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NOTE

Comparative evaluation of selected strains of lactobacilli for the development of antioxidant activity in milk

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Abstract Species of *Lactobacillus* are widely used as starter cultures in the dairy industry and have been reported to play a significant role in the production of bioactive peptides in fermented dairy products. The aim of the present study was to screen selected strains of *Lactobacillus* for their ability to generate antioxidant activity during the fermentation of milk and to correlate their proteolytic activity with antioxidative peptide production. Skim milk fermented with 19 strains of *Lactobacillus* from ten species was evaluated for antioxidant activity by 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) decolourization assay. The whey fraction of fermented skim milk showed radical scavenging activity ranging from 0.12 to 0.49 mmol·L^{-1} Trolox equivalent antioxidant capacity. The proteolytic activity of the different strains ranged from 0.40 to 2.25 mmol·Leu·L^{-1}. Seven strains including *Lactobacillus delbrueckii* subsp. *bulgaricus* NCDC08, *Lactobacillus plantarum* NCDC25, *Lactobacillus casei* subsp. *casei* NCDC17, *Lactobacillus rhamnosus* NCDC24, *Lactobacillus paracasei* subsp. *paracasei* NCDC63, *Lactobacillus fermentum* NCDC141 and *Lactobacillus helveticus* NCDC288 exhibited a positive correlation between antioxidant and proteolytic activity. On hydrolysis of casein with cell-free extract from these seven strains, *L. rhamnosus* NCDC24, *L. casei* subsp. *casei* NCDC17 and *L. paracasei* subsp. *paracasei* NCDC63 showed a significant ($P<0.05$) increase in radical scavenging activity. The low molecular weight peptides (<3 kg·mol^{-1}) represented 60–70% of the total radical scavenging activity for these three strains. The development of antioxidant activity was found to be strain specific and that these strains of

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Lactobacillus may be useful in developing functional foods to combat oxidative stress.

比较和评价乳杆菌菌株在发酵乳制品中的抗氧化活性

摘要 乳酸菌作为发酵剂广泛地应用于乳品工业，而且乳酸菌对发酵乳制品中生物活性肽的产生起到了关键的作用。本文对筛选的乳酸菌在乳发酵过程中产生抗氧化物质的活性以及蛋白水解活性与抗氧化肽产物的相关性进行了研究。用来源于10个种的19株乳杆菌发酵脱脂乳，采用2,2'-二氮-双(3-乙基苯并噻唑-6-磺酸)(ABTS)褪色法测定这些菌株的抗氧化活性。发酵脱脂乳乳清部分的清除自由基活性为0.12~0.49 mmol·L^{-1} Trolox当量的抗氧化能力(TEAC)；不同菌株蛋白水解活性为0.40~2.25 mmol·Leu·L^{-1}。德氏乳杆菌保加利亚亚种NCDC08(*Lactobacillus delbrueckii* subsp. *bulgaricus* NCDC08)、植物乳杆菌NCDC25(*Lactobacillus plantarum* NCDC25)、干酪乳杆菌干酪亚种NCDC17(*Lactobacillus casei* subsp. *casei* NCDC17)、鼠李糖乳杆菌NCDC24(*Lactobacillus rhamnosus* NCDC24)、副干酪乳杆菌副干酪亚种NCDC63(*Lactobacillus paracasei* subsp. *paracasei* NCDC63)、发酵乳杆菌NCDC141(*Lactobacillus fermentum* NCDC141)和瑞士乳杆菌NCDC288(*Lactobacillus helveticus* NCDC288)七株菌发酵产物的抗氧化能力和蛋白水解活性呈正相关。用这7株菌的无细胞抽提物来水解酪蛋白，发现鼠李糖乳杆菌NCDC24(*L. rhamnosus* NCDC24)、干酪乳杆菌干酪亚种NCDC17(*L. casei* subsp. *casei* NCDC17)和副干酪乳杆菌副干酪亚种NCDC63(*L. paracasei* subsp. *paracasei* NCDC63)这三株菌水解产物的自由基清除能力显著地增加($p<0.05$),而且在这些具有自由基清除活性的水解物中60%~70%为小分子量的肽(<3 kg·mol^{-1})。因此，乳杆菌菌株抗氧化活性的发现有益于开发具有潜在抗氧化特性的功能食品。

