Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.067

RESEARCH ARTICLE

# Antioxidant properties of *Lactobacillus brevis* of Horse Origin and Commercial Lactic Acid Bacterial Strains: A Comparison

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ARTICLE HISTORY (18-122)

Received: April 04, 2018
Revised: May 27, 2018
Accepted: May 28, 2018
Published online: August 06, 2018

**Key words:**
Antioxidants
DPPH
*Lactobacillus brevis*
Reactive oxygen species
SOD

**ABSTRACT**

Oxidative stress due to assembly of excessive reactive oxygen species (ROS) is responsible for damage of biomolecules that may lead to cell death. Search of symbiotic bacteria with antioxidant properties is an active area of research for medical and veterinary practitioners. Present study is conducted to compare antioxidant and probiotic potential of four strains of *Lactobacillus brevis* (MG882399, MG882400, MG882401 and MG882402) with one reference strain of antioxidant property possessing bacteria, *L. acidophilus* ATCC 4356, and two commercial probiotic bacteria, *Bifidobacterium longum* BB536 and *L. rhamnosus* GG ATCC 53103. The antioxidant potential of intact cells, cell free supernatant (CFS) and cell lysate of all strains was investigated for scavenging of α-diphenyl-β-picrylhydrazyl, Superoxide dismutase, Hydroxyl radical and inhibition of lipid peroxidation. Cell lysate was noticed to possess least antioxidant activity while intact cells and CFS were found to show similar antioxidant potential. Among our strains *L. brevis* MG882402 was found superior in all tests and displayed better probiotic and antioxidant competence as compared to *B. longum*, *L. rhamnosus* and *L. acidophilus*. It was selected for further evaluation through *in vivo* procedures.



**To Cite This Article:** Noureen S, Riaz A, Saif A, Arshad M, Qamar MF and Arshad N, 2018. Antioxidant properties of *Lactobacillus brevis* of horse origin and commercial lactic acid bacterial strains: a comparison. Pak Vet J, 38(3): 306-310. http://dx.doi.org/10.29261/pakvetj/2018.067

## INTRODUCTION

ROS are produced during metabolism through partial reduction of oxygen. They are also produced by exogenous factors as radiation, X-ray exposure, tobacco smoke and ecological contamination (Ardestani and Yazdanparast, 2007). Oxidative metabolism is important for the existence, energy production and proper cell functioning (Poli *et al.*, 2004). While excessive ROS assembly in the body leads to oxidative stress. Oxidative stress is responsible for chain reactions that harms DNA, protein and lipids which lead to cell death and tissue necrosis. Antioxidants cease these chain reactions by oxidizing ROS (Adesulu *et al.*, 2018).

The adverse effect of ROS can be minimized naturally by defense mechanisms consisting of enzymatic antioxidants *viz.*, glutathione peroxidase (GPX), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT), along with non-enzymatic antioxidants including glutathione (GSH) and vitamins (Valko *et al.*, 2006). The antioxidant potential of some foods from animal and plant source *viz.*, milk, egg yolk, maize and have been extensively documented (Chen *et al.*, 2003; Davalos *et al.*, 2004).

Similarly, beneficial microbes are also known to possess antioxidative defense mechanism. The secretion of antioxidant metabolites from such strains provide stability to the strain and benefit to the host (Pascual *et al.*, 2008). Among such microbes, Lactic acid producing bacteria (LAB) are generally considered as safe food-grade microorganisms. They have various valuable properties such as antimicrobial, anti-cholesterol, antioxidant, anti-inflammatory, anti-tumorigenic (Mikelsaar *et al.*, 2004; Pascual *et al.*, 2008; Bukhari *et al*., 2017). New strains of LAB with novel functional properties are of interest to both health practitioners and the food industry. The antioxidative properties of LAB *in vitro* and *in vivo* has been reported by some authors (Stecchini *et al.*, 2001; Oh *et al.*, 2018). The species of *Lactobacillus* genera have best survival rate in the human intestinal tract

and provide good antioxidant activity by producing antioxidant metabolites including phenolic compounds (Kachouri *et al*., 2015). However, these properties show strain specificity therefore, more and more strains need to be characterized. The aim of this study was to compare antioxidant properties of four strains of *Lactobacillus* spp. isolated from horse fecal samples and compare their efficacy with one reference strain, *L. acidophilus* ATCC 4356 and two commercial probiotic strains, *L. rhamnosus GG* ATCC 53103 and *B. longum* BB536. In order to establish the location of antioxidant component we decided to use intact cell, cell free extract (CFS) and cell lysate for determining antioxidant property.

