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# ANTIOXIDANT ACTIVITY OF DIFFERENT MILK FERMENTED WITH LOCALLY ISOLATED LACTIC ACID BACTERIA

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## ABSTRACT

**The study was conducted to determine the antioxidant activity of three media (cow, buffalo and goat's milk) fermented with six locally isolated *Lactobacillus* strains (*L. casei* BIOTECH 1064, *L. paracasei* BIOTECH 10363, *L. paracasei* BIOTECH 10371, *L. paracasei* BIOTECH 10372, *L. paracasei paracasei* BIOTECH 10369 and *L. plantarum* 1074). The initial assessment of milk media showed that buffalo's milk had significantly ($P<0.05$) higher antioxidant activity (34.37%) compared to cow (26.77%) and goat's milk (30.19%). The highest antioxidant activity was observed in cow's milk with *L. paracasei* BIOTECH 10363 (79.07%) after fermentation. At 37°C for 72 hours, *L. paracasei* BIOTECH 10363 displayed the highest viable count (10.31 log cfu/mL), titratable acidity (1.08%) and antioxidant activity (84.23%) and the lowest pH (3.83) in cow's milk. The results reveal that the antioxidant activity, lactic acid content, viable count and pH were influenced by incubation time and temperature. The findings of the present study show that *L. paracasei* BIOTECH 10363 could be utilized for the production of dairy-based functional food with antioxidative properties.**

Key words: antioxidant activity, DPPH radical scavenging activity, fermented milk, lactic acid bacteria, *Lactobacillus paracasei*

## INTRODUCTION

Oxidative stress results when there is a disturbance between pro-oxidant/antioxidant balance in favor of oxidant factors. The uncontrolled generation of oxidants may lead to cell death and tissue damage through oxidation of lipids, proteins, and DNA (Power *et al.*, 2013). Oxidative processes are also considered as one of the major sources of deterioration in the food system. These events can occur during the manufacturing, storage, distribution and final preparation of food products (Wąsowicz *et al.*, 2004). Among the food components, lipids are the most susceptible to oxidation due to their chemical instability. Lipid oxidation can cause rancidity such as off-flavors, reduced nutritional value, and may produce toxic substances, which can compromise the health of the consumers (Osuntoki and Korie, 2010; Ahmed *et al.*, 2016).

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To reduce oxidative damage, supplementation of synthetic or natural antioxidants has been practiced. Synthetic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), n-propyl gallate and ferulic acid, exhibit strong antioxidant activity against several oxidation systems (Abubakr *et al.*, 2012). However, the use of synthetic antioxidants has some undesirable side-effects including urticaria and dermatitis (Race, 2009), leading to restriction or prohibition of its use as food additives in some countries (Thorat *et al.*, 2013).

Recently, food-derived biologically active compounds with antioxidant activities have been identified from a wide range of food sources including milk proteins (Nongonierma and FitzGerald, 2013). Some of these compounds have been shown to have noteworthy antioxidative activities including scavenging of free radicals (Chiozzi *et al.*, 2016), inhibition of lipid peroxidation (Wu *et al.*, 2003), and chelation of transition metal ions (Timón *et al.*, 2014). The occurrence of these compounds originates from the degradation of milk proteins during fermentation by the proteolytic enzyme of lactic acid bacteria (Korhonen and Pihlanto, 2006). This suggests that bacterial fermentation can increase the antioxidant activity of milk. Little work has been conducted on the antioxidant activity of milk fermented with locally isolated lactic acid bacteria (LAB). Therefore, the objectives of this study were to 1) evaluate the physi-cochemical and antioxidant activity of locally sourced milk media (cow, buffalo and goat's milk); 2) determine the antioxidant activity of different milk media fermented with locally isolated *Lactobacillus* strains and 3) determine the effects of incubation time and temperature on viable count, titratable acidity, pH, and antioxidant activity of selected milk x *Lactobacillus* strain combination.

