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Physiological characterization and *in vitro* evaluation of lactic acid bacteria from fermented foods

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Abstract
The research on probiotic isolates has received great attention for many years, resulting in the development of various food products that possess diverse health benefits. in the present study tests on physiological characterization and invitro evaluation of probiotic isolates from various fermented foods resulted in identification of lactic acid bacteria isolates in the fermented foods. Among the sixteen isolates that were studied the lactic acid bacteria isolates present in kombucha, buttermilk (Sangam), mozzarella cheese and strawberry yoghurt drink showed superior characteristics in terms of probiotic properties like acid and bile tolerance, antibiotic resistance and antibacterial activity These isolates contained majorly the LAB species such as *Lactobacillus delbrueckii* ssp. *bulgaricus, Lactococcus lactis, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus*. These strains could be used in the development of starter cultures for the production of fermented products in a controlled environment in future.

Keywords: Physiological, characterization, evaluation, bacteria, fermented

Introduction
Bacteriocins are proteinaceous molecules that can inhibit bacterial growth at the ribosomal level. At the end of the exponential growth phase, bacterial cells are generated and their range of action can vary depending on the species that produce them. The producer strains are immune to their bacteriocins because they have genes that encode immunity mechanisms that distinguish "self" from "non-self" (Draper *et al*., 2009) [20]. The bacteriocins are used as sources for preventive drugs and therapeutics (Michon *et al.*, 2016) [43].
LAB fermentation of milk products is generally proteolytic in nature due to the instability of milk proteins. Proteolytic system of LAB is important for growth of microorganisms and in native free amino acid of milk. Proteolytic system involved in casein utilization of LAB cell add to development of organoleptic properties of fermented milk products (Yamina *et al*., 2013) [74].
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) [3]. *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) [72].
LAB is available in environment like soil, water, sewage, plant, animal and human environments (Mounier *et al*., 2017) [45]. Environments that are full of carbohydrates are generally ideal for growth of LAB. Human and animal cavities are also good places for their growth (Pang *et al.*, 2014). The LAB can be isolated and then used against natural microbial populations from many raw fruits and vegetables (Remize *et al.*, 2019) [56].
There are hundreds of traditionally fermented foods prepared by using various methods over the world. In food and dairy industries probiotic bacteria play a crucial role in the fermentation. *Lactobacilli* inhabit the gastrointestinal tract and promote antimicrobial activities and participate in the host's defense system. During fermentation process, LAB produces a number of compounds such as carbon dioxide, hydrogen peroxide, organic acids and also polysaccharides. These metabolic compounds exhibit activity against microbes, inhibit the activity of spoilage causing bacteria and preserve the nutritive qualities of the food for prolonged shelf life (Ruethaiwan *et al.*, 2012) [58].
Microorganisms convert the chemical composition of raw materials during fermentation,

which enrich the nutritional value in some fermented foods, and impart health-benefits to the consumers (Steinkraus, 2002; Farhad *et al*., 2010; Tamang, 2015) [64, 22, 23, 66, 67].

Materials and Methods
Physiological Characterization of Effective Isolates
Growth of Isolates at Different Temperatures
The isolates were tested for their ability to grow in MRS broth at 30, 35,40,45,50 for 5 days and incubating for 24–48 h. For this, 10 mL of MRS broth tubes were inoculated 1% of Lactobacilli cultures. The development of turbidity in culture tubes was recorded as the ability of isolates to grow at different temperatures and results were noted as positive or negative (Tambekar and Bhutada, 2010) [68, 69].

Oxygen Requirement of the Isolates
All the isolates were inoculated in MRS medium in semi-solid condition were kept differently under oxygenated condition; the inoculation of culture in the media tubes, after incubation of isolates at 30℃ in 24-48hrs. Observation of growth in surface of media indicates isolates were aerobic, then observation of growth of bacteria in bottom of the tube indicates anerobic and then the growth observed below the top layer or middle of the media indicates that the isolates were grown in micro-aerophilic condition **(**Lee *et al.* 2011) [57].

NaCl tolerance test
NaCl tolerance of isolated Lactobacillus was determined by using MRS broth with 2%, 4% and 6% of NaCl concentration. Fresh culture was inoculated and incubated at 37℃ for 48 h. Only media was used as negative control. Results were determined by observing the turbidity after 24 h and 48 h and no growth was observed in negative control (Mannan *et al.,* 2017) [41].