Keywords *Lactobacillus* · Skim milk · ABTS · Whey · Cell-free extract

关键词 乳酸菌 · 抗氧化活性 · 蛋白水解活性 · 无细胞抽提物

1 Introduction

There is strong evidence that reactive oxygen species (ROS) and free radicals play an important role in many degenerative diseases such as cancer, atherosclerosis and diabetes (Beckman and Ames 1998). Foods containing antioxidative materials may be useful for the prevention of these diseases. Formation of free radicals, such as super-oxide anion radical and hydroxyl radical, is an unavoidable consequence in aerobic organisms during respiration. These radicals are very unstable and react rapidly with other groups or substances in the body, leading to cellular and tissue damage. The body has its own defence system against ROS which utilizes antioxidant compounds and enzymes. These defence systems are not effective enough to totally prevent the damage. Therefore, food supplements containing antioxidants may be used to help the human body to reduce oxidative damage (Oxman et al. 2000; Kullisaar et al. 2003).

Species of *Lactobacillus* are widely used as starter cultures in fermented dairy products and have been associated with health-related properties such as probiotics. Among the range of bioactivities from lactobacilli, recent research has also demonstrated their antioxidant properties (Osuntoki and Korie 2010; Qian et al. 2011; Virtanen et al. 2007).

A k-casein derived peptide with 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity was found in milk fermented with *Lactobacillus delbruckii* subsp.

bulgaricus (Kudoh et al. 2001). Farvin et al. (2010) identified antioxidant peptides from yogurt, which comprised of a few N terminal fragments of α_{s1}-, α_{s2}- and k-casein and several fragments from β-casein.

There are only limited studies on antioxidant properties of fermented milk. The objectives of the present study were to evaluate the antioxidant activity of skim milk during fermentation with selected lactobacilli and to assess their potential towards the generation of antioxidative peptides.

2 Material and methods

2.1 Bacterial strains and cultivation media

A total of 19 *Lactobacillus* strains were procured from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal. The strains included *Lactobacillus brevis* (NCDC01), two *Lactobacillus delbrueckii* subsp. *bulgaricus* (NCDC08, 293), four *Lactobacillus acidophilus* (NCDC14, 15, 195, 340), *Lactobacillus casei* subsp. *casei* (NCDC17), two *Lactobacillus casei* (NCDC297, 298), two *Lactobacillus paracasei* subsp. *paracasei* (NCDC 22, 63), three *Lactobacillus fermentum* (NCDC 141, 156, 214), *Lactobacillus rhamnosus* (NCDC 24), *Lactobacillus plantarum* (NCDC25) and two *Lactobacillus helveticus* (NCDC 288, 292). The cultures were propagated in sterile de Man–Rogosa–Sharpe (MRS) broth. One set of cultures was stored at −80 °C in MRS broth containing 20% glycerol. Each strain was subcultured twice in MRS broth and incubated at 37 °C for 24 h before use.

2.2 Culturing of lactobacilli

Each strain of *Lactobacillus* was first precultured (1%, *v*/*v*) in sterilized (121 °C for 15 min) skim milk at 37 °C for 24 h. These cultures were then used for fermentation of fresh pasteurized skim milk for 24 h at 37 °C corresponding to cell count 10^7–10^8 CFU.mL^{-1}. Simultaneously, an uninoculated sterilized skim milk was also incubated under similar conditions as control. For each strain, the experiment was conducted in triplicate. Aliquots were drawn after 24 h of fermentation for measurement of proteolytic and antioxidant activities.