## MATERIALS AND METHODS

Six potential probiotic LAB strains (see Table 1), were purified using the four quadrant streaking plate method. One colony from each culture was picked and punctured to an MRS agar (HiMedia®, Mumbai, India) stab using sterilized inoculating needle and incubated for 18 to 24 hours at 37°C. The stabs served as the stock culture. For routine analysis, the

Table 1. Strains of Lactic Acid Bacteria used in the study.

| Strain[1] | Accession Number | Source |
|---|---|---|
| *Lactobacillus casei* | BIOTECH 1064[2] | Fermented Mungbean |
| *Lactobacillus paracasei* | BIOTECH 10363[2] | Tapuy (Philippine Rice Wine) |
| *Lactobacillus paracasei* | BIOTECH 10371[2] | Commercial drink |
| *Lactobacillus paracasei* | BIOTECH 10372[2] | Commercial drink |
| *Lactobacillus paracasei paracasei* | BIOTECH 10369[2] | Kuyog, Bicol, Philippines |
| *Lactobacillus plantarum* | 1074[3] | Kesong puti (Soft White Cheese) |

[1]Strains can be labeled as potential probiotics as they displayed desirable functional, safety and technological properties (Malilay, 2018).
[2]Strains were obtained from the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), UPLB.
[3]Strain was acquired from Dairy Training and Research Institute, CAFS, UPLB.

stock cultures were activated twice in MRS broth (HiMedia®, Mumbai, India). Briefly, 1% (v/v) of each strain was inoculated in MRS broth which was then incubated for 18 to 24 hours at 37°C. For inoculum density standardization, the cells were harvested through centrifugation (5,000 x g, 10 minutes, 4°C) using a refrigerated centrifuge (Hermle Z 326K Model, Hermle Labortechnik, Wehingen, Germany) and washed twice with sterile quarter-strength Ringer's solution. The culture was adjusted to give a turbidity equivalent to a 0.5 McFarland standard. This suspension provided viable counts of approximately $10^7$ to $10^8$ colony-forming unit (cfu) per mL for each strain when pour plated in MRS Agar.

Reconstituted skim milk (12%) was prepared and sterilized at 10 psi for 10 minutes. After cooling to 37°C, the activated strains were singly inoculated (1% v/v) into 10 mL of 12% sterile RSM and incubated for 24 hours at 37°C to generate starter cultures. Raw cow, buffalo and goat's milk were acquired from the Dairy Training and Research Institute (DTRI), Philippine Carabao Center (PCC) at UPLB and Univet Nutrition & Animal Healthcare Company (UNAHCO) Goat Farm, respectively. Milk samples were subjected to organoleptic, alcohol precipitation (APT), clot-on-boiling (COB) and California mastitis (CMT) tests to determine their initial quality. Only raw milk samples that meet the set standards for these tests were used. Fat and protein content of milk samples were also determined using Ekomilk-Ultra Milk Analyzer (Eon Trading, Bulgaria) and by performing the AOAC (2005) Kjeldahl method, respectively. Titratable acidity (expressed as % lactic acid) was determined using AOAC (2005) titration method and the pH was measured using the digital pH meter model 3505 (Jenway, Staffordshire, UK). The antioxidant activity was evaluated using the standard DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay.

Milk pasteurization was carried out using the high-temperature, short-time (HTST) method (72.5°C for 15 seconds). After cooling to 37°C, starter cultures were inoculated at 2% (v/v) into 600 mL pasteurized cow, buffalo and goat's milk. Single strain fermentations were performed at 37°C for 48 hours. Antioxidant activity (measured as DPPH-radical scavenging activity) was determined after 48 hours of fermentation. Milk media x LAB combination with the highest antioxidant activity was chosen for the next phase of the study. Based on the result, cow's milk x *L. paracasei* BIOTECH 10363 combination was selected to further evaluate its fermentation characteristics (viable count, titratable acidity, and pH) and antioxidant activity at different incubation time (24, 48, and 72 hours) and temperature s (32, 37, and 42°C). The viable counts were obtained using the standard pour plating method. Titratable acidity and pH of fermented milk samples were also determined. The antioxidant activity was measured in fermented milk samples using the DPPH radical scavenging activity assay.