Carbohydrate Fermentation
The probiotic isolates were tested for their carbohydrate fermentation. The new tubes from overnight cultures of lactic acid bacteria were prepared. Ten ml media was dispensed and Durham's tube was inserted invertably in each of test tubes. A colony of each culture was inoculated and incubated at 37°C for 24 h containing Phenol red (0.05g/l) as pH indicator. Only media was used as negative control. A change in color from red to yellow was observed after inoculation and incubation which indicates formation of gas. The carbohydrates utilized in this test were Arabinose, Cellobiose, Fructose, Glucose, Lactose, Maltose, Mannitol, Mannose, Raffinose, Rhamnose, Salicin, Sorbitol, Sucrose and Trehalose, Xylose (Mannan *et al*., 2017) [41].

In Vitro Evaluation of LAB
Acid Tolerance of LAB
MRS broth at pH 2,4 and 6 were prepared by adjusting with 10N HCl and 1N NaOH. Fresh bacterial cultures were inoculated into respective MRS broth in test tubes and incubated at 37℃ for 48 h. Only media was used as negative control. Results were obtained by observing turbidity of the culture media after 24 h and 48 h and no growth was observed in negative control (Mannan *et al*., 2017) [41].

Bile Salt Tolerance
The medium with varying concentrations of bile salt (1.0, 1.5 and 2.0%) was inoculated with each selected bacterial culture and incubated at 37℃ for 48hrs. Then 0.1ml inoculums was transferred to MRS agar by pour plate method and incubated at 37 ℃ for 48hrs. The growth of LAB cultures on agar plates was used to designate isolates as bile salt tolerant (Tambekar and Bhutada, 2010) [68, 69].

Antibiotic Susceptibility
Resistance of LAB strains isolated from fermented foods testing against several antibiotics including Ampicillin (10 µg), Chloramphenicol (25 µg), Penicillin-G (1 unit), streptomycin (10 µg), Sulphatriad (300 mcg) and tetracycline (25 mcg) was determined using antibiotic disks. Each strain was activated in MRS broth, and 1% inoculum (McFarland standard) was added to MRS agar at 45°C–50°C and poured into plates. Then, antibiotic discs were placed at the centre of the medium and plates were incubated at 37°C for 24–48 h. The inhibition zones around the disks if present were measured (Ispirli and Dertli, 2017) [31].

Antimicrobial Activity
The LAB strains were screened for antimicrobial activity against *Escherichia coli* and *staphylococcus aureus* by agar diffusion method. The cell free neutralized supernatants of LAB isolates were screened for bacteriocin activity by agar spot method. For the detection of antibacterial activity of the strains of *Lactobacillus spp*., MRS containing only 0.2% glucose was used. The test was performed as per the method described by Ozlem Erdogrul and Feryal Erbulur (Suganya *et al*., 2013) [65].

Physiological Characterization of Isolates
Growth at Different Temperatures
The probiotic isolates were collected and grown at different temperatures and the results are presented in Table 1.

Table 1: Temperature tolerance of isolates at different temperature

S. No	Isolate	Growth temperatures				
		20 ℃	35 ℃	40 ℃	45℃	50 ℃
1.	JBM	-	+	+	+	-
2.	SBM	-	-	-	-	-
3.	HCU	+	+	+	+	-
4.	SCU	-	-	-	-	-
5.	DB	+	+	+	-	-
6.	ABM	-	-	-	-	-
7.	HP	+	-	-	-	-
8.	IB	-	-	-	-	-
9.	KM	+	-	-	-	-
10.	JLS	-	-	-	-	-
11.	MY	+	-	-	-	-
12.	MZC	+	-	-	-	-
13.	PY	+	-	-	-	-
14.	SA	+	+	+	+	-
15.	SYD	+	-	-	-	-
16.	MMC	-	+	+	+	-

JBM=Jersey butter milk, SBM= Sangam butter milk, HCU= Home made Curd, SCU= Sangam curd, DB= Dosa batter, ABM= Amul butter milk, HP= Health potion drink, IB= Idly batter, KM= Kombucha, JLS= Jersey lassi, MY= Mango yoghurt, MZC= Mozzarella cheese, PY= Peach yoghurt, SA= Saurkraut, SYD= Strawberry yoghurt drink, MMC= Milky mist cheese

The isolates from HCU, DB, HP, KM, MY, MZC, PY, SA, SYD and MMC showed growth at 20 ℃; the five isolates JBM, HCU, DB, SA, MMC showed growth at 35℃ and 40 ℃; None of the isolates could grow at 50 ℃.
These results agree with that of Stamer (1979) [62], Ardhana and Graham (2003) [6] who concluded that the optimum

temperature for the growth of lactic acid bacteria is 30-45°C. According to Whitman *et al.* (2009) [15] each genus of lactic acid bacteria has a different optimum growth temperature.

