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Lactobacillus fermentum strains with significant probiotic and antioxidant potential

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Abstract

The growing demand for dairy products amended with probiotics has led to the exploration of new beneficial microorganisms such as probiotics with beneficial properties. In the present work, the probiotic and antioxidant potential of *Lactobacillus fermentum* strains isolated from dairy products were evaluated. Strains were investigated for their probiotic properties by performing different tests such as survival in pepsin, low pH, and bile salt, antibacterial activity, and antioxidant potential. These strains were further evaluated for their utilisation in yogurt formation as a probiotic. The isolated strains were identified as *L. fermentum* Y1, *L. fermentum* Y2, and *L. fermentum* C by 16S rRNA sequencing. All strains showed greater survival ability in simulated gastric conditions (pH 2.2 + pepsin) and in the presence of 0.3% bile salt. The highest antibacterial activity was exhibited by *L. fermentum* Y1 against *Bacillus cereus*. Among these three strains, *L. fermentum* Y1 had the highest reducing power, and *L. fermentum* C had the highest DPPH scavenging activity. All *Lactobacillus* strains as a single inoculum or in consortium showed significant ($p < 0.05$) probiotic properties by maintaining pH, titratable acidity, solid content, and high water holding capacity in comparison to the control in the cow yogurt and homogenised milk. The isolated *Lactobacillus* strains may be a potential source of probiotics in commercial yogurt preparation.

Introduction

Nowadays, it is a big challenge to provide a safe and healthy food to consumers that is not only beneficial for health but also effective to inhibit chronic diseases and disorders (Abdelazez *et al.,* 2018). In this regard, the use of microorganisms such as probiotics in fermented foods to improve health and reducing the risk of chronic diseases *i.e.,* heart disease, diabetes, hypertension, obesity, and kidney toxicity remains a challenge for researchers (Abdelazez *et al.,* 2017; Ahmad *et al.,* 2018).

Probiotics have been used widely in the fermented product industry as they play a vital role to modify the ecology of the intestine, and provide the host with immunity. According to FAO/WHO, probiotics are live microorganisms which when administered in an adequate amount would confer health benefit on the host. On the other hand, medicines such as antibiotics have many side effects which prompt scientists to explore for other alternatives (Amir *et al.,* 2016). Most of the microorganisms that are used as probiotics are lactic acid bacteria *e.g., Lactobacillus, Bifidobacterium*, and other microorganisms such as yeasts (Drago *et al.,* 2015).

Ellie Metchnikoff, a Russian researcher, was the first person who proposed the beneficial effects of probiotics in 1905. He believed that normal flora of the gut caused adverse effects with the production of toxin called autotoxication. These drastic effects can be decreased with the consumption of a fermented product composed of rod-shape bacteria, *Lactobacillus*, which will then decrease the toxicity by balancing the microflora (McFarland, 2015). Several other *in vitro* and *in vivo* studies reported that probiotics help in maintaining gut epithelial barrier physiology by enhancing mucus secretion which decreases the attachment and blocks the proliferative activity of pathogens in the intestinal epithelial cells (Vaziri *et al.,* 2015). The production of antimicrobial substances and enhancement of digestion process provide strength to the immune system and stimulate vitamin production (Horvath *et al.,* 2016). Probiotics also demonstrated antioxidant properties by the production of an antioxidant called superoxide dismutase (SOD), non-enzymatic antioxidant glutathione (GSH), and antioxidant biomolecule exopolysaccharide. All these depict the importance of probiotic strains. *Lactobacillus* spp., Gram-positive and non-spore-forming bacteria, have been found as a valuable probiotic source. These bacteria are predominant human and animal microflora (Afify *et al.,* 2012). Due to the

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production of lactic acid, these bacteria are used in fermentation (Bassyouni *et al.,* 2012).

Yogurts are formed by probiotics. According to Codex Alimentarius, yogurts are milk fermented by mixing cultures of *Streptococcus thermophiles* or any *Lactobacillus* species (Codex Alimentarius, 2003). Today, probiotics are used in the production of yogurt either as single microbial culture *i.e., L. acidophilus, Bifidobacteria*, and *L. casei*; or as a combination of these bacteria because they do not only improve the flavour and texture of the product, but also inhibit food spoilage due to their antimicrobial activities. Many studies showed that probiotics do not retain their physicochemical properties or viability when they are used as a starter culture in the fermented dairy products (Champagne *et al.,* 2005; Mani-Lopez *et al.,* 2014).

Therefore, the present work aimed to isolate the potential *Lactobacillus* strains, and evaluate their probiotic and antioxidant quality, and their utilisation as a starter culture in single or in consortium form in the production of yogurt.