2.3 Proteolytic activity

Proteolytic activity of fermented milk samples was measured using orthopthaldehyde (OPA) method (Church et al. 1983, 1985). To 2.5 mL fermented milk, 0.5 mL distilled water and 0.5 mL of 0.72 mol·L^{-1} trichloroacetic acid (TCA) were added. After 10 min, the solution was filtered, using Whatman no. 4 filter paper. To 3 mL OPA reagent, 150 μL of sample (TCA filtrate) was added and incubated for 2 min at room temperature; the absorbance was measured at 340 nm using dual beam spectrophotometer (Specord 200, Analytik Jena AG, Germany). A calibration curve of leucine (0–2.5 mmol·L^{-1}) was prepared, and the results were expressed as millimoles per litre leucine.

2.4 Antioxidant activity

Whey fraction from fermented skim milk/control milk was obtained by adjusting the pH to 4.6 using 1.0 mol·L^{-1} hydrochloric acid (HCl) and centrifugation at 10,000 rpm for 10 min at 4 °C (Kubota 6800, Tokyo, Japan). The clear supernatant was passed through polyvinylidene fluoride (PVDF) 0.45 μm filter and used for measurement of antioxidant activity.

Antioxidant activity of samples was measured using the 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) spectrophotometric decolourization method described by Re et al. (1999). To 5 mL ABTS stock solution (5 mmol·L^{-1}), 88 μL of 140 mmol·L^{-1} potassium persulphate was added. The mixture was stored at ambient temperature for 12–16 h. The solution of $ABTS^{\cdot+}$ was diluted with phosphate buffer saline pH 7.4 to the final absorbance of 0.70±0.02 at 734 nm (Specord 200 spectrophotometer, Analytik Jena AG, Germany). Thirty microlitres of sample was added to 3 mL of diluted $ABTS^{\cdot+}$ solution. This mixture was shaken for 10 s at 30 °C, and the decrease in the absorbance was recorded at 734 nm for 6 min. Appropriate solvent blanks were run in each assay. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used as a reference standard. The percentage inhibition was calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data. The antioxidant activity of sample was expressed in terms millimoles per litre of Trolox equivalent antioxidant capacity (TEAC).

$$\text{Inhibition}\,\% = \frac{\left(A_{734\text{control}} - A_{734\text{sample}}\right)}{A_{734\text{control}}} \times 100$$

2.5 Preparation of cell-free extract

In order to explore the contribution of antioxidant activity due to intracellular proteases and peptidases, cell-free extract of seven proteolytic strains: *L. delbrueckii* subsp. *bulgaricus* NCDC08, *L. plantarum* NCDC25, *L. rhamnosus* NCDC24 casei subsp. *casei* NCDC17, *L. paracasei* subsp. *paracasei* NCDC63, *L. fermentum* NCDC141 and *L. helveticus* NCDC288 were prepared following the method of Pan et al. (2005). The cultures were grown in MRS media at 37 °C for 24 h. Cells were harvested by centrifugation at 10,000 rpm for 20 min at 4 °C and washed three times with 50 mmol·L^{-1} Tris–HCl buffer (pH 7.0). Washed cells were suspended in same buffer and ultrasonicated (Sonicator, VCX 750, Sonics and Materials Inc., Newtown, CT, USA) with glass beads (0.3 mm diameter) intermittently for 30 min (holding for 5-min interval after each 5-min exposure). The temperature was maintained around 10 °C by keeping the cell suspension in ice bath. The cell debris and glass beads were removed by centrifugation at 12,000 rpm for 15 min at 4 °C. The clear supernatant of CFE was freeze-dried (Ecospin3180C, Hanil Science International); assessed for the soluble protein content and proteinase activity, by dissolving in 50 mmol·L^{-1} Tris–HCl buffer pH 6.5 (10 mg.mL^{-1}) and used as crude enzyme preparation for hydrolysis of casein.