The present findings were in line with Patil *et al.* (2010) [52] who reported that *L. fermentum* and *L. plantarum* were able to grow at temperatures of 15,35,40°C. Bansal *et al.* (2013) [11] investigated that lactic acid bacteria like *Lactobacillus delbruckii, Lactobacillus fermentum, Lactobacillus acidophilus* and *Lactobacillus lactis* from curd, paneer whey and sauerkraut showed temperature tolerance at 45°C. Mohsin and Gaurav (2013) stated that one of lactic acid bacteria isolate from curd was unable to grow at 45°C while another isolate was able to grow at that temperature.

In an investigation by Safriani (2015) [59] three lactic acid bacteria isolates from fermented milk were found to survive better at 37°C. Another study identical to Misganaw and Teketay (2016) found that rod-shaped lactic acid bacteria isolates grew at 37°C and 45°C. At 45°C, the growth rate of three isolates was seen to be slow. In some cases, isolates were grown at 30°C while in others they were not. Assefa *et al*. (2017) [8] reported that the majority of isolates showed growth at 37°C. In general, it was found that 45°C was the optimal temperature for growing LAB cultures derived from fermented products. (Miyaji *et al.*, 2019; Das *et al.*, 2019) [18].

Oxygen requirement

The isolates were grown in aerobic, microaerobic and anaerobic conditions. The details of the data are furnished in Table 2. Growth was indicated by a positive (+) sign and absence of growth was indicated by a negative (–) sign.

Table 2: Oxygen requirement of isolates

S. No	Isolate	Oxygen requirement		
		Aerobic	Micro aerobic	Anaerobic
1.	JBM	-	-	+
2.	SBM	-	+	-
3.	HCU	+	-	-
4.	SCU	+	-	-
5.	DB	+	-	-
6.	ABM	-	+	-
7.	HP	+	-	-
8.	IB	-	-	+
9.	KM	+	-	-
10.	JLS	+	-	-
11.	MY	+	-	-
12.	MZC	+	-	+
13.	PY	+	-	-
14.	SA	-	+	-
15.	SYD	+	-	-
16.	MMC	+	-	-

The data is represented in the above table where lactic acid bacteria isolates were grown in different oxygen environments of MRS broth at 30°C for 24 hours. The isolates from HCU, SCU, DB, HP, KM, JLS, MY, MZC, PY, SYD and MMC showed growth in an aerobic environment. The isolates from SBM, ABM and SA showed growth in a micro aerobic environment. In anaerobic environment growth was observed in isolates such as JBM, IB and MZC.

Some fermentative bacteria had low oxygen availability because they were anaerobes, while others required oxygen for metabolic activities even though they were aerobes (Wikipedia, 2012). Lee *et al.* (2011) [57] reported that *Lactobacilli* were microaerobic indicating that they could grow in the presence of low concentrations of atmospheric oxygen.

Kavitha *et al.* (2016) [34] concluded that the oxygen requirements of *Lactobacilli* were exposed to growth in oxygenic, reduced oxygen and anoxygenic environments at 37°C for 24 h. All the isolates of lactic acid bacteria showed turbidity in the medium, indicating that the occurrence of growth in both aerobic and micro-aerobic conditions. In anaerobic conditions turbidity was not observed in isolates.

Effect of NaCl at 2%, 4% and 6.5% concentrations

The determination of the NaCl tolerance for probiotic isolates observed in MRS broth was supplemented with different concentrations of Nacl (2, 4 and 6.5%). Growth was indicated by a positive (+) sign and absence of growth was indicated by a negative (–) sign. The results are presented in Table 3.