The water-soluble peptide extracts (WSE) of raw and fermented milk samples were prepared prior to the conduct of antioxidant activity assay using the method of Donkor *et al.* (2007) with slight modifications. Aliquots (35 mL) were collected from the milk samples and the pH was adjusted to 4.6 by adding 1M HCl. The suspension was centrifuged (5,000 x g, 4°C, 20 minutes) and the supernatant was filtered on a Whatman™ quantitative filter paper grade 40 (GE Healthcare Life Sciences, Pittsburgh, Pennsylvania, USA.). The filtrate was stored at ≤20°C for further analysis.

The DPPH radical scavenging activity was evaluated in raw and fermented milk samples using the method of Son and Lewis (2002) with some modifications. DPPH radical (Sigma-Aldrich, St. Louis, Missouri, USA) solution (0.004%, w/v) in 95% methanol (Ajax

Finechem Pty Ltd, Australia) was prepared. WSE sample (2 ml) was mixed with 2 ml of the methanolic solution containing DPPH radicals. The mixture was allowed to stand in a dark room for 30 minutes at room temperature. The absorbance of each sample was measured at 517 nm. Methanol served as a blank, while DPPH solution in methanol served as the control. The antioxidant activity was expressed as the percentage of DPPH activity.

$$\text{DPPH Activity (\%)} = \frac{A_{517nm}^{control} - A_{517nm}^{sample}}{A_{517nm}^{control}} \times 100$$

All determinations were performed in triplicate. Data on the physico-chemical properties and antioxidant activity of raw milk samples were analyzed using one-way ANOVA for a Completely Randomized Design (CRD). Data on the antioxidant activity of different milk media fermented with selected LAB were analyzed using a two-factor Factorial ANOVA in CRD. The treatments were arranged in a 6x3 factorial design: six *Lactobacillus* strains and three milk media (cow, buffalo and goat's milk). Data on the fermentation characteristics (viable count, titratable acidity, and pH) and antioxidant activity of *L. paracasei* BIOTECH 10363 in cow's milk fermented at different incubation conditions were analyzed using one-way Repeated Measure ANOVA with three time-points (24, 48 and 72 hours). All statistical analyses were carried out using the Statistical Analysis System (SAS) release 9.4 (SAS Institute, Inc., Cary, NC) software. Comparison of treatment means was analyzed using the Least Significant Difference (LSD) at 5% level of significance. Results were presented as mean ± standard deviation.

## RESULTS AND DISCUSSION

The physico-chemical properties and antioxidant activity of three media (cow, buffalo and goat's milk) are presented in Table 2. Buffalo's milk had significantly ($P$<0.05) higher protein content (4.28%) compared to goat (3.71%) and cow's milk (3.24%). The values obtained in cow and goat's milk are comparable with the findings of Tatar *et al.* (2015). On the other hand, the protein content obtained in buffalo's milk was slightly lower than the value (4.85%) obtained by Mayilathal *et al.* (2017). Milk proteins, especially casein

Table 2. Physico-chemical properties and antioxidant activity of cow, buffalo and goat's milk

| Parameters | Milk Media | | | *P*-value |
|---|---|---|---|---|
| | Cow | Buffalo | Goat | |
| Protein (%) | 3.24±0.02$^{c}$ | 4.28±0.02$^{a}$ | 3.71±0.03$^{b}$ | <0.0001 |
| Fat (%) | 3.27±0.15$^{c}$ | 9.94±0.14$^{a}$ | 4.49±0.24$^{b}$ | <0.0001 |
| pH | 6.73±0.01$^{b}$ | 6.92±0.00$^{a}$ | 6.62±0.01$^{c}$ | <0.0001 |
| Acidity (% lactic acid) | 0.13±0.01$^{b}$ | 0.15±0.01$^{b}$ | 0.17±0.01$^{a}$ | 0.0037 |
| Antioxidant Activity (%) | 26.77±0.37$^{c}$ | 34.37±0.49$^{a}$ | 30.19±0.58$^{b}$ | <0.0001 |

Data are presented as mean ± standard deviation.