2.6 Protein quantification

The protein content of the cell-free extract was estimated following the method of Smith et al. (1985) using bicinchoninic acid (BCA) reagent. To 3 mL of BCA reagent, 150 μL of appropriately diluted sample was added. The reaction mixture was incubated at 37 °C for 30 min, and the absorbance was measured at 570 nm (Specord 200 spectrophotometer, Analytik Jena AG, Germany). A standard curve of bovine serum albumin (50–250 $\mu g \cdot mL^{-1}$) was prepared.

2.7 Determination of proteinase activity

Proteinase activity of cell-free extract was determined according to Twing (1984) with some modifications. The reaction mixture contained 20 μL of 0.4% fluorescein isothiocyanate casein and 30 μL of cell-free extract in 50 $mmol \cdot L^{-1}$ Tris–HCl buffer pH 6.5. The reaction mixture was incubated at 37 °C for 60 min, then 120 μL of 5% TCA was added and the mixture was centrifuged at 14,000 rpm for 10 min. Sixty microlitres of the supernatant was neutralized with 3 mL of 500 $mmol \cdot L^{-1}$ Tris–HCl buffer (pH 8.5), and the fluorescence was measured using a spectrofluorometer (Varian, SF330, Cary Eclipse, India) with an excitation wavelength of 490 nm and an emission wavelength of 525 nm. One unit of proteolytic activity was defined as the amount of cell-free extract yielding 1% of total initial casein fluorescence as TCA-soluble fluorescence after 60 min of hydrolysis and was expressed as units per milligram protein.

2.8 Hydrolysis of casein

Bovine casein was prepared by isoelectric precipitation of skim milk at pH 4.6. The precipitated casein was recovered and washed with distilled water. The pH of the wet casein was raised to 7.0 with NaOH (1.0 $mol \cdot L^{-1}$) solution with continuous stirring using a magnetic stirrer, and the sodium caseinate preparation was freeze-dried. Twenty-five millilitres of 2.5% sodium caseinate was incubated with cell-free extract in the (*E*/*S*) ratio 1:1 (*w*/*w*) for 12 h at 37 °C. The resulting mixture was heated at 90 °C for 5 min, and pH was adjusted to 4.6 with 5.5 $mol \cdot L^{-1}$ lactic acid. Precipitated casein was removed by centrifugation at 10,000 rpm for 10 min. The clear supernatant was collected; the pH was adjusted to 7.0 and then filtered through PVDF 0.45 μm filter. The clear supernatant (4 mL) was subjected to ultrafiltration (3 $kg \cdot mol^{-1}$ cutoff) using Amicon ultracentrifugal filter device (Amicon Ultra-4, Millipore, India). The tubes were centrifuged at 7,000 rpm for 30 min at 4 °C, and the permeate fraction was collected. Antioxidant activity of hydrolysate and permeate fraction was measured for sample treated with cell-free extract from each of the seven selected strains as earlier described in this paper.

2.9 Statistical analysis

The results were expressed as mean±SD values. Statistical analysis of the results was performed by calculating Pearson correlation coefficient (*r*) for

proteolytic activity vs. antioxidant activity. One-way analysis of variance was applied for the antioxidant activity of hydrolysate and ultrafiltered permeate based on Snedecor and Cochran (1994). This was done to test for any significant difference ($P<0.05$) in the mean value of antioxidant activity between the strains.