Table 3: Effect of NaCl at Different Concentrations

S. No	Isolate	NaCl concentration		
		2%	4%	6.5%
1.	JBM	-	+	+
2.	SBM	+	+	+
3.	HCU	+	+	+
4.	SCU	-	+	+
5.	DB	-	+	-
6.	ABM	+	+	+
7.	HP	-	+	+
8.	IB	+	+	-
9.	KM	+	+	-
10.	JLS	-	-	-
11.	MY	-	-	-
12.	MZC	-	+	+
13.	PY	-	-	-
14.	SA	+	+	+
15.	SYD	-	-	-
16.	MMC	-	+	+

The isolates such as SBM, HCU, ABM, IB, KM and SA showed growth at 2% concentration of NaCl. The growth was not observed in isolates such as JBM, SCU, DB, JLS, MY, MZC, PY, SYD and MMC at 2% of Nacl. The isolates such as JBM, HCU, SBM, SCU, DB, ABM, HP, IB, KM, MZC, SA and MMC showed growth at a 4% of NaCl. Isolates from JLS, MY, PY and SYD could not grow at 4% of NaCl. The positive results were obtained in JBM, SBM, HCU, SCU, ABM, HP, MZC, SA and MMC isolates for 6.5% of NaCl. Isolates such as IB, KM, JLS, MY, PY and SYD showed negative for growth at 6.5% of NaCl.

Lactic acid bacteria were found to survive in high salt concentrations as reported by Hoque *et al.* (2010) [29] who used NaCl compound to inhibit the growth of lactic acid bacteria. The present results were similar to those of Kamel *et al.* (2012) who reported that the isolates of lactic acid bacteria were tolerated at 2% NaCl and 6.5% NaCl concentrations. Seifu *et al.* (2012) [60] investigated that genus of *Lactobacillus* from Ethiopian fermented milks did not show growth at 4 and 6.5% of NaCl.

Kuikui *et al.* (2015) reported that some of lactic acid bacteria isolates from cereal fermented foods tolerated 6.5% of NaCl some did not have tolerance at 6.5% of NaCl. All the lactic acid bacteria isolate from fermented foods were able to grow at 2% and 4% NaCl concentrations, but they could not grow at 6% NaCl as documented by Rahman *et al.* (2015) [55].

Bennani *et al.* (2017) [12] reported that the isolates from fermented dairy products like milk and cheese showed tolerance and grew at a concentration of 6.5% NaCl.

Sugar Fermentation Process of the LAB Isolates with Different Sugars

The results on sugar fermentation of probiotic isolates by different sugars and is presented in Table 4

In a study conducted by Emanuel *et al.* (2005) [21] it was reported that the lactic acid bacteria isolate identified was *Lactobacillus plantarum* based on the result sugar fermentation. Zakpaa *et al.* (2009) [75] reported that *Lactobacillus* isolates from meat products like sausages, glucose, fructose and sucrose showed positive reactions for sugar fermentation.

Lim *et al.* (2008) reported that *Lactobacillus plantarum* from kimchi (Korean fermented food) showed positive reactions for arabinose, glucose, fructose, mannose, sorbitol, mannitol, salicin, cellobiose, maltose, melibiose and trehalose and *L. plantarum* showed negative reactions for raffinose and rhamnose. A probiotic isolate from traditional yoghurts, *Lactobacillus delbrueckii ssp. bulgaricus* showed positive reactions for fermentation of sugars as reported by Azadnia *et al*. (2011) [10].

Suganya *et al.* (2013) [65] stated that glucose, lactose, mannose, and ribose from different brands of curd samples showed positive results in sugar fermentation test.

Mamata *et al*. (2017) [40] reported that lactic acid bacteria isolate showed fermentation of lactose and fructose but not glucose, whereas another isolate fermented all the tested sugars like glucose, fructose, sucrose, lactose, maltose, mannitol, sorbitol and salicin except arabinose.

Kingsley *et al*. (2020) [35] stated that *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus delbruckii, Lactococcus lactis* from fermented cassava tubers showed positive reactions for glucose, fructose, sucrose, maltose, mannose, sorbitol, galactose, lactose and salicin.

Premasiri *et al.* (2020) investigated lactic acid bacteria from milk for fermentation of different sugars ribose, glucose, trehalose, mannose, mannitol, sucrose, fructose and galactose and found positive results.