## MATERIALS AND METHODS

**Chemicals:** deMan- Rogosa and Sharpe (MRS) (Oxide), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich), $CaCO_3$ (Analar) and L-cystein (Aldrich), Ethanol (Aldrich), 1-10-phenanthroline (BioM), $FeSO_4$, Hydrogen peroxide (Sigma), Pyrogallol (BioM), Thiobarbituric acid (TBA) (Sigma), Sodium perborate (SPB) (Riedet-de-Haem), Tween 20 (Alfa Aesar), Trichloroacetic acid (TBA) (Analar), Butylated hydroxytoluene (BHT), Linoleic acid (Alfa Aesar).

**Strain identification:** Four strains of *Lactobacillus* spp. isolated from horse fecal samples were used in this study. All microbes were grown on MRS agar plates supplemented with $CaCO_3$ and L-cystein for 72 hours at 37°C for the conformation of their lactic acid property. They were subjected to gram staining, spore staining, motility and catalase activity. These strains were screened for probiotic properties through growth at different physical conditions (temperature and pH), and tolerance to NaCl, bile salt and lysozyme. Moreover., 16S rRNA gene sequencing was performed for their species identification. *L. acidophilus* (ATCC 4356), *B. longum* (BB 536) and *L. rhamnosus* (GG ATCC 53103) were purchased through local vender.

**Determination of antioxidant potential of bacterial strains preparation of cells, supernatant and cell lysate:** Strains were inoculated in sterile MRS broth (pH 6.5±0.2) and incubated at 37°C in shaking incubator (Irmeco) for 3 days. The supernatant was separated by centrifugation at 10,000 rpm for 10 minutes. The CFS was separated and kept at 4°C for further analysis. Cells were washed three times using PBS and were re-suspended in same solvent. The OD of suspension was adjusted to 1.00±0.03 at 600 nm corresponding to $10^9$ cfu/ml. The suspension was divided in two parts. One part was used as intact cell while other part was subjected to sonication for the preparation of cell lysate. Sonication was performed in ice-water bath for 60 minutes. Ultrasonic disrupted cells were separated by centrifugation for 10 min at 10,000 rpm and supernatant was used as cell lysate (Lin and Yen 1999).

**Assessment of radical scavenging capacity:** Antioxidant ability was determined *in vitro* by assessment of DPPH, superoxide anion, hydroxyl radical scavenging capacity and inhibition of lipid peroxidation following the protocols described by Lin and Chang (2000), Yaping *et al.* (2003), Wang *et al.* (2009) and Lin and Chang (2000) respectively. Tests were performed independently on cells, CFS and their cell lysates. Experiments were performed in triplicate from each sample. The antioxidant activity of strains was broadly divided into three categories: high (>60%), medium (50≥60%) and low (<50%).

**Statistical analysis:** All results are presented as a group Means±SEM. Comparison among isolates was performed using one-way ANOVA ($P<0.05$, significant) accompanied by Tukey's test. Data evaluation was accomplished in the statistical package "IBM SPSS 21.0 for Windows 8.1".

## RESULTS

**Morphological and probiotic characteristics of selected strains:** The strains were gram positive, non-motile, catalase negative and non-spore forming. These strains were able to grow on wide range of temperature (25 to 55ºC) and pH (2 to 8) and could tolerate bile salt (upto 2%) and Lysozyme (10 ppm). Similar characteristics were noticed in *L. acidophilus* ATCC 4356, *L. rhamnosus* GG ATCC 53103 and *B. longum* BB536. Our strains were identified as *L. brevis* on the basis of 16S rRNA gene sequencing, their NCBI accession numbers are MG882399, MG882400, MG882401 and MG882402. The strains were deposited in Fungal Culture Bank of Pakistan (FCBP), Institute of Agriculture Sciences, University of the Punjab, Lahore, for their availability to other researchers. The FCBP has assigned them stock No. FCBI-694 to 697. Differences among strains were observed while recording NaCl tolerance. *L. brevis* (MG882399 and MG882400) and *B. longum* BB536 (Reference strain) could not grow in the presence of 10% NaCl (Table 1).