3 Results

3.1 Antioxidant activity of lactobacilli

The radical scavenging activity of whey fraction of skimmed milk fermented for 24 h at 37 °C using 19 strains of lactobacilli from ten species is presented in Table 1. The antioxidant activity was measured by the ability of whey to scavenge $ABTS^{\cdot+}$ cation radicals. In this method, the extent of scavenging of a preformed free radical relative to that of a standard antioxidant compound (Trolox, an analogue of Vitamin E) is determined. The antioxidant activity of the different lactobacilli varied between 0.12 and 0.49 mmol·L^{-1} (TEAC) (after deducting for the endogenous activity of unfermented skimmed milk). *L. rhamnosus* NCDC24

Table 1 Proteolytic and antioxidant activity of lactobacilli

Strains	Proteolytic activity[a] (mmol·L^{-1} Leu)	Antioxidant activity[a] (mmol·L^{-1}) (TEAC)	Correlation coefficient (r)[b]
L. brevis NCDC01	0.40±0.02	0.13±0.01	0.50
L. delbrueckii subsp. *bulgaricus* NCDC08	1.84±0.35	0.44±0.12	0.94
L. delbrueckii subsp. bulgaricus NCDC293	1.49±0.09	0.36±0.05	0.80
L. acidophilus NCDC14	1.62±0.22	0.23±0.02	0.79
L. acidophilus NCDC15	1.66±0.28	0.25±0.02	0.82
L. acidophilus NCDC340	1.25±0.05	0.21±0.01	0.81
L. acidophilus NCDC195	1.00±0.03	0.34±0.04	0.50
L. casei subsp. casei NCDC17	1.93±0.40	0.26±0.01	0.99
L. paracasei subsp. *paracasei* NCDC22	0.73±0.04	0.12±0.01	0.52
L. paracasei subsp. *paracasei* NCDC63	1.92±0.08	0.35±0.03	0.98
L. plantarum NCDC25	2.02±0.10	0.24±0.04	0.99
L. rhamnosus NCDC24	2.25±0.09	0.49±0.07	0.99
L. casei NCDC297	1.45±0.07	0.23±0.01	0.76
L. casei NCDC298	1.47±0.18	0.14±0.01	0.72
L. fermentum NCDC141	1.66±0.16	0.27±0.01	0.93
L. fermentum NCDC156	1.25±0.08	0.29±0.05	0.60
L. fermentum NCDC214	0.96±0.07	0.14±0.02	0.80
L. helveticus NCDC288	1.70±0.05	0.32±0.11	0.96
L. helveticus NCDC292	0.89±0.16	0.31±0.07	0.78

[a] Mean±SD ($n=3$)

[b] Proteolytic activity vs. antioxidant activity

showed the highest radical scavenging activity, followed by *L. delbrueckii* subsp. *bulgaricus* NCDC08, while *L. paracasei* subsp. *paracasei* NCDC22 showed the least antioxidant activity. The antioxidant activity ranged between 0.31 and 0.36 mmol·L^{-1} (TEAC) for five strains (*L. helveticus* NCDC288, 292; *L. acidophilus* NCDC195; *L. delbrueckii* subsp. *bulgaricus* NCDC08; *L. paracasei* subsp. *paracasei* NCDC63).

3.2 Proteolytic activity of lactobacilli

Proteolytic activity of different lactobacilli during fermentation of skim milk for 24 h at 37 °C is presented in Table 1. Among the 19 strains of lactobacilli, the proteolytic activity measured using the OPA method ranged between 0.40 and 2.25 mmol·L^{-1}. Among the different species of *Lactobacillus*, the proteolytic activity of *L. rhamnosus* NCDC24 was the highest while *L. brevis* NCDC01 exhibited the lowest proteolytic activity. The proteolytic activity of the other strains ranged from 1.45 to 2.02 mmol·L^{-1} leucine, with *L. plantarum* NCDC25 exhibiting relatively higher proteolytic activity than *L. helveticus* (NCDC288, 292).