Materials and methods

Isolation and identification of Lactobacillus from dairy samples

Dairy samples (cow milk and fresh homemade yogurt) were obtained from different rural areas (Multan, Lodhran, Laar) of Southern Punjab. The samples were collected in sterilised tubes and polythene bags, and then brought to the laboratory for further processing. The selective growth media namely de Man, Rogosa, and Sharpe (MRS; Oxoid) agar and broth were used for the isolation of *Lactobacillus*. Serial dilution (10^{-1} to 10^{-6}) of all samples were prepared in peptone water, and incubated at 25°C for 30 min to increase the recovery and efficiency of bacteria. After that, 0.1 mL aliquot of both original sample and dilution of each samples were spread on MRS agar which contained natamycin as fungicide to prevent fungal contamination. These plates were incubated at 37°C for 48 h under anaerobic conditions (Pedersen, 1992; Narwade *et al.,* 2015). After incubation, individual colonies were selected, and the purified bacterial strains were identified by morphological (Gram-staining and endospore test), physiological (catalase test), and biochemical tests (carbohydrate fermentation, indole test, citrate utilisation test, H_2S production test, and methyl red test) (Pyar and Peh, 2014). After that, 16S rRNA sequencing was performed to molecularly identify the selected strains.

Tests for probiotic properties

Acid and bile salt tolerance test

For determination of acid tolerance, the isolated strains were grown in MRS broth at 37°C for 24 h. Before inoculation, different pH's of 2, 3.5, 4.5, and 7.4 were adjusted with 1% HCl. After incubation, the growth of inoculated strains was checked by taking absorbance at 600 nm in comparison with the control (un-inoculated broth). The test was performed in triplicates. To determine the ability of bile salt tolerance, MRS broth was prepared with 0.3% bile salt and without bile salt. After media sterilisation, isolated strains were inoculated, and incubated at 37°C for 48 h. The degree of bile salt resistance was interpreted by comparing the optical density of tested strain with the control. The OD was taken at 620 nm (Mohanty and Ray, 2016).

Bile salt hydrolysis and exposure to gastric simulants

Bile salt hydrolysis activity of isolated strains detected on MRS media was amended with 0.2% sodium deoxy taurocholate by streak plate method. The bile salt hydrolysis effect of selected strains was showed by different colony morphology as compared to the control after 24 h of incubation at 37°C (Bi *et al.,* 2016).

Gastric simulants involved acidic environment of the stomach with pepsin enzyme. Fresh MRS media was prepared with pepsin composition of 0.3 and 0.5% NaCl. Artificial gastric juice (pH 2) was used, and 0.1 M NaOH or 1 M HCl was used for pH adjustment . After the preparation of gastric juice, the bacterial culture was inoculated in media, and incubated at 37°C for 24 h aerobically. Bacterial growth was evaluated by taking absorbance at 600 nm in comparison with control (Isa and Razavi, 2017).

Antimicrobial activity test

The antimicrobial activity of isolated strains was analysed by well diffusion method. Pathogenic bacteria that were used as indicators included methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus, E. coli, Klebsiella* spp., *Enterobacteriaceae* 7623, *Pseudomonas aeruginosa, Bacillus subtilis, B. cereus*, and *Proteus* spp. obtained from MMG Department, University of the Punjab. Fresh pathogenic strains were inoculated in L. broth and incubated at 37°C for 24 h. After incubation, the OD was adjusted by McFarland standard method as 0.1. Then, the culture of pathogenic strains was swabbed with sterile cotton swab homogenously onto plates of Muller Hinton agar. Each well was filled with 70 µL supernatant of the selected strains.

After 24 h of incubation, the diameter of the inhibition zone was measured. The test was performed in triplicates (Mohankumar and Murugalatha, 2011).

Assays for antioxidant activity

Preparation of CFS for antioxidant activity

In antioxidant assay, the cell lysate, cell secretion, or cell-free supernatant (CFS) were usually used. Here, only CSF was used. For the preparation of CFS, strains were grown at 37°C for 24 h. Grown cells were transferred into the Eppendorf tube, and centrifuged at 10,000 rpm at 4°C for 10 min. After centrifugation, CFS was filtered through 0.22 µm pore size filter paper before used for further testing and analysis (Xing *et al.,* 2015).

Reducing power of strain

A 0.5 mL of CFS was mixed with equal volume (0.02 mM, 0.5 mL) of phosphate buffer solution (pH 6.5) and 1% potassium ferricyanide (0.5 mL). The mixture was incubated at 50°C for 20 min. After rapidly cooling, the addition of 0.5 mL TCA (10%, w/v) was done, and centrifugation of reaction mixture was carried out at 3,000 rpm for 10 min, then the supernatant was mixed with 1 mL of ferric chloride (0.1%), and the absorbance of solution was measured at 700 nm. The greater value of absorbance reflects high reducing potential of the reaction mixture. The control was prepared with the same method but without CFS (Xing *et al.,* 2015).

