# Antioxidant Activity of Some Yogurt Starter Cultures

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**ABSTRACT :** Several commercial yogurt starter cultures (*L. acidophilus* LA5, *L. casei* 01, *L. acidophilus* LA100, *L. bulgaricus* LB207 and *L. rhamnosus* GG744) were investigated for antioxidant activity by using *in vitro* assays. From the results of the present work, all strains tested showed varying degrees of antioxidant activity. *L. bulgaricus* LB207 showed the highest antioxidant activity, with a linoleic acid peroxidation inhibition of 81.3%. Hydroxy radical scavenging activity, ferrous iron chelating activity, reducing power and superoxide dismutase (SOD) activity were also studied. *L. bulgaricus* LB207 showed the highest hydroxy radical scavenging activity and *L. casei* 01 showed the highest chelating activity. *L. bulgaricus* LB207 and *L. acidophilus* LA100 showed good reducing power. All the strains in this study showed low SOD activity. The results of the present work suggest that antioxidant activity of *L. bulgaricus* LB207 due to its strong hydroxy radical scavenging activity and reducing power. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 2 : 255-258)***

**Key Words** : Yogurt Starter Culture, Antioxidant Activity, Lipid Peroxidation

## INTRODUCTION

Oxidative stress occurs when abnormally high levels of reactive oxygen species (ROS) are generated. When ROS is not counterated, cell death and tissue damage can result (Battino et al., 1999). In order to prevent oxidative damage, recently much attention has been focused on the use of antioxidants, especially natural antioxidants.

Microbial cells have a number of antioxidant defense mechanisms whose specific role is to remove or inactivate ROS to protect biological system. Recent studies showed that a number of lactic acid bacteria exerted an antioxidant action (Kaizu et al., 1993; Ahotupa et al., 1996; Korpela et al., 1997; Lin and Yen, 1999a-c; Amanatidou et al., 2001). Therefore, toxicity of ROS could be eliminated or counteracted by lactic acid bacteria (Stecchini et al., 2001).

Yogurt has been reported to have health benefits. The related nutritional and health attributes of cultured yogurt included hypoallergenic effects, enhancement of bioavailability of calcium and other nutrients, improvement of lactose intolerance, control of gastrointestinal infections, stimulation of immunological systems, anticarcinogenic effect, growth stimulation, reduction of serum cholesterol and longevity (Chandan, 1989). Lin and Yen (1999a) supposed that the longevity effect may be, in least part, due to the antioxidant activity of lactic starter culture. However, until now there is little information regarding the antioxidant activity of lactic acid bacteria.

Therefore, this study tried to examine the antioxidant activity of some commercial yogurt starter cultures by using various *in vitro* assays.

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## MATERIALS AND METHOD

### Strains

*L. acidophilus* LA5, *L. casei* 01, *L. acidophilus* LA100, *L. bulgaricus* LB207 and *L. rhamnosus* GG744 were obtained as frozen stock culture from Department of Food Biotechnology, Sungkyunkwan University (Suwon). The strains were grown for 48 h at 37°C in MRS broth (Difco). All strains were serially transferred at least three times prior to use in this study.

### Preparation of cell lysate

The cells were harvested by centrifugation at 4°C for 30 min (4,000×g) and the pellet was washed twice with 20 mM sodium phosphate buffer (SPB, pH 7.4) and then resuspended in SPB. Washed cell suspension was disrupted at 4°C by ultrasonic disruption. Cell debris were removed by centrifugation (7,000×g for 10 min at 4°C), and filtration (0.45 μm, Millipore). Protein concentration was measured by Bradford method (Bio-Rad Laboratories) after adjusting to 1 mg/ml.

### Antioxidant activity

The antioxidant activity was determined by TBA (thiobarbituric acid) method as described previously (Lin and Chang, 2000; Kim et al., 2003). Reaction mixture contained 0.6 ml of 20 mM SPB, 1 ml linoleic acid emulsion (0.1 ml linoleic acid; 99%, Sigma, 0.2 ml Tween 20 and 19.7 ml deionized water) and 0.2 ml of sample. Lipid peroxidation was started by addition of $FeSO_4$ (0.2 ml, 0.01%) and $H_2O_2$ (0.2 ml, 0.56 mM), and incubated in water bath for 6 h at 37°C. Trichloroacetic acid (TCA; 0.2 ml, 4%), TBA (2 ml, 0.8%) and butylated hydroxytoluene (BHT; 0.2 ml, 0.4%) were then added, and the mixture was boiled at 100°C for 20 min. The antioxidant activity was

**Table 1.** Antioxidant activity of yogurt starter cultures

| Strains | Antioxidant activity (%) | α-Tocopherol equivalent (μg) |
|---|---|---|
| *L casei* 01 | 68.17±1.16[b] | 31.03 |
| *L. acidophilus* LA100 | 65.32±0.98[b] | 22.15 |
| *L. rhamnosus* GG 744 | 72.87±1.83[b] | 54.09 |
| *L. acidophilus* LA5 | 38.27±9.41[c] | 0.90 |
| *L. bulgaricus* LB 207 | 81.30±3.60[a] | 146.55 |

The cell lysate was adjusted to 1 mg protein/ml.
[a, b] Values in the same column with different superscripts letters are significantly different ($p<0.05$).

evaluated from the extracted peroxide by measuring the absorbance at 532 nm using Jasco V530 spectrophotomer (Japan), and expressed as the percentage of inhibition of linoleic acid peroxidation with a control containing no sample. α-Tocopherol is the standard for the expression of α-tocopherol equivalents.