3.3 Correlation of antioxidant and proteolytic activity

The antioxidant activity of all the strains of *Lactobacillus* assessed increased with an increase in the level of proteolysis. Most of the strains showed a positive correlation coefficient (r) (0.72–0.99) between proteolytic activity and radical scavenging activity (Table 1). Among them, seven strains (*L. delbrueckii* subsp. *bulgaricus* NCDC08, *L. plantarum* NCDC25, *L. casei* subsp. *casei* NCDC17, *L. rhamnosus* NCDC24, *L. paracasei* subsp. *paracasei* NCDC63, *L. fermentum* NCDC141 and *L. helveticus* NCDC288) exhibited a high positive correlation coefficient (r>0.90). However, *L. brevis* NCDC01 showed a low proteolytic activity of 0.40 mmol·L^{-1} leucine with the antioxidant activity corresponding to 0.13 mmol·L^{-1} Trolox equivalents, whereas *L. casei* (NCDC298) showed a high proteolytic activity with relatively low antioxidant activity.

3.4 Hydrolysis of casein

The total proteinase activity of cell-free extract from seven selected strains of *Lactobacillus* ranged from 2.25 to 5.66 U.mg^{-1} protein (Fig. 1). The proteolytic activity was observed to be the lowest for *L. plantarum* NCDC25 while the highest for *L. helveticus* NCDC288. The radical scavenging activity of casein hydrolysates produced by cell-free extracts of potential lactobacilli ranged from 0.98 to 1.62 mmol·L^{-1} Trolox (Fig. 2). The total antioxidant activity of *L. rhamnosus* NCDC24, *L. casei* subsp. *casei* NCDC17 and *L. paracasei* subsp. *paracasei* NCDC63 was significantly higher (P<0.05) than other strains. Furthermore, antioxidant activity of ultrafiltered permeates (3 kg·mol^{-1} cutoff) for these three strains represented 60–70% of total antioxidant activity, mainly due to the casein breakdown peptides. On hydrolysis with cell-free extract, a 3–4-fold increase in antioxidant activity was observed as compared to fermentation with intact cells.

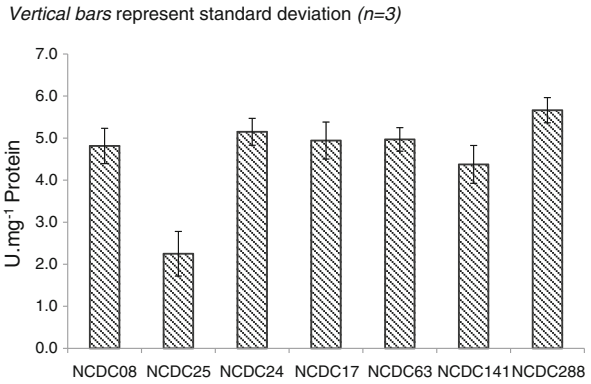

Fig. 1 Proteolytic activity of cell-free extract of selected lactobacilli

4 Discussion

In the present study, 19 strains from ten species of *Lactobacillus* were assessed for antioxidant activity during the fermentation of skim milk. All the strains showed radical scavenging activity from 0.12 to 0.49 mmol·L^{-1} (TEAC). As the bacterial cells and casein were removed from the sample, the observed radical scavenging activity may be attributed to extracellular metabolic components, products of cell lysis or hydrolyzed milk proteins (Kumar et al. 2010).

Based on proteinase activity measurement for CFE, lower proteolytic activity of *L. plantarum* NCDC25 was observed compared to *L. helveticus* NCDC288, which is in accordance with observations of Sasaki et al. (1995). However, on fermentation of milk at 37 °C for 24 h, the higher proteolytic activity observed for *L. plantarum* NCDC25 compared to *L. helveticus* NCDC288 may be due to the relative slower growth rate of *L. helveticus* NCDC288 (data not shown) at the prevailing temperature. Recently, Kholif et al. (2011) also showed similar results with greater proteolytic activity of *L. plantarum* NRRL B4004 than *L. helveticus* CNRZ 593N during growth for 24 h at 37 °C.