**Table 1:** Comparison of biochemical and probiotic characteristic of field, reference and probiotic strains

| Strain | Gram Staining | Catalase | Motility | Sporulation | Tolerance | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Temperature (°C) | | | | PH | | | NaCl (%) | | Bile (%) | | Lys* |
| | | | | | 25 | 35 | 45 | 55 | 2 | 6 | 8 | 9 | 10 | 1 | 2 | |
| *L. brevis* MG882399 | + | - | - | - | + | + | + | + | + | + | + | + | - | + | + | + |
| *L. brevis* MG882400 | + | - | - | - | + | + | + | + | + | + | + | + | - | + | + | + |
| *L. brevis* MG882401 | + | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + |
| *L. brevis* MG882402 | + | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + |
| *B. longum* BB536 | + | - | - | - | + | + | + | + | + | + | + | + | - | + | + | + |
| *L. acidophilus* ATCC 4356 | + | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + |
| *L. rhamnosus GG* ATCC 53103 | + | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + |

Footnote: + (positive/growth), - (negative/no growth), *: Lysozyme (10ppm).

**Scavenging activity of DPPH radical:** Intact cells and CFS of all microbes were noticed to have higher DPPH activity as compared to cell lysate. Among the four field isolates, the intact cell of *L. brevis* MG882402 showed highest DPPH antioxidant activity. This strain was also better than reference and probiotic strains used in this study (*L. acidophilus* ATCC 4356, *B. longum* BB536 and *L. rhamnosus* GG ATCC 53103). Among ATCC strain *L. rhamnosus* GG ATCC 53103 displayed medium activity. In supernatant *L. brevis* MG882399 and MG882402 revealed highest DPPH scavenging activity. In cell lysate all strains displayed low activity. On the basis of good DPPH activity in extracellular matrix, our isolates could be arranged as *L. brevis* MG882402=*L. brevis* MG882399>*L. brevis* MG882400=*L. brevis* MG882401 (Table 2).

**Scavenging activity of Hydroxyl radical:** Intact cells of *L. brevis* (MG882399, MG882400 and MG882402) displayed high hydroxyl radical scavenging activity which was similar to one of our probiotic strains, *L. rhamnosus* GG ATCC 53103. Similar results were observed while analyzing CFS. The cell lysate of all field strains and reference strains was found to possess least hydroxyl radical scavenging activity. On the basis of best OH activity in intact cells and CFS, our isolates could be arranged as *L. brevis* MG882402=*L. brevis* MG882399= *L. brevis* MG882400>*L. brevis* MG882401 (Table 3).

**SOD radical scavenging activity:** Consistent with the results of DPPH and OH ion scavenging assays, the high SOD activity was observed in intact cells and supernatant (Table 4). The intact cells and cell free supernatant of *L. brevis* MG882402 was comparable with *B. longum* and *L. rhamnosus* GG ATCC 53103 respectively. In cell lysate SOD activity of all strains was low. On the basis of good SOD activity in supernatant, our isolates could be organized as *L. brevis* MG882402=*L. brevis* MG882401> *L. brevis* MG882399=*L. brevis* MG882400.

**Lipid peroxidation inhibition activity:** *L. brevis* MG882402 presented strongest inhibition of lipid peroxidation in intact cells as compared to other *L. brevis* isolates and reference strains. The supernatant of three strains of *L. brevis* MG882399, MG882400 and MG882402 displayed similar inhibition of lipid peroxidation which was graded as high. Among probiotic strains *L. rhamnosus* GG ATCC 53103 displayed highest activity. Comparable with above mentioned parameters, the cell lysate of all strains showed very low lipid peroxidation inhibition activity. On the basis of good inhibition of lipid peroxidation activity in CSF, our isolates were organized as *L. brevis* MG882402=*L. brevis* MG882400=*L. brevis* MG882399>*L. brevis* MG882401 (Table 5).

**Table 2:** DPPH radical scavenging activity (%)

| | DPPH Scavenging activity | | |
|---|---|---|---|
| Strains | Intact cell | Supernatant | Cell lysate |
| *L. brevis* MG882399 | 64.63±0.21$^{b}$ | 62.03±1.07$^{ab}$ | 17.91±1.41 |
| *L. brevis* MG882400 | 65.42±0.50$^{b}$ | 55.02±0.42$^{d}$ | 23.00±2.26 |
| *L. brevis* MG882401 | 61.74±0.43$^{c}$ | 56.17±0.57$^{cd}$ | 20.91±5.33 |
| *L. brevis* MG882402 | 74.30±0.07$^{a}$ | 62.98±1.93$^{a}$ | 26.26±2.05 |
| *B. longum* BB536 | 55.92±0.30$^{e}$ | 57.74±0.95$^{bcd}$ | 20.92±5.33 |
| *L. acidophilus* ATCC 4356 | 45.75±0.75$^{f}$ | 55.58±2.4$^{d}$ | 23.76±1.71 |
| *L. rhamnosus GG* ATCC 53103 | 58.10±0.88$^{d}$ | 60.32±.96$^{abc}$ | 18.81±2.23 |

Data are presented as Mean±SEM. Values having different superscript letter in same column are significantly different at P<0.05.