DPPH free radical scavenging assay

One millilitre of CFS was mixed with 2 mL of DPPH (0.05 mM in ethanol) solution. DPPH was mixed with deionised water, and used as the control. The mixture was placed in the dark at room temperature for 30 min. After incubation, the mixture was centrifuged at 8,000 rpm for 10 min. DPPH scavenging ability of strains was evaluated by measuring the absorbance of solution at 517 nm for three times. Trolox was used as standard. The scavenging ability was determined using Eq. 1:

$$\text{Scavenging ability} = [(A_{Blank} - A_{Sample}) / A_{Blank}] \times 100 \quad \text{(Eq. 1)}$$

where, A = Absorbance (Li *et al.,* 2012).

Resistance to hydrogen peroxide

MRS media was prepared with different concentrations of hydrogen peroxide (0.4, 1 mM). Sterilised media was inoculated with isolated strains and incubated for 24 h at 37°C. The control was prepared with the same method but without bacterial inoculum. Following incubation, resistance of strain to hydrogen peroxide was measured by reading absorbance spectrophotometrically at 600 nm, and compared with the control (Li *et al.,* 2012).

Preparation of probiotic yogurt

For the production of yogurt, cow milk and commercial homogenised milk were used. Briefly, 80 mL of cow milk and homogenised milk samples were taken and distributed in four separate parts, and later pasteurised at 85°C for 30 min. After cooling at 43°C, yogurt starter culture was added with inoculation of selected probiotic strains namely *L. fermentum* Y1, *L. fermentum* Y2, *L. fermentum* C, and the combination of *L. fermentum* Y1 + *L. fermentum* Y2 + *L. fermentum* C, while the control had no probiotic strains but contained yogurt starter culture. Pure fresh culture of probiotics was prepared. Then, the pellets of these strains were suspended in milk after centrifugation at 8,000 rpm for 10 min at 4°C. Inoculated milk was added in the test tubes of cow and homogenised milk in equal quantity separately, and incubated at 42°C. After 24 h, yogurt samples were stored at 4°C, and their physicochemical properties were evaluated (Mani-Lopez *et al.,* 2014).

Physiochemical properties of yogurt

Measurement of pH and titratable acidity

The pH value of yogurt and milk samples was measured by using a pH meter at 0 and 15 days of storage (Yeganehzad *et al.,* 2007).

The titratable acidity percentage of different yogurt samples was measured with titration method by using standardised 0.1 N NaOH and 0.5% phenolphthalein as indicator. Briefly, 1 g of yogurt sample was placed in a glass dish, added with 2 mL of water, mixed properly, and then placed on a shaker. The sample was then titrated by placing NaOH drop by drop, and the process was stopped when a pink colour was observed. The percentage of lactic acid was calculated by using Eq. 2 (Yeganehzad *et al.,* 2007).

$$\text{Lactic acid \%} = V\,(NaOH) \times 0.09 / s_{sample} \quad \text{(Eq. 2)}$$

where, V = volume of 0.1 N NaOH used in titration, and s_{sample} = mass of the yogurt sample used.

Total solid contents and water holding capacity

Total solid contents were determined using the method described by Nguyen *et al.* (2014). The first initial weight of samples was taken and placed in

a hot dry oven at 99°C for 40 min. After drying, samples were cooled at room temperature, and re-weighed. The final weight of samples after drying was considered as solid contents. Total solid contents were calculated using Eq. 3 (Nguyen *et al.,* 2014):

Total solid contents = Initial weight of sample (before drying) – Final weight of sample (after drying) (Eq. 3)

To measuring the water holding capacity, 10 g of each sample were taken and centrifuged at 3,000 rpm at 10°C for 60 min. After centrifugation, supernatant was discarded, and weight of the remaining pellet was taken. Water holding capacity was expressed as the percentage relative to the initial weight of the sample (Sengupta *et al.,* 2014).

Statistical analysis

All experiments were performed in triplicates. The results were expressed as mean ± standard deviation. Duncan test was used to determine whether statistical differences occurred between groups by using IBM SPSS Statistics software. $p < 0.05$ was considered as statistically significant for all analysis.

Results

In the present work, bacteria were isolated from dairy products to evaluate their probiotic and antioxidant properties. Strains were isolated by the dilution plating method, and selected based on the identified probiotic potentials. Isolates were characterised as *Lactobacillus* based on morphological, biochemical, and physiological characterization. All isolates were rod-shaped, Gram-positive, catalase-negative, non-spore former, non-motile, and can ferment different types of sugar (lactose, glucose, fructose, dextrose, sucrose, and mannitol).

Isolates were sent for molecular identification by 16S rRNA gene analysis at Macrogen, Korea, and identified as *L. fermentum* Y1 (AS 1) (yogurt sample from Lodhran), *L. fermentum* Y2 (AS 5) (yogurt sample from Multan), and *L. fermentum* C (AS 3) (cow milk sample from Laar). Similarities were shown by the phylogenetic tree (Figure 1).