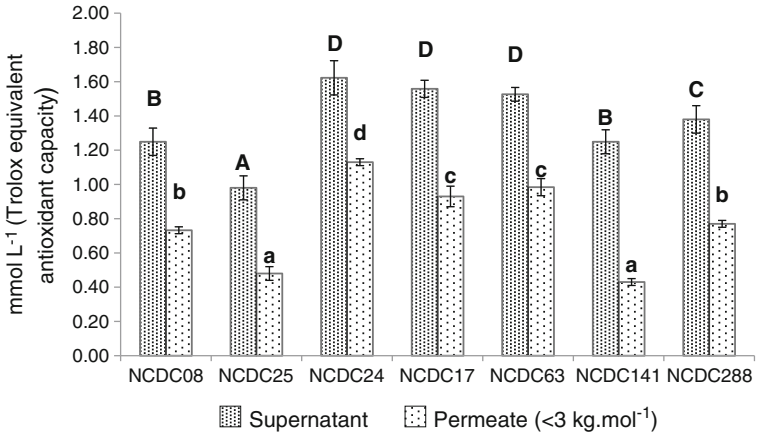

Fig. 2 Antioxidant activity of casein hydrolysate prepared using cell-free extract from lactobacilli

In general, a good correlation was observed between proteolytic and antioxidant activity among the selected strains of *Lactobacillus* assessed, indicating that the generation of antioxidant peptides with proteolysis contributes towards the development of antioxidant activity. Furthermore, the radical scavenging activity among the different species of *Lactobacillus* was observed to be strain specific. Our results corroborates the findings of Virtanen et al. (2007) which showed increased levels of proteolysis by lactobacilli (*Lactobacillus jensenii* ATCC 25258 and *L. acidophilus* ATCC 4356) resulted in a high antioxidant activity in the whey.

Furthermore, the results observed in our experiments are in accordance with Annuk et al. (2003) who demonstrated that obligatory homofermentative lactobacilli produced high antioxidant activity, whereas this was highly strain dependent among the facultative and obligatory heterofermentative lactobacilli.

Hydrolysis of casein using cell-free extract of seven selected strains of *Lactobacillus* resulted in differences in the distribution of peptides based on molecular mass. This may be due to differences in their proteinase–endopeptidase activity (Sasaki et al. 1995). The lower antioxidant activity observed for hydrolysate from *L. plantarum* NCDC25 was in accordance with their low proteolytic activity. However, the hydrolysates from three strains (*L. rhamnosus* NCDC24, *L. casei* subsp. *casei* NCDC17 and *L. paracasei* subsp. *paracasei* NCDC63) having high proportion of low molecular weight peptides (<3 $kg \cdot mol^{-1}$) showed higher antioxidant activity. This was in agreement with observations of our previous study (Kumar et al. 2010) and those of other workers (Moosman and Behl 2002; Qian et al. 2011) that low molecular weight peptides formed on hydrolysis of casein greatly contribute towards antioxidant activity. High antioxidant activity observed for the *L. rhamnosus* NCDC24, *L. casei* subsp. *casei* NCDC17 and *L. paracasei* subsp. *paracasei* NCDC63 could be possibly attributed to certain specific proteolytic enzymes associated with these strains.

5 Conclusion

Distinct differences were observed among different strains of lactobacilli towards the development of antioxidant activity during milk fermentation, indicating their strain-specific characteristic. Seven strains including *L. delbrueckii* subsp. *bulgaricus* NCDC08, *L. plantarum* NCDC25, *L. casei* subsp. *casei* NCDC17, *L. rhamnosus* NCDC24, *L. paracasei* subsp. *paracasei* NCDC63, *L. fermentum* NCDC141 and *L. helveticus* NCDC288 showed high positive correlation between proteolytic activity and antioxidant activity during fermentation of milk. On hydrolysis of casein with their cell-free extract, *L. rhamnosus* NCDC24, *L. casei* subsp. *casei* NCDC17 and *L. paracasei* subsp. *paracasei* NCDC63 resulted in significant antioxidant activity, suggesting that the fermented products produced by using these potential organisms may play an important role to combat oxidative stress.

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