**Table 3:** Hydroxyl radical scavenging activity (%)

| | Hydroxyl radical scavenging activity | | |
|---|---|---|---|
| Strains | Intact cell | Supernatant | Cell lysate |
| *L. brevis* MG882399 | 60.28±3.56$^{ab}$ | 62.75±0.78$^{a}$ | 14.75±1.61$^{c}$ |
| *L. brevis* MG882400 | 62.64±1.25$^{ab}$ | 60.95±0.06$^{a}$ | 14.92±2.22$^{c}$ |
| *L. brevis* MG882401 | 53.26±0.84$^{b}$ | 57.17±0.66$^{ab}$ | 38.63±2.40$^{a}$ |
| *L. brevis* MG882402 | 66.43±8.20$^{a}$ | 62.20±0.62$^{a}$ | 40.16±0.84$^{a}$ |
| *B. longum* BB536 | 57.28±1.14$^{ab}$ | 54.85±2.51$^{b}$ | 24.70±2.503$^{b}$ |
| *L. acidophilus* ATCC 4356 | 58.02±1.42$^{ab}$ | 58.84±4.58$^{ab}$ | 43.21±5.70$^{a}$ |
| *L. rhamnosus GG* ATCC 53103 | 61.78±1.50$^{ab}$ | 60.23±0.49$^{ab}$ | 10.52±0.52$^{c}$ |

Data are presented as Mean±SEM. Values having different superscript letter in same column are significantly different at P<0.05.

**Table 4:** Superoxide dismutase scavenging activity (%)

| | Superoxide dismutase scavenging activity | | |
|---|---|---|---|
| Strains | Intact Cells | Supernatant | Cell lysate |
| *L. brevis* MG882399 | 51.56±3.15$^{bc}$ | 42.01±3.42$^{c}$ | 35.51±2.33$^{c}$ |
| *L. brevis* MG882400 | 45.50±2.57$^{c}$ | 41.74±0.75$^{c}$ | 40.34±0.68$^{b}$ |
| *L. brevis* MG882401 | 52.63±2.75$^{bc}$ | 55.45±0.49$^{ab}$ | 46.34±0.30$^{a}$ |
| *L. brevis* MG882402 | 66.24±3.23$^{a}$ | 61.17±6.00$^{a}$ | 48.81±1.23$^{a}$ |
| *B. longum* BB536 | 58.79±2.79$^{ab}$ | 50.37±40.0$^{bc}$ | 46.56±0.89$^{a}$ |
| *L. acidophilus* ATCC 4356 | 50.23±1.72$^{bc}$ | 43.47±2.05$^{c}$ | 29.57±1.01$^{d}$ |
| *L. rhamnosus GG* ATCC 53103 | 53.80±3.21$^{bc}$ | 56.24±0.81$^{ab}$ | 27.18±0.43$^{d}$ |

Foot note: Data are presented as Mean±SEM. Values having different superscript letter in same column are significantly different at P<0.05.

**Table 5:** Lipid peroxidation inhibition (%)

| | Inhibition of lipid peroxidation | | |
|---|---|---|---|
| Strains | Intact cells | Supernatant | Cell lysate |
| *L. brevis* MG882399 | 57.44±0.55$^{bc}$ | 60.95±0.06$^{ab}$ | 14.76±1.61$^{c}$ |
| *L. brevis* MG882400 | 58.85±1.16$^{ab}$ | 62.75±0.78$^{a}$ | 15.95±2.32$^{c}$ |
| *L. brevis* MG882401 | 51.92±1.88$^{c}$ | 57.16±0.66$^{b}$ | 37.63±2.38$^{a}$ |
| *L. brevis* MG882402 | 64.44±0.05$^{a}$ | 63.19±0.53$^{a}$ | 40.59±0.48$^{a}$ |
| *B. longum* BB536 | 56.23±3.70$^{bc}$ | 44.85±2.50$^{c}$ | 24.70±2.50$^{b}$ |
| *L. acidophilus* ATCC 4356 | 31.85±0.99$^{d}$ | 35.39±1.47$^{d}$ | 44.15±2.04$^{a}$ |
| *L. rhamnosus GG* ATCC 53103 | 58.95±1.42$^{ab}$ | 60.23±0.49$^{ab}$ | 10.52±0.51$^{c}$ |

Data are presented as Mean±SEM. Values having different superscript letter in same column are significantly different at P<0.05.