Lactobacillus strains were grown in various pH of 2, 3.5, 4.5, and 7.4 to determine the tolerance of strains towards low and neutral pH. All strains showed good probiotic property by expressing the ability of survival at low pH (Figure 2a) and tolerance of 0.3% ox gall bile salt. The same concentration of bile salt (0.3%) is present in the intestine. The three strains exhibited maximum growth at pH 7.4 and high percentage of bile salt tolerance (Figure 2a). *Lactobacillus fermentum* Y1, *L. fermentum* Y2, and *L. fermentum* C exhibited 21.07, 21.15, and 53.93% tolerance of bile salt, respectively. The growth of *Lactobacillus* at acidic and basic pH (2 - 7.4) was also reported. All isolates possessed bile salt hydrolase enzyme and were positive for bile salt hydrolysis, which was shown by the white precipitation on growth (Figure 2b).

Different strains showed significant

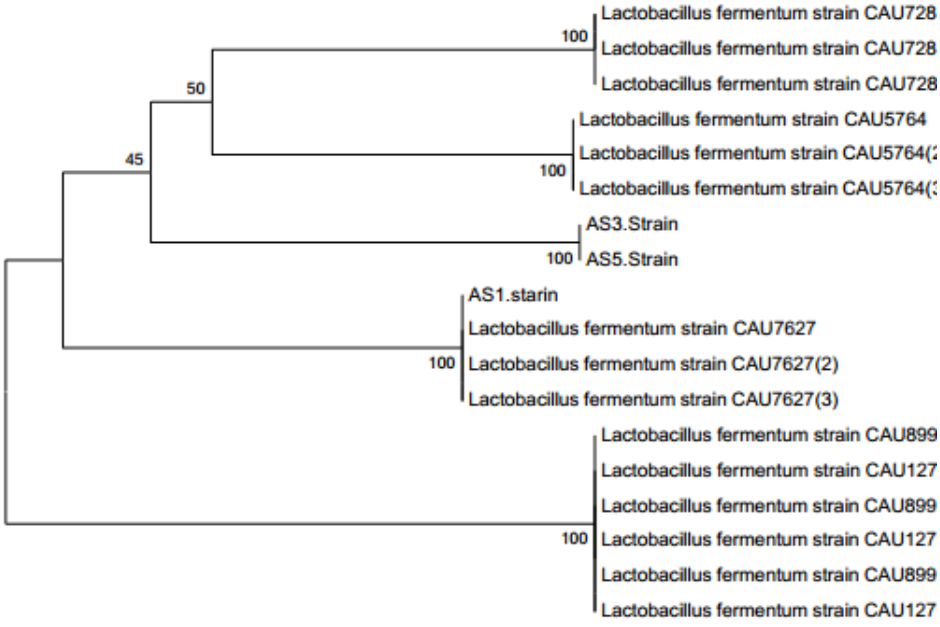

Figure 1. Phylogenetic tree of *Lactobacillus* fermentum strains showing the relationship with isolated strains based on 16S rRNA gene sequencing.

($p < 0.05$) variability in the percentage of growth when exposed to simulated gastric juice. Great survival in the presence of pepsin was shown by *L. fermentum* C, while *L. fermentum* Y1 and *L. fermentum* Y2 had low rate of survival in simulated gastric juice, as shown in Figure 2c.

The strains showed varied antimicrobial activities against the tested pathogenic bacteria. *Lactobacillus fermentum* Y1 showed effective antibacterial activity against methicillin-resistant *Staphylococcus aureus* (15 mm), *Bacillus cereus* (18 mm), *Enterococcus* spp. (12 mm), and *Klebsiella pneumonia* (13 mm). Antibacterial activity of isolated strain are shown in Table 1. The highest effective strain was *L. fermentum* Y2 against *Enterobacteriaceae* 7623 and *Enterococcus* with an inhibition zone of 13 and 11 mm, respectively. *Lactobacillus fermentum* C showed greatest antibacterial activity against pathogenic strains (*Pseudomonas aeruginosa, Enterococcus* spp.,

