	+	+	+	-	-	-
SYD	-	-	+	-	-	-	-	-	+	-
MMC	-	-	-	-	+	-	+	-	-	+

The present findings were similar with those of Jamuna and Jeevaratnam (2004) who stated that LAB isolated from fermented foods like appam batter and pickles showed negative and positive results for Aescualin fermentation. Tanasupawat *et al.,* (2006) reported that some of the LAB isolates from traditional Thai fermented sausages were positive and some were negative for Aescualin fermentation. Similar results were obtained by Ashmaig *et al.,* (2009) who stated that LAB isolate from traditional Sudanese fermented milk showed positive and negative results.

Sneha *et al.,* (2017) found that LAB strains from curd and juiceof fruit showed both positive and negative results. Bennani *et al.,* (2017) concluded that isolates of LAB from cow milk showed both negative and positive reactions for Aescualin fermentation.

Nitrate reductase test

In the nitrate reductase test, presence of the nitrate enzyme, the test reagent KNO3 is converted to NO_2, which reacts with the indicator sulphanilic acid and naphthylamine to form nitrous derivatives with a characteristic red color.

The isolates such as SA, KM, SBM, HP, JBM, JLS, MZC, MMC, and PY showed positive reactions for nitrate reductase test. These results were similar to the results of Monica *et al.,* (2012) who investigated that LAB isolates from some fermented foods showed s positive while some showed negative results. Zakpaa *et al.,* (2009) reported that only one LAB isolate from fermented foods like fermented meat products showed a positive result and only one isolate showed a negative result.

The isolates such as ABM, MY, SCU, SYD, HCU, IB, and DB were all negative. Kavitha *et al.,* (2016) reported that *Lactobacillus acidophilus* from dahi samples showed a negative reaction. The results of the present study were in line with those of Sneha *et al.,* (2017) who reported negative results for LAB from curd and fruit juice.

Citrate utilization test

The positive results were showed for HP, MZC, PY and IB while negative results were obtained for SA, KM, SBM, ABM, MY, SCU, JBM, JLS, SYD, HCU, MMC and DB for citrate utilization test. Monica *et al.,* (2012) and Mamata *et al.,* (2017) reported that some of lactic acid bacteria isolates from non-dairy probiotic drinks showed positive and some showed negative.

The present findings were similar to the findings of Ankita and Jayanti (2015) who reported that the probiotic properties of *Lactobacillus Spp.* from selected regional dairy products showed negative citrate utilization test. Kavitha *et al.,* (2016) found that *Lactobacillus acidophilus* from a dahi sample showed a negative reaction. Ankur *et al.,* (2017) stated that lactic acid bacteria isolated from fermented foods were negative for citrate utilization. Timothy (2017) reported that *Lactobacillus fermentum* and *Lactobacillus bulgaricus* from curd and milk showed negative reactions.

Starch hydrolysis

Determination of amylase production potentials of LAB isolates was carried out using starch hydrolysis test procedures adapted by Sun *et al.,* 2010.

The isolates such as HP, MZC, PY, IB, SA, KM, SBM, ABM, MY, SCU, JBM, HCU, MMC, DB isolates showed negative results. JLS and SYD isolates showed positive results in starch hydrolysis test. The present findings were in line with those of Abhijit *et al.,* (2012), who reported *that lactobacillus* strains

obtained from pickled vegetables and fermented beverages showed a positive reaction.

Ankita and Jayanti (2018) reported that the probiotic properties of *Lactobacillus Spp.* from selected regional dairy products showed negative results in starch hydrolysis tests. Timothy *et al.*, (2017) determined the amylase production of LAB isolates from curd and milk. Isolates of LAB showed that some positive and negative results in starch hydrolysis test.

Voges Prausker's test

The VP test is used to determine if an organism produces acetyl methyl carbinol from glucose fermentation. If present, acetyl methyl carbinol is converted to diacetyl in the presence of alpha-naphthol, strong alkali and atmospheric oxygen.

The isolates such as SBM, SCU, JLS, MMC, IB tested positive. Isolates such as SYD, HP, MZC, PY, KM, ABM, MY, JBM, HCU, MMC, DB showed negative reaction in Voges Prausker's test. These results were in line with those of Sung Mee *et al.*, (2008) who stated that *L. plantarum* from kimchi fermented products showed a negative reaction.

The common characters of *Lactobacillus* species showed negative in Voges Prausker's test reaction conducted by Guessas and Kihal (2004). Hossain *et al.*, (2010) reported that *Lactobacillus* isolates from yoghurt showed a negative reaction. The present study results are similar to those of Rhaiem *et al.*, (2016) who reported the absence of colour changes occurring in LAB strains of the Voges Proskauer test. Another study conducted by Arindam and Chandan (2017) reported that some LAB isolates were positive and some isolates showed negative reaction. Ngene *et al.*, (2017) stated that *Lactococcus lactis, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus casei, and Lactobacillus plantarum* strains from yoghurt showed negative reactions for Voges Prausker's test.

In present investigation, thirty bacterial isolates were collected from both dairy and non-dairy samples. All the isolates were characterized on the basis of colony morphology and biochemical characteristics. Sixteen bacterial isolates were identified as *Lactobacillus* by morphological, cultural and biochemical characteristics. Further study of selected *Lactobacillus* isolates by 16S rDNA gene sequencing is being undertaken for its species identification.

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