Table 4: Fermentation of sugar by Lactic Acid Bacteria isolates

Isolate	Fermentation of different sugars															
	Arabinose	Cellobiose	Fructose	Glucose	Lactose	Maltose	Mannitol	Mannose	Melibiose	Raffinase	Rhamnose	Salicin	Sorbitol	Sucrose	Trehalose	Xylose
SA	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+
KM	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+
SBM	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+
ABM	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+
HP	-	-	+	+	+	+	-	+	-	+	-	-	-	+	-	+
MY	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-
SCU	+	-	+	+	+	+	-	+	+	+	-	+	+	+	+	-
JBM	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
JLS	-	-	+	+	+	+	-	+	+	+	+	-	+	+	-	+
MZC	-	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+
SYD	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
HCU	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+
MMC	-	+	+	+	+	-	-	+	-	+	+	+	+	+	-	+
PY	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	-
IB	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+
DB	+	-	+	+	+	+	+	-	+	+	+	+	-	+	-	+

In Vitro Evaluation of LAB

In vitro evaluation of LAB was carried out by acid and bile tolerance, antibiotic susceptibility and anti-bacterial activity.

Acid Tolerance of LAB

Results of acid tolerance of probiotic isolates observed at different pH levels are presented in Table 5. Growth was indicated by a positive (+) sign, and absence of growth was indicated by a negative (–) sign.

Table 5: Acid Tolerance of LAB

S. No	Isolate	Varying levels of pH		
		pH2	pH4	pH6
1.	JBM	-	+	+
2.	SBM	-	+	+
3.	HCU	-	+	+
4.	SCU	-	+	+
5.	DB	-	+	+
6.	ABM	-	+	+
7.	HP	+	+	+
8.	IB	+	+	+
9.	KM	-	+	+
10.	JLS	-	+	+
11.	MY	-	+	+
12.	MZC	-	+	+
13.	PY	-	+	+
14.	SA	-	+	+
15.	SYD	-	+	+
16.	MMC	-	+	+

The two probiotic isolates from HP and IB were grown at acidic pH 2 while the isolates from JBM, SBM, HCU, SCU, DB, ABM, HP, IB, KM, JLS, MY, MZC, PY, SA, SYD and MMC showed growth at pH 4 and 6.

Adebayo (2008) [1] reported that fermented foods were considered as potential source for isolation of lactic acid bacteria and they have the ability to produce high levels of lactic acid and as well survive under high acidic conditions.

According to Charteris *et al.* (1998) enteric *Lactobacilli* were able to tolerate pH 2.0 for several minutes and also survived in high acidic (pH 1.0) conditions.

Some lactic acid bacterial strains could grow at pH 4 and some isolates showed no growth at pH 6 as observed in the study conducted by Mutukumira *et al.* (2002) [46]. Another study by Hoque *et al*. (2010) [29] found that *Lactobacillus* growth occurs at pH 2.5 to 5.0.

The present findings were similar to those of Pundir *et al.* (2013) [54] who reported that *Lactobacillus* isolated from fermented products survived at pH 2.5 to pH 6.0.

Another study by Onilude *et al*. (2013) [49] concluded that lactic acid bacterial isolates from Nigerian fermented food products survived at pH 6. Mannan *et al.* (2017) [41] reported that isolated *Lactobacillus* showed maximum growth at pH 2. Therefore, these bacteria exhibit survival in a highly acidic environment. Assefa *et al.* (2017) [8] found that lactic acid bacterial isolates from dairy samples survived at pH 4.

Ali *et al.* (2018) [5] reported that all lactic acid bacteria species were found to have varying levels of tolerance to acidic environments and also revealed that pH 4 was found most favourable medium as the viability increased at that pH. The most inhibitory medium for lactic acid bacteria as the viability decreased at pH2.

Bile Salt Tolerance of LAB

The results of the probiotic isolates survived in different concentrations of bile salts are presented in Table 6. Growth was indicated by a positive (+) sign and absence of growth was indicated by a negative (–) sign.

Table 6: Bile Salt Tolerance of Isolates at Different Levels

S. No	Isolate	Bile salt concentration at different levels			
		0.5%	1.0%	1.5%	2.0%
1.	JBM	+	+	+	+
2.	SBM	+	+	+	+
3.	HCU	+	+	+	+
4.	SCU	+	+	+	+
5.	DB	+	+	+	+
6.	ABM	+	+	+	+
7.	HP	+	+	+	+
8.	IB	+	+	+	+
9.	KM	+	+	+	+
10.	JLS	+	+	+	+
11.	MY	+	+	+	+
12.	MZC	+	+	+	+
13.	PY	+	+	+	+
14.	SA	+	+	+	+
15.	SYD	+	+	+	+
16.	MMC	+	+	+	+

All isolates survived at 0.5,1.0,1.5 and 2.0% of bile salts. The current results of resistance against bile salt were supported by the findings of Gilliland (1979) [25], who reported that *Lactobacilli* isolated from milk products showed high tolerance to bile salts. Similar results were found in a study conducted by Patel *et al.* (2004) [51].