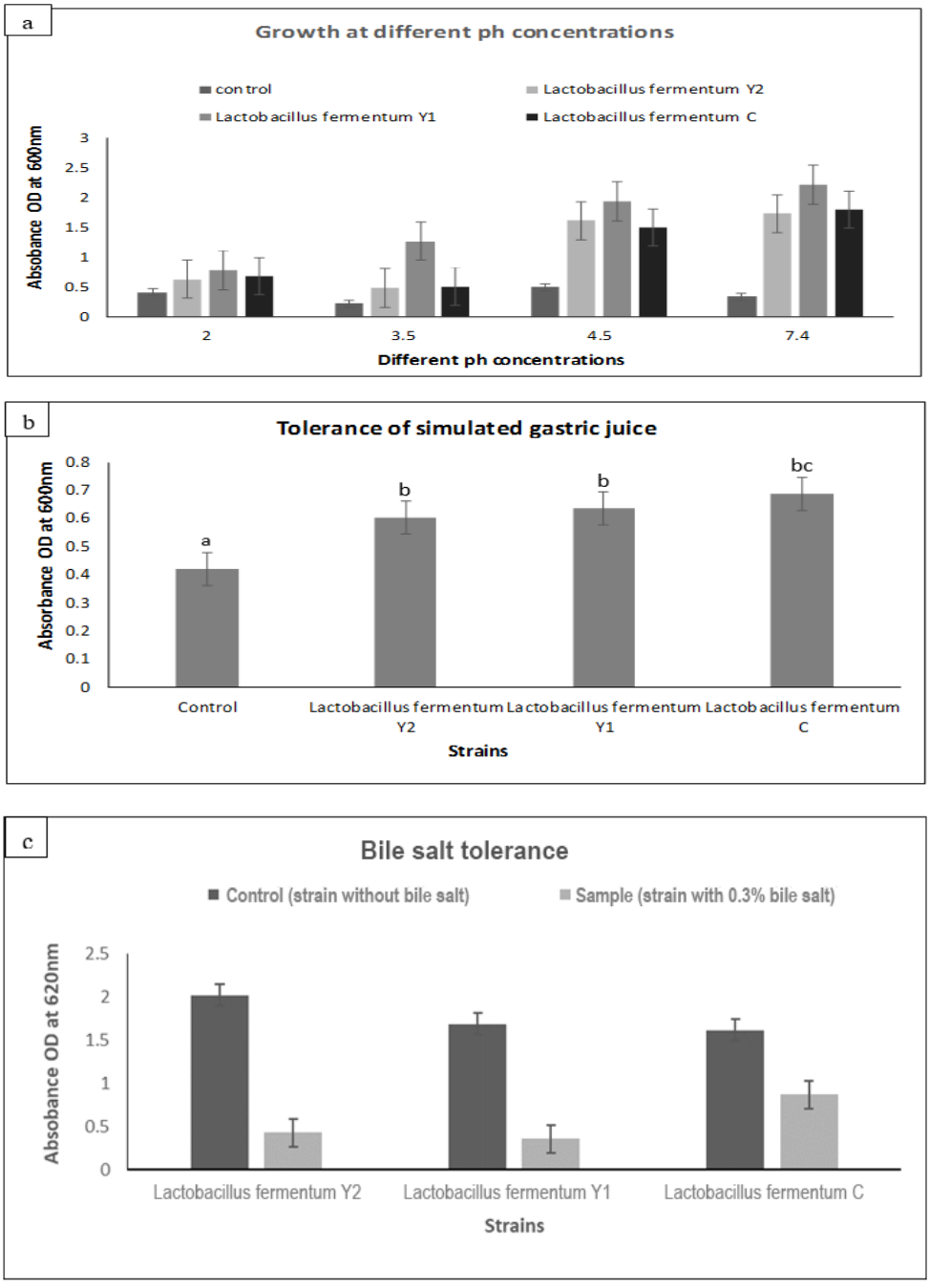

Figure 2. Probiotic properties of *Lactobacillus fermentum* strains. (a) Optimal growth at different pH's (2, 3.5, 4.5, and 7.4); (b) Survival abilities in a simulated gastric juice at pH 2.0; and (c) Bile salt (0.3%) tolerance. Values are mean ± standard deviation of triplicates ($n = 3$). The significant ($p = 0.05$) difference among strains and control was expressed by different letters based on Duncan's multiple range test.

MRSA 7, and *E. coli*) as compared to the other strains with zones of inhibition of 15, 16, 14, and 11 mm, respectively. *Lactobacillus fermentum* C exhibited the highest activity against *Enterococcus* spp. (16 mm), and the least activity against *E. coli* (11 mm).

All strains could reduce Fe^{3+} ions (Figure 3a). The highest value of OD indicated high reducing power. *Lactobacillus fermentum* Y1 showed the highest reducing power (OD = 2.802), followed by *L. fermentum* C (OD = 2.170), and *L. fermentum* Y2 (OD = 1.040).

DPPH is a stable free radical, and mostly used for determining the free radical scavenging activity of antioxidants. The scavenging activity of antioxidants is showed by reducing the radical form of DPPH into non-radical form. The free radical-scavenging activity of each sample was compared with Trolox as standard or positive control at 517 nm. Different concentrations of Trolox was used (1, 0.8, 0.6, 0.4, and 0.2 mM). Results indicated that the CFS of *L. fermentum* C had the highest scavenging activity against DPPH by 70%. The least scavenging activity was shown by *L. fermentum* Y2 at 55% (Figure 3b).