**Hydroxy radicals scavenging activity**

The hydroxy radical scavenging activity was determined by the method described in Kullisaar et al. (2002). The hydroxy radicals generated via the Fenton reaction using terephthalic acid (THA) as a chemical dosimester (Barreto et al., 1995). THA (2 ml, 10 mM) solution in SPB was added 0.1 ml of sample. Then the hydroxy radicals were generated by adding 0.1 ml $CuSO_4 \times 5\ H_2O$ and 0.1 ml hydrogen peroxide (the final concentration of the latter is 0.01 mM). The resultant THA-adduct was detected by using fluorescence spectrophotometer (Bio-TEK instruments SMF25, Switzerland) at 312 nm excitation and 426 nm emission. The hydroxy radical scavenging activity was expressed as the inhibition rate of THA-adduct generation by sample.

**Chelating activity on ferrous iron**

The chelating activity was measured by the method of Yen and Wu (1999). The $Fe^{2+}$ was assayed by measuring the formation of ferrous iron-ferrozine complex. The reaction mixture consisted of 4.6 ml deionized water, ferrous chloride (0.1 ml, 2 mM, Sigma) and ferrozine (0.2 ml, 5 mM, Sigma), and 0.1 ml of sample was added. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm. The percentage of chelating activity was defined as follows.

$$\text{Chelating activity (\%)} = [1-(\text{absorbance of sample})/(\text{absorbance of control})]\times 100$$

**Reducing power**

The reducing power was determined by Oyaizu's method (1986). 0.5 ml of sample was mixed with 0.5 ml of 200 mM potassium phosphate buffer (pH 6.4) and 0.5 ml of 1% potassium ferricyanide (Sigma). The reaction mixture was incubated at 37°C for 20 min. After 0.5 ml of 10% TCA was added, the mixture was centrifuged at 4°C for 5 min (3,000 g). 0.5 ml of the upper layer was mixed with 0.5 ml of 0.1% ferric chloride (Sigma), and the absorbance was determined at 700 nm. A higher absorbance indicates a higher reducing power.

**SOD activity**

The activity of superoxide dismutase (SOD) was measured by using a commercially available kit (RANSOD, Randox Laboratories, UK). The 50 μl of cell lysate was added to 1.7 ml mixed substrate, incubated at 37°C and 250 μl xanthine oxidase was added. The initial absorbance read after 30 s and the final absorbance after 3 min at 505 nm. The activity of SOD was expressed as U/g of protein [one unit is the degrees of inhibition that superoxide radical react with INT (2-4-iodophenyl-3-4-nitrophenol-5-phenyltetrazolium chlrolide) to form a red formazan dye].

**Statistical analysis**

The values are expressed as means±SD. Statistical analysis was performed by one way analysis of variation (ANOVA), and significant differences were detected ($p<0.05$) by Duncan's multiple range tests using a PC statistical package (SAS, release 8.01, SAS Institute Inc., Cary, NC).

## RESULTS AND DISCUSSION

**Antioxidant activity**

Lipid peroxidation is a key process in many pathological effects such as DNA damage, carcinogenesis, mutagenesis and aging (Halliwell and Gutteridge, 1999). Therefore lipid peroxidation protection is essential. The antioxidant activity of yogurt starter cultures was evaluated by determining their inhibitory activity on lipid peroxidation, a commonly used system for antioxidant activity.

As shown in Table 1, the strongest antioxidant activity was 81.3% for *L. bulgaricus* LB207 and that was 146.55 μg of α-tocopherol equivalent. *L. rahamnosus* GG744, *L. casei* 01 and *L. acidophilus* LA100 showed high antioxidant activity, lipid peroxidation inhibition of 72.87%, 68.17% and 65.32%, respectively. The lowest antioxidant activity was 38.27% for *L. acidophilus* LA5. These results indicated that yogurt starter culture has a good antioxidant effect on inhibition of lipid peroxidation. Kaizu et al. (1993) reported that hemolysis of erythrocytes was inhibited in rats by administering intracellular extract of lactobacilli. The authors of this report considered that the extract was absorbed from the small intestine and acted in the blood and tissue. It is reasonable to expect a significant number of yogurt cultures to be lysed during transit through the

**Table 2.** Hydroxy radical scavenging activity and ferrous iron chelating activity of yogurt starter cultures

| Strains | Hydroxy radical scavenging activity (%) | Ferrous iron chelating activity (%) |
|---|---|---|
| *L casei* 01 | 71.3±4.85$^{ab}$ | 72.06±3.37$^{a}$ |
| *L. acidophilus* LA100 | 70.78±5.21$^{b}$ | 66.66±2.36$^{ab}$ |
| *L. rhamnosus* GG 744 | 51.85±2.76$^{c}$ | 66.01±3.51$^{ab}$ |
| *L. acidophilus* LA5 | 67.18±4.65$^{b}$ | 60.32±3.69$^{b}$ |
| *L. bulgaricus* LB 207 | 75.0±4.65$^{a}$ | 46.32±6.44$^{c}$ |

The cell lysate was adjusted to 1 mg protein/ml.
$^{a-c}$ Values in the same column with different superscripts letters are significantly different ($p<0.05$).

**Table 3.** Superoxide dismutase activity of yogurt starter cultures

| Strains | SOD activity (U/g of protein) |
|---|---|
| *L casei* 01 | 6.67±1.16$^{c}$ |
| *L. acidophilus* LA100 | 10.66±0.98$^{a}$ |
| *L. rhamnosus* GG 