These results of the present study are similar Ashraf *et al.* (2009) [7] who found that *Lactobacilli* were capable of survival in the environment of the gastrointestinal tract, which has characteristic features of having an acidic pH and high concentrations of bile salts.

The present findings are in line with Bhattacharya and Das (2010) [13] who demonstrated that lactic acid bacteria from fermented products showed growth in 0.5% and 1% bile salt concentrations. Similar results were also obtained by Mohsin and Gaurav (2013) who reported that isolates of lactic acid bacteria from curd were resistant to 0.5% bile salt concentration.

The LAB strains *L. rhamnosus* strains from cheese showed growth in the presence of 1.0%, 1.5% and 2.0% of bile salt concentration in the study conducted by Grazia *et al.* (2005) [27]. Tambekar (2010) [68, 69] reported that the isolates of probiotic bacteria showed tolerance at bile salt tolerance at 2.0%.

Similar results were reported by Pundir *et al.* (2013) [54] who concluded that the selected LAB isolates were able to survive in 0.5, 1.0, 1.5 and 2.0% bile salt concentrations. Kabore *et al.* (2012) [33] reported that lactic acid bacteria from fermented seeds were able to survive at 0.3% of bile salts.

Antibiotic Sensitive Assay

The results of the probiotic isolates treated with different antibiotics are presented in Table 7. Antibiotic resistance was indicated by 'R' and susceptibility was indicted by 'S'

Table 7: Antibiotic resistance assay of probiotic isolates

S. No	Isolate	Antibiotic Susceptibility/Resistance					
		Ampicillin	Chloramphenicol	Penicillin-G	Streptomycin	Sulphatriad	Tetracycline
1.	SA	S	S	S	S	S	S
2.	KM	S	R	S	R	R	R
3.	SBM	S	S	S	S	S	S
4.	ABM	S	S	S	S	S	S
5.	HP	S	S	S	S	S	S
6.	MY	S	S	S	S	S	S
7.	SCU	S	S	S	S	S	S
8.	JBM	S	S	S	S	S	S
9.	JLS	S	S	S	S	S	S
10.	MZC	S	S	R	R	S	S
11.	SYD	S	R	R	S	S	R
12.	HCU	S	S	S	S	S	S
13.	MMC	S	S	S	S	S	S
14.	PY	S	S	S	S	S	S
15.	IB	S	S	S	S	S	S
16.	DB	S	S	S	S	S	S

All sixteen probiotic isolates expressed susceptibility towards the Ampicillin. Isolates from MZC and SYD showed antibiotic resistance to chloramphenicol while the remaining isolates showed susceptibility. Isolates from MZC and SYD showed antibiotic resistance to penicillin while the remaining isolates showed susceptibility. The probiotic isolates KM and MZC showed resistance towards streptomycin while the remaining isolates showed susceptible conditions. Except KM probiotic isolate all probiotic isolates were susceptible to Sulphatriad.

Antibiotic resistance can change according to product variety or lactic acid bacteria species. Florez *et al.* (2005) [24] investigated antibiotic resistance of lactic in traditional cheese, *Lactobacilli* isolates such as *L. lactis* were shown resistant to antibiotics such as chloramphenicol and tetracycline.

The results were in line with studies conducted by Danielsen and Wind (2003) [17], Coppola *et al.* (2005) [27] and Zhou *et al.* (2005) [77]. Ammor *et al.* (2007) [2] found that *Lactobacillus* isolates showed susceptibility to chloramphenicol. Mathur and Singh (2005) [42] determined the antibiotic resistance of *Lactobacillus* which was isolated from yoghurt and other dairy products and showed resistance to chloramphenicol.

Korhonen *et al.* (2008) [36] reported that *Lactobacillus* species showed resistance to tetracycline. Liu *et al.* (2009) [38] stated *that Lactobacilli* and *Lactococci* strains from fermented milk were resistant to streptomycin. The antibiotic properties exist because of the ability of lactic acid bacteria to synthesise bacteriocines and lactic acid. Probiotic bacteria produce peptides, which have antibiotic activities (Ashraf *et al*., 2009) [7].