Resistance to hydrogen peroxide was determined spectrophotometrically (Figure 3c). Two different concentrations (0.4, 1 mM) were used. *Lactobacillus fermentum* Y1 showed more growth at 0.4 mM, and *Lactobacillus fermentum* C was more viable at 1 mM. *Lactobacillus fermentum* Y2 showed less growth as compared to two other strains at both concentrations of hydrogen peroxide (0.4, 1 mM).

Yogurt production by using probiotic starter culture enhances the physicochemical properties. But yogurt production by using different dairy sources *i.e.,* cow milk and commercial homogenised milk affected their physiochemical properties. The physical properties of yogurt were checked after 24 h of storage. Yogurt of homogenised milk with *L. fermentum* Y1 showed significant ($p < 0.05$) properties such as low pH (4.4), titratable acidity (80%), and solid content (0.915%) with high water holding capacity (45%); while the consortium of all strains also enhanced the physical properties in homogenised milk sample of yogurt as compared to cow milk yogurt; but in comparison with the control, all strains enhanced the physical properties of yogurt. The results are shown in (Table 2).

Discussion

The use of food supplements with beneficial microorganisms has increased globally due to their dynamic effects to prevent diseases and ensuring good health. In the present work, bacterial strains were isolated from dairy products to evaluate their probiotic and antioxidant properties. These strains were identified as *L. fermentum* Y1, *L. fermentum* Y2, and *L. fermentum* C. Ishola and Adebayo-Tayo (2012) and Gharbi *et al.* (2019) reported that *L. fermentum* was a predominant bacterium isolated from fermented dairy products and human microbiota. Biochemical characterisation of isolates revealed that all isolates were rod-shaped, Gram-positive, catalase-negative, non-spore former, non-motile, and can ferment different types of sugar (lactose, glucose, fructose, dextrose, sucrose, and mannitol). These results are similar to the studies of Bassyouni *et al.* (2012), Amer *et al.* (2017), and Kumar and Kumar (2015).

The gastrointestinal tract has a highly acidic condition in which the pH ranges from 1 - 2.5. The pH gradually increases downwards from stomach to small intestine (2 - 7.4). Probiotic bacteria must pass through the alimentary canal, and survive these harsh conditions, before they can be beneficial by colonisation in gastrointestinal tract. Therefore, it is

Table 1. Antibacterial activity of isolated strains (diameter of inhibition zone in mm).

Pathogenic strain	Isolate's name		
	***Lactobacillus fermentum* Y2**	***Lactobacillus fermentum* Y1**	***Lactobacillus fermentum* C**
Pseudomonas aeruginosa	-	-	15 ± 0.2
Enterobacteriaceae 7623	11 ± 0.2	-	-
Enterococcus spp.	14 ± 0.0	12 ± 0.2	16 ± 0.0
Klebsiella pneumonia	-	13 ± 0.0	-
Bacillus cereus	-	18 ± 0.1	-
Methicillin-resistant *Staphylococcus aureus* 7	-	-	14 ± 0.1
Methicillin-resistant *Staphylococcus aureus* 8	-	15 ± 0.1	-
Escherichia coli	-	-	11 ± 0.3

Values are mean ± standard deviation of triplicates ($n = 3$).