$^{abc}$Means within row with different superscript are significantly different ($P$<0.05).

and whey proteins, are considered to be one of the richest sources of peptides with wide range of biological activities including antioxidative properties (Korhonen and Pihlanto, 2006; Dziuba *et al.*, 2009).

The amount of fat in buffalo's milk (9.94%) was also significantly ($P$<0.05) higher than goat (4.49%) and cow's milk (3.27%). This was expected since buffalo's milk is known for its high fat content with very large fat globules (Walstra *et al.*, 2006), where the average content of fat in buffalo's milk is 8.3% and can be as high as 15% under normal conditions (Varrichio *et al.*, 2007). Buffalo's milk had the highest initial pH (6.92) compared to cow (6.73) and goat's milk (6.62). The obtained pH values are in accordance with the previous report (Mahmood and Usman, 2010). In terms of titratable acidity, it was significantly higher in goat's milk (0.17%) than that of the buffalo (0.15%) and cow's milk (0.13%). The initial acidity of milk usually varies within the range of 0.14 to 0.17% under normal conditions (Schmidt *et al.*, 1996).

In terms of antioxidant activity, the buffalo's milk (34.37%) was significantly higher ($P$<0.05) compared to other milk media. At 26.77%, cow's milk exhibited the lowest activity. The antioxidant activities of buffalo and cow's milk obtained in this study were slightly higher than those obtained by Khan *et al.* (2017). Their findings revealed that the antioxidant activity (DPPH radical scavenging activity) of raw, pasteurized and boiled cow's milk were 24.3, 23.8, and 23.6%, respectively, whereas, the antioxidant activity of raw, pasteurized and boiled buffalo's milk were 31.8, 31.5, and 30.4%, correspondingly. They also reported that pasteurization and boiling of milk did not have any significant effect on free radical scavenging activity of both cow and buffalo's milk. The difference in the antioxidant activity of different milk media could be attributed to the variation in the concentration of natural antioxidants present. Casein, whey, sulfur-containing amino acids, selenium, zinc, catalase, glutathione peroxidase, superoxide dismutase, α-tocopherol, ascorbic acid, carotenoids, and conjugated linoleic acid are some of the compounds that contribute to the inherent antioxidant activity of milk (Attaie *et al.*, 1996; Usta and Yilmaz-Erzan, 2013). In a study conducted by Ahmad *et al.* (2013), buffalo's milk exhibited a higher amount of ascorbic acid (3.66 mg/100mL) and α-tocopherol (5.5 mg/100mL) compared to a cow's with ascorbic acid and α-tocopherol content of 0.94 mg/100mL and 2.1 mg/100mL, respectively. Moreover, Ahmad *et al.* (2008) reported that buffalo's milk had a higher concentration of sulfur-containing amino acids, selenium, and zinc compared to cow's milk. This shows that the nature and composition of milk are considered significant factors in its antioxidant activity (Soleymanzadeh *et al.*, 2016).

The antioxidant activities of different milk media fermented with six potential probiotic LAB are shown in Table 3. All tested strains showed varying degrees of DPPH radical scavenging activity. The antioxidant activity of LAB strains when cow's milk was used as a substrate ranged from 64.68 to 79.07%. *L. paracasei* BIOTECH 10363 exhibited significantly higher ($P$<0.05) DPPH radical scavenging activity (79.07%) compared to other strains. At 64.68%, *L. casei* BIOTECH 1064 showed the lowest activity in cow's milk. When buffalo's milk was used, the antioxidant activities of LAB strains ranged from 71.16% to 77.06%. *L. paracasei* BIOTECH 10363 still displayed the highest antioxidant activity (77.06%), but it is not significantly different from the activity shown by *L. paracasei* BIOTECH 10371 (76.52%). When goat's milk was used as a substrate, *L. paracasei* BIOTECH 10369 and *L. paracasei* BIOTECH 10363 exhibited the highest DPPH-radical scavenging activity of 73.53% and 73.24%, respectively. At 68.58%, *L. casei* BIOTECH 1064 displayed the lowest

Table 3. Interactive effects of milk source and lactobacilli strains on antioxidant activity of fermented milk samples incubated at 37°C for 48 hours.

| Bacterial strains | Antioxidant Activity (%) | | |
|---|---|---|---|
| | Cow | Buffalo | Goat |
| *L. casei* BIOTECH 1064 | 64.68±0.41$^{i}$ | 71.16±0.25$^{g}$ | 68.59±0.04$^{h}$ |
| *L. paracasei* BIOTECH 10363 | 79.07±0.30$^{a}$ | 77.06±0.12$^{b}$ | 73.24±0.14$^{de}$ |
| *L. paracasei* BIOTECH 10371 | 70.49±0.86$^{g}$ | 76.52±0.01$^{b}$ | 70.43±1.09$^{g}$ |
| *L. paracasei* BIOTECH 10372 | 64.91±0.50$^{i}$ | 74.70±0.34$^{c}$ | 72.88±0.47$^{ef}$ |
| *L. paracasei paracasei* BIOTECH 10369 | 72.87±0.17$^{def}$ | 75.13±0.26$^{c}$ | 73.53±0.23$^{d}$ |
| *L. plantarum* 1074 | 70.73±0.12$^{g}$ | 72.40±0.26$^{f}$ | 72.69±0.89$^{ef}$ |

Data are presented as mean ± standard deviation.
$^{a\text{-}i}$Means with different superscript are significantly different ($P$<0.05).

activity in goat's milk. The results indicate the strain-specificity of LAB in generating antioxidative compounds during fermentation of different milk media.

The cow's milk fermented with *L. paracasei* BIOTECH 10363 displayed the highest DPPH radical scavenging activity (79.07%) among treatments. Bacterial cells, metabolic substances liberated from the cell or hydrolyzed milk components are known to result in increased antioxidant activity of fermented milk (Virtanen *et al.*, 2007). In this study, bacterial cells and casein were removed by centrifugation to obtain the water-soluble peptide extract. This suggests that the obtained antioxidant activity of fermented milk samples originates from cell lysis products, extracellular metabolites, or hydrolyzed milk components. The occurrence of these metabolites in fermented milk could be due to the hydrolysis of milk proteins by the proteolytic system of LAB. This proteolytic system is mainly comprised of one or more cell wall proteinases and a number of intracellular peptidases (Hafeez *et al.*, 2014). Through this system, it may produce a number of bioactive peptides that can promote various physiological functions in human health including antioxidative activities. Based on the results, cow's milk x *L. paracasei* BIOTECH 10363 combination was selected for the next phase of the study.

The overall probiotic viable count, titratable acidity, pH and antioxidant activity of cow's milk fermented with *L. paracasei* BIOTECH 10363 at different incubation temperatures (32, 37 and 42°C) were measured over a period of 72 hours. A one-way repeated measures ANOVA was used to compare the effect of incubation time on milk samples fermented at different temperatures. The results demonstrate that the mean viable count, titratable acidity, pH and antioxidant activity of cow's milk fermented at different temperatures differed significantly ($P$<0.05) between three time-points (24, 48 and 72 hours). At 37°C for 72 hours, *L. paracasei* BIOTECH 10363 displayed the highest viable count, titratable acidity and antioxidant activity and the lowest pH in cow's milk.

The optimum temperature for the growth of probiotic strains is at 37°C (Shortt, 1999). This coincides with the result of this study where the highest increase in the viable count of *L. paracasei* BIOTECH 10363 was obtained in cow's milk fermented at 37°C. Table 4 further illustrates that milk with *L. paracasei* BIOTECH 10363 incubated at 37°C displayed increasing viability with longer incubation time. A similar trend was observed

with samples incubated at 32°C, however, samples incubated at 42°C showed a decreasing trend in the viable probiotic count with longer incubation time. The highest viable count (10.31 log cfu/mL) was observed in milk samples fermented at 37°C for 72 hours. Similarly, the highest increase in the number of *L. acidophilus* La-5, a commercial probiotic strain, was achieved at 37°C with an increase in the cell number up to 0.48 log cycles (Khosravi-Darani *et al.*, 2015). It has been suggested that the fermentation temperature of fermented products containing probiotics should be at 37 to 40°C (Saarela *et al.*, 2000).

An increase in the growth of *L. paracasei* BIOTECH 10363 resulted in an increase in lactic acid content with a concurrent reduction in pH. This is expected since lactic acid is the major metabolite produced by the lactic acid bacteria as the product of carbohydrate metabolism. An appreciable increase in titratable acidity and a decrease in pH were noted in milk inoculated with *L. paracasei* BIOTECH 10363 at all given temperatures (Tables 5 and 6). The highest lactic acid content (1.08%) and the lowest pH value (3.83) were obtained in samples fermented at 37°C for 72 hours where the highest cell number of *L. paracasei* BIOTECH 10363 was also observed. On the other hand, the lowest lactic acid content and the highest pH values were observed in all milk samples fermented at 32°C where the viable probiotic count was also the lowest. Incubation temperatures lower than the optimal temperature cause a slower reduction in pH (De Brabandere and De Baerdemaeker, 1999). This may explain the slower rate of acid production observed in milk samples fermented at 32°C.

The occurrence of antioxidative peptides in fermented milk originates from the degradation of milk proteins by the proteinase of LAB in a process known as proteolysis. The extent of proteolysis appeared to be strain-specific and time-dependent. According to Agyei *et al.* (2013), the synthesis and activity of LAB proteinase are affected by fermentation conditions such as incubation temperature, extracellular pH, agitation, and the presence of oxygen. The effect of incubation temperature on the bioactivity of fermented milk has already been reported in previous studies. According to Rana and Bajaj (2015), the antimicrobial activity of *Lactobacillus* strains at an incubation temperature of 37°C was significantly higher than those incubated at 42°C. The authors concluded that the observed difference might be due to a higher growth rate at 37°C. This is in agreement with the result of this study where the highest antioxidant activity (84.23%) was observed in milk samples fermented at 37°C for 72 hours where the highest cell number of *L. paracasei* BIOTECH 10363 was also obtained. Table 7 further demonstrates that milk incubated at 37°C displayed an increasing antioxidant activity with longer incubation time. A similar trend was observed in samples incubated at 32°C, however, samples incubated at 42°C showed a decreasing trend with

Table 4. Viable count of *L. paracasei* BIOTECH 10363 in cow's milk fermented at different incubation time and temperature.

| Parameter | Incubation Temperature (°C) | Incubation Time (hours) | | |
|---|---|---|---|---|
| | | 24 | 48 | 72 |
| Viable Count (log cfu/mL) | 32 | 8.31±0.01$^{b}$ | 8.71±0.09$^{b}$ | 8.88±0.05$^{b}$ |
| | 37 | 8.68±0.06$^{b}$ | 9.06±0.02$^{a}$ | 10.31±0.06$^{a}$ |
| | 42 | 9.69±0.38$^{a}$ | 9.01±0.06$^{a}$ | 8.88±0.07$^{b}$ |

Data are presented as mean ± standard deviation.
$^{ab}$Means within column with different superscript are significantly different ($P$<0.05).

Table 5. Titratable acidity of cow's milk with *L. paracasei* BIOTECH 10363 fermented at different incubation time and temperature.

| Parameter | Incubation Temperature (°C) | Incubation Time (hours) | | |
|---|---|---|---|---|
| | | 24 | 48 | 72 |
| Acidity (% lactic acid) | 32 | 0.25±0.02$^{b}$ | 0.70±0.02$^{c}$ | 0.78±0.04$^{c}$ |
| | 37 | 0.40±0.02$^{a}$ | 0.87±0.03$^{a}$ | 1.08±0.02$^{a}$ |
| | 42 | 0.43±0.01$^{a}$ | 0.76±0.01$^{b}$ | 1.00±0.02$^{b}$ |

Data are presented as mean ± standard deviation.
$^{abc}$Means within column with different superscript are significantly different ($P$<0.05).

Table 6. pH of cow's milk with *L. paracasei* BIOTECH 10363 fermented at different incubation time and temperature.

| Parameter | Incubation Temperature (°C) | Incubation Time (hours) | | |
|---|---|---|---|---|
| | | 24 | 48 | 72 |
| pH | 32 | 5.58±0.02$^{a}$ | 4.27±0.02$^{a}$ | 3.96±0.01$^{a}$ |
| | 37 | 4.96±0.01$^{b}$ | 3.88±0.01$^{c}$ | 3.83±0.01$^{b}$ |
| | 42 | 4.86±0.04$^{c}$ | 4.01±0.02$^{b}$ | 3.87±0.01$^{c}$ |

Data are presented as mean ± standard deviation.
$^{abc}$Means within column with different superscript are significantly different ($P$<0.05).

Table 7. Antioxidant activity of cow's milk with *L. paracasei* BIOTECH 10363 fermented at different incubation time and temperature.

| Parameter | Incubation Temperature (°C) | Incubation Time (hours) | | |
|---|---|---|---|---|
| | | 24 | 48 | 72 |
| Antioxidant Activity (%) | 32 | 67.54±0.15$^{c}$ | 77.52±0.41$^{b}$ | 80.47±0.43$^{b}$ |
| | 37 | 71.09±0.25$^{b}$ | 78.99±0.74$^{a}$ | 84.23±0.52$^{a}$ |
| | 42 | 73.90±0.66$^{a}$ | 78.28±0.10$^{ab}$ | 77.72±0.48$^{c}$ |

Data are presented as mean ± standard deviation.
$^{abc}$ Means within column with different superscript are significantly different ($P$<0.05).

longer incubation period. The decrease in bioactivity could be attributed to thermal inactivation of biosystems at temperature higher than the optimum growth temperature of microorganisms (Agyei *et al.*, 2012). The findings reveal that the optimum incubation conditions for *L. paracasei* BIOTECH 10363 for maximum antioxidant activity in cow's milk were at 37°C for 72 hours.

This research may seem to be the first report that screens the antioxidant activity of cow's milk fermented with *L. paracasei* BIOTECH 10363. The results of the present study show that bacterial fermentation increased the antioxidant activity (DPPH radical scavenging activity) of the three media (cow, buffalo and goat's milk). The antioxidant

activity of fermented milk was influenced by milk source and LAB strains. Furthermore, incubation time and temperature significantly influenced the antioxidant activity, lactic acid content, viable count and pH of cow's milk fermented with *L. paracasei* BIOTECH 10363. Based on the findings of this study, *L. paracasei* BIOTECH 10363 could be used as a starter or adjunct culture for the production of different types of fermented dairy products with antioxidative properties. Further works, however, should be done to isolate, identify and quantify the compound responsible for the observed antioxidant activity. Conduct of other antioxidant analyses in milk samples is also recommended to significantly demonstrate the said activities. Moreover, studies can be done to evaluate the sensory quality and consumer acceptability of the fermented milk samples.

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