The antibiotic resistance was showed by lactic acid bacteria potential strains from curd and milk samples to chloramphenicol (Murugan *et al*., 2013) [65]. Similar results were found also by Suganya *et al.* (2013) [65] who reported *that lactobacillus* isolates from curd showed resistance to chloramphenicol.

Nawaz *et al.* (2011) [47] found that some lactic acid bacterial strains from fermented products were susceptible to antibiotics such as Ampicillin, streptomycin and tetracycline. Another study conducted by Monica *et al.* (2016) [43] reported that that lactic acid bacterial strains *Lactobacillus brevis, Lactobacillus casei, Lactobacillus plantarum, and Lactobacillus buchneri sp.* from traditional fermented foods and beverages showed antibiotic resistance to penicillin G and tetracycline.

Goswami *et al.* (2017) [26] isolated LAB from kahudi (fermented mustard pickle) which showed antbiotic resistance to Sulphatriad. Turhan *et al.* (2018) [70] reported that lactic acid bacteria such as *Lactobacillus spp* from traditional Turkish fermented dairy products showed antibiotic resistance to tetracycline.

Antimicrobial Activity of LAB

The results on antimicrobial activity of probiotic isolates towards *Staphylococcus aureus* and *Escherichia coli* results are presented in Table 8.

Table 8: Anti-microbial activity of LAB

S. No	Isolate	Pathogenic bacteria	
		Staphylococcus aureus	*Escherichia coli*
1.	SA	++	++
2.	KM	++	++
3.	SBM	++	++
4.	ABM	++	+
5.	HP	++	+
6.	MY	+	+
7.	SCU	+	+
8.	JBM	++	+
9.	JLS	+	++
10.	MZC	++	++
11.	SYD	++	++
12.	HCU	-	_
13.	MMC	_	_
14.	PY	+	+
15.	IB	+	++
16.	DB	-_	-_

Degree of Inhibition: + =Moderate inhibition zone, ++ = strong inhibition zone, - = No inhibition zone

The probiotic isolates such as SA, KM, SBM, ABM, HP, JBM, MZC and SYD showed strong inhibition zones against *staphylococcus aureus.* Isolates such as SCU, JLS, PY, MY and IB isolates showed moderate inhibition zones against *staphylococcus aureus.* No inhibition zone was observed in HCU, MMC and DB.

The probiotic isolates such as SA, KM, SBM, JLS, MZC, SYD and IB isolates showed a strong inhibition zone against *Escherichia coli.* Moderate inhibition zones against *Escherichia coli* were observed in ABM, HP, SCU, MY, PY and JBM. Isolates such as HCU, MMC and DB showed no inhibition zone against *Escherichia coli.* The dominant lactic acid bacteria had unique antimicrobial and probiotic characteristics because they can control the growth of undesired microorganisms as reported by Leroy and De Vuyst (2004) [39] and Vinderola and Reinheimer (2003) [71].

The present results are similar to those reported by Joshi *et al.* (2006) [32], who reported that the isolated strain of *Lactobacillus* genus from carrot fermentation produced bacteriocin with maximum antimicrobial activity against *Escherichia coli* and *staphylococcus aureus.* Ashraf *et al.* (2009) [7] reported that all *Lactobacilli* inhibited the growth of *Escherichia coli* and *staphylococcus aureus,* except *L. delbrucekii* that exhibited significantly low antimicrobial effect.

Awaisheh and Ibrahim (2009) [9] reported that lactic acid bacteria isolates from fermented vegetables and dairy samples showed antibacterial activity against *Escherichia coli.*

Parveen *et al.* (2011) [50] reported that *Lactobacillus spp.* exhibited varying degrees of inhibitory activity against strains of *Escherichia coli, Staphylococcus aureus.*

Zhang *et al.* (2013) [76] found that *L. plantarum* strains from fermented Chinese sauerkraut showed antimicrobial activity against *Escherichia coli.* The isolate possessed a wide spectrum of inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* reported by Islam *et al.* (2020) [30].

Conclusion

Present study concluded that among sixteen isolates were studied the lactic acid bacteria isolates present in kombucha, buttermilk (Sangam), mozzarella cheese and strawberry yoghurt drink showed superior characteristics in terms of probiotic properties. These isolates contained majorly the LAB species such as *Lactobacillus delbrueckii* ssp. *bulgaricus, Lactococcus lactis, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus*. These strains could be used in the development of starter cultures for the production of fermented products in a controlled environment in future.

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