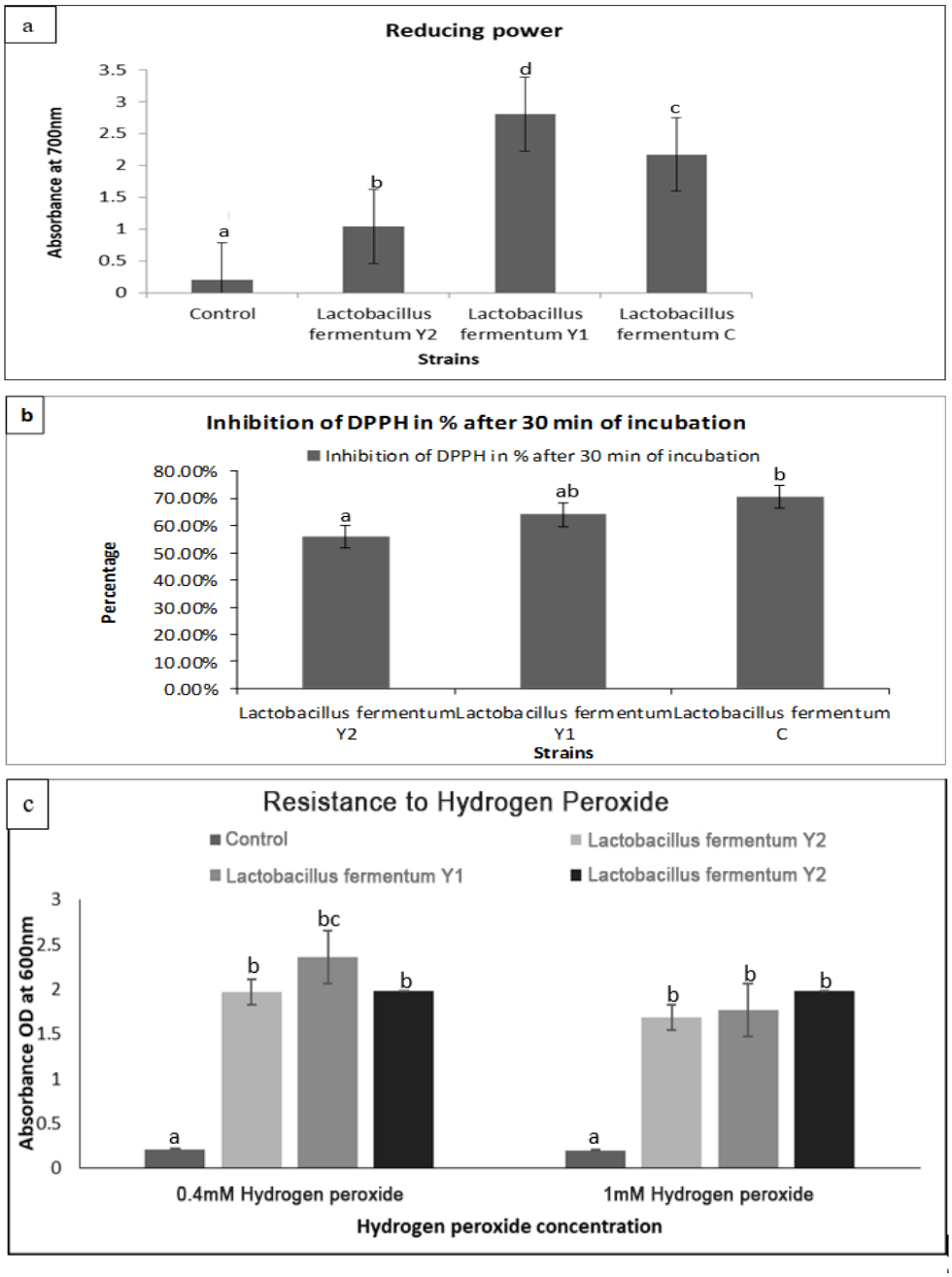

Figure 3. Antioxidant assays of *Lactobacillus fermentum* strains. (a) Reducing power by taking OD at 700 nm after incubation at 37°C; (b) Scavenging effect on DPPH free radical; and (c) Resistance at different hydrogen peroxide concentrations (0.4, 1 mM) incubated at 37°C for 24 h. The significant ($p = 0.05$) difference among *L. fermentum* strains and control was expressed by different letters after applied Duncan's multiple range test. The same letters do not differ significantly at $p < 0.05$.

necessary that potential probiotic isolates should be screened for their probiotic properties by growing at low pH (Shi *et al.,* 2018). The strains isolated and characterised in the present work showed good probiotic property by surviving at the low pH level tested. Ragul *et al.* (2017) also reported on the viability of *Bacillus* sp. at pH 2 for 3 h which is a good probiotic characteristic. High acid tolerance is related to the strain's ability H+ATPase activity (Matsumoto *et al.,* 2004).

Other researchers also observed the growth of *Lactobacillus* in 0.3% bile salt, and its survival for 24 h (Shakibaie *et al.,* 2017; Shi *et al.,* 2018) which is similar to that observed in the present work. The tolerance to bile salts is considered as an important function of probiotics to pass along the intestinal tract. In general, bile salts are capable of disorganising the structure of the cell membrane, thus reducing microbial viability. This problem is solved by the development of hydrolytic enzymes (Bi *et al.,* 2016). Lactobacilli can break down and reduce the toxic effects of the combined bile salts (Bao *et al.,* 2010;

Table 2. Physical properties of yogurt samples after 24 h.

No.	Source	Sample	pH		Titratable acidity (%)	Solid content (%)	Water holding capacity (%)
			Initial	Final			
1	Cow milk	Control	6.9	4.39	0.65[a]	0.901[a]	30[a]
2	Cow milk	*Lactobacillus fermentum* Y2	6.9	4.41	0.72[b]	0.903[a]	35[b]
3	Cow milk	*Lactobacillus fermentum* Y1	6.9	4.45	0.72[b]	0.912[c]	38[c]
4	Cow milk	*Lactobacillus fermentum* C	6.9	4.55	0.69[ab]	0.907[b]	36[b]
5	Cow milk	(*Lactobacillus fermentum* Y1 + *Lactobacillus fermentum* Y2 + *Lactobacillus fermentum* C)	6.9	4.38	0.78[c]	0.909[b]	39[c]
6	Homogenised milk	Control	7.2	4.30	0.61[a]	0.903[a]	33[a]
7	Homogenised milk	*Lactobacillus fermentum* Y2	7.2	4.47	0.71[c]	0.907[b]	36[b]
8	Homogenised milk	*Lactobacillus fermentum* Y1	7.2	4.40	0.80[d]	0.915[d]	45[c]
9	Homogenised milk	*Lactobacillus fermentum* C	7.2	4.56	0.68[b]	0.908[b]	36[b]
10	Homogenised milk	(*Lactobacillus fermentum* Y1 + *Lactobacillus fermentum* Y2 + *Lactobacillus fermentum* C)	7.2	4.41	0.79[cd]	0.913[c]	42[c]

The probiotic and control yogurts were fermented with and without *Lactobacillus*. Lowercase superscripts indicate significant differences in each parameter between the control and probiotic yogurt at each time point ($p < 0.05$).