## DISCUSSION

Free radical scavenging activity of an antioxidant is crucial due to the deleterious nature of ROS. Although almost all organisms possess antioxidant defense and repair system, however, under certain conditions this system is unable to prevent the entire damage caused by ROS (Pham *et al.*, 2008). Therefore, exogenous antioxidant is frequently supplemented for health benefits. However, simulated antioxidants e.g., butylated hydroxyl anisole and butylated hydroxyl toluene have been reported to show cytotoxicity (Son *et al.*, 2018). Hence more attention is being paid to find safer antioxidant from natural sources (Das and Goyal. 2015; Leite *et al.*, 2015). Beneficial microbes could be a sources of natural antioxidant. Lactic acid bacteria *viz*., *B. longum, L. rhamnosus, L. brevis* and *L. acidophilus* present in intestinal microbial ecosystem are reported to help in the promotion of host health. Nevertheless, their effects are strain specific (Wouters *et al.*, 2013). The main features determining existence of such bacteria in the intestine include their acid and bile tolerance and competition with microbial flora (probiotic properties). Regarding antioxidant ability, the beneficial microbes offer health benefits through (i) secretion/release of intra or extracellular metabolites of antioxidant nature, or (ii) by directly playing role in scavenging reactive oxygen sp.

Current study describes antioxidant potential of four strains of *L. brevis* (MG882399, MG882400, MG882401 and MG882402), isolated from fecal samples of horse, and their comparison with *L. acidophilus* ATCC 4356, *L. rhamnosus GG* ATCC 53103 and *B. longum* BB536. *L. acidophilus* ATCC 4356 is the reference strain with known antioxidant potential while *L. rhamnosus GG* ATCC 53103 and *B. longum* BB536 are commercial probiotics.

Probiotics are usually given orally, therefore the strains must have the ability to survive passage through stomach and duodenum where pH is low and bile is added. Therefore, resistance to the low pH, tolerance to bile salt are considered important properties of beneficial microbes (Yadav *et al.*, 2016; Khan *et al.*, 2018). All the isolated strains were able to tolerate pH (02-08) and presence of bile salt (0.5-2.0%) even for 24 hours of incubation at 37°C. NaCl is an inhibitory substance which inhibit growth of many types of bacteria, therefore high salt tolerance is considered as a criteria of probiotic property. So, it was essential to test the NaCl tolerance of the isolates (Menconi *et al.*, 2014). Hoque *et al.* (2010) observed the NaCl (1-9%) tolerance of their *Lactobacillus* spp. isolated from yoghurts. The strain *L. brevis* MG882402 could survive at 10% NaCl concentration. Similarly, probiotic microorganisms as well as their products, are influenced by different enzymes while passing through the intestine. Lysozyme is one of these enzymes which can affect survival of microorganisms. The beneficial microbes should be able to resist these conditions. In order to check resistance of *L. brevis* to this enzyme they were exposed to grow in the presence of lysozyme (10 ppm). All the isolates, used in this study, were found lysozyme tolerant indicating their ability to stay in intestinal environment. Our findings are consistent with Zamani (2016) who reported lysozyme tolerance in *L. plantarum* upto 10ppm. All these data indicate that our strains may survive the passage and stay in intestinal environment.

Later on, antioxidant activity was determined in intact cells, CFS and in cell lysate. DPPH is a free radical, its scavenging activity is widely used to check the antioxidant potential of natural products. (Yang and Guo, 2008). In our findings *L. brevis* MG882402 was noticed to show highest DPPH scavenging activity in intact cell followed by CSF. The CSF of *L. brevis* MG882402 and *L. brevis* MG882499 and *L. rhamnosus GG* ATCC 53103 were having similar DPPH activity. The commercial strains displayed lower level of DPPH activity in cells. Among commercial strains best activity was noticed by *L. rhamnosus GG* ATCC 53103. The better antioxidant activity of *L. brevis* MG882402 might be attributed by the cell surface proteins and extracellular metabolites (peptides) released by bacterial strain in medium (Li *et al.*, 2012). These findings are consistent with Shen *et al.* (2011) who reported *B. animals* had the highest radical-scavenging activity (73.11%) in supernatant. Intact cells were also showing high activity. Ou *et al.* (2009) reported 71.3% DPPH activity of *B. longum* in intact cell. Lin and Chang (2000) investigated *L. acidophilus* ATCC 4356 43.2% and 20.8% in intact cell and cell lysate respectively.

The hydroxyl radicals are very reactive free radicals, contribute in the beginning of lipid peroxidation and react with other molecule in living cells. Some LAB strains, such as *Streptococcus thermophilus* 821, *B. longum* 15708 and *L. casei* KCTC 3260, have already been reported to possess capability of removing transition metal ions that might otherwise participate in hydroxyl-radical generation (Lee *et al.*, 2005; Li *et al.*, 2012). In this study among the field strains intact cells of all strains except *L. brevis* MG882401 displayed similar OH scavenging activity suggesting that level of OH radical scavenging factors is comparable in these strains. Consistently, OH radical scavenging activity in CFS of our strains was similar to that in *L. acidophilus* ATCC 4356 and *L. rhamnosus GG* ATCC 53103 while it was low in *B. longum* 15708 suggesting the superiority of our strain over commercial probiotic strain. Our results are consistent with Kim *et al.* (2006) and Shen *et al.* (2011) who reported OH ion scavenging activity of *L. acidophilus* KCTC 3111 in intact cell and *B. animals* 01 in both supernatant and cell lysate.

Superoxide radicals are formed in the mitochondria as a byproduct of electron transport chain reactions and are dangerous to the cell (Kim *et al.*, 2006). The superoxide dismutase enzyme can stop this process. Like DPPH, intact cells of *L. brevis* MG882402 showed highest superoxide scavenging activity which was comparable with *B. longum* BB 536. The supernatant of *L. brevis* MG882401 and *L. brevis* MG882402 had highest SOD activity which was comparable to *L. rhamnosus* GG ATCC 53103. Other strains were found ≤50% SOD activity. High efficiency of *L. brevis* MG882401, MG882402 could be due to the more availability or quantity of antioxidative enzymes in CSF and their amount on cell surface. These findings further strengthen our view that MG882402 is superior to other strains used in the study. Wang *et al.* (2009) reported *L. fermentum* with 80.56% superoxide anion scavenging capacity.

During oxidative stress lipids are main targets by free radicals that initiate lipid peroxidation which decrease the membrane fluidity by altering membrane properties (Aruoma, 1998). Current study revealed that *L. brevis* MG882400 and *L. brevis* MG882402 exhibited the highest inhibition of lipid peroxidation in both intact cell and supernatant which was equivalent to *L. rhamnosus GG* ATCC 53103. While other reference and probiotic strains display lower lipid peroxidation potential, demonstrating that both intact cells and extracellular cell-free extracts of *L. brevis* MG882400 and *L. brevis* MG882402 were effective in impeding the oxidative damage caused by $H_2O_2$. However, the CSF exhibited a greater inhibitory effect on comparison with the intact cells and cell lysate. It suggests that the high concentration of antioxidant components is released by the strain, which could be later purified for use. Other researchers also reported lipid peroxidation inhibition activity of *L. acidophilus* ATCC 4356, *L. fermentum* E-3, *L. acidophilus* KCTC 3111 in intact cell and cell lysate (Lin and Chang, 2000; Kim *et al.*, 2006). In contrast to other researchers we could not find good antioxidant activity in cell lysate of any strain.

In culmination, strains of *L. brevis* MG882399, MG882400, MG882401 and MG882402 were found to have antioxidant and probiotic potential. *L. brevis*

MG882402 displayed highest antioxidant potential in all radical scavenging assays and was graded as best one. Further *in vivo* trials targeting on probiotic application of *L. brevis* MG882402 are recommended.

**Acknowledgments:** This work sponsored by HEC indigenous fellowship 2012, 17-5(2Bm1-438) grant.

**Authors contribution:** Planning, designing and supervising of study: NA and MA; Experimentation: SA, AR and AS; Data analysis: NA and SA; Manuscript drafting: SA, AR and MFQ. All authors read and approved the final manuscript.

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