Wang *et al.,* 2010). All isolates possessed bile salt hydrolase enzyme, and were positive for bile salt hydrolysis, which was evidenced by the white precipitation during growth. Different strains showed significant ($p < 0.05$) variability in the percentage of growth when exposed to simulated gastric juice. Similar results were described in the study that reported the viability of bacteria at pH 1.5 and the growth of *Lactobacillus* strains in the presence of pepsin enzyme. The same bacterial cell viability *in vitro* of low pH and high concentrations of bile salt tolerance experiments was exhibited in a study conducted by Haghshenas *et al*. (2015). Our finding also displayed the potential of strains with high growth at low pH and high concentrations of bile salts.

Lactobacilli are important for their survival rate as well as their antibacterial activity against different pathogenic bacteria. The isolated *Lactobacillus* strains showed antibacterial activity against different tested pathogenic bacteria. The highest antibacterial activity was exhibited by *L. fermentum* Y1 against *Bacillus cereus*. Antibacterial activity exhibited by isolates against *S. aureus* and *B. cereus* was also reported in previous studies (Englerova *et al.,* 2017). The antibacterial effect may be due to the increased production of lactic acid through the fermentation process. The fermentation reduces pH of the medium which may reduce or inhibit the growth of many enteropathogens and foodborne pathogens (Kivanc *et al.,* 2011). Alternative antibacterial strategies in the treatment, prevention of gastrointestinal infections, and modification of gut microflora may be the future application of probiotics (Suskovic *et al.,* 2010). Tham *et al.* (2012) also demonstrated similar results with *Lactobacillus* strains that exhibited good antibacterial potential.

Previous studies suggested that probiotics exert beneficial effects through various mechanisms, important among which is antioxidant activity. The activity of *Lactobacillus* as a probiotic in oxidative stress has been demonstrated by different authors using different assays (DPPH scavenging assay, reducing power assay, and resistance to hydrogen peroxide) (Wang *et al.,* 2009). All strains isolated in the present work showed good antioxidant property with *L. fermentum* Y1 giving the highest reducing power (OD = 2.802 at 700 nm). *Lactobacillus fermentum* C showed the highest scavenging activity against DPPH by 70%. The least scavenging activity was represented by *L. fermentum* Y2 at 55%. Inhibition of DPPH by 77.34% was reported in a previous study (Yu *et al.,* 2015). Significant scavenging ability by CFS (96.74 - 91.72%) to DPPH was reported by previous study (Xing *et al.,* 2015). Our results about the tolerance of strains to harmful oxidative free radicals are in agreement with Li *et al.* (2012). *Lactobacillus fermentum* Y1 showed more growth at 0.4 mM, and *L. fermentum* C was more viable at 1 mM. *Lactobacillus fermentum* Y2 showed less growth as compared to two other strains at both concentrations of hydrogen peroxide (0.4, 1 mM). Resistance to 1 mM hydrogen peroxide ranged between 83.6%, and survival of strains at 4 mM has also been confirmed (Kullisaar *et al.,* 2002).

Yogurt production using probiotic starter culture enhanced its physicochemical properties. The physical properties of yogurt were checked after 24 h of storage. Yogurt of homogenised milk with

L. fermentum Y1 showed significant ($p < 0.05$) properties such as low pH (4.4), titratable acidity (80%), and solid content (0.915%) with high water holding capacity (45%), while the consortium of all strains also enhanced the physical properties in homogenised milk sample of yogurt as compared to cow milk yogurt, but in comparison with the control, all strains enhanced physical properties of yogurt. These results are similar to the study of Mani-Lopez *et al.* (2014) in which after storage, there was a decrease in pH and increase in titratable acidity, and solid contents were higher in homogenised milk as more solid contents in yogurt with more calcium constituents were (Mani-Lopez *et al.,* 2014). The isolated strains could be health-promoting beneficial bacteria with potential antibacterial and antioxidant properties. Therefore, these strains may be used as a potential probiotic candidate in the starter culture and in the development of functional food. Similar kind of results which also support that of the present work were reported by Shi *et al.* (2018). Nevertheless, further study is warranted for commercialisation of this beneficial input.

Conclusion

From the results obtained in the present work, it can thus be concluded that dairy products are good sources of *Lactobacillus* strains with probiotic and antioxidant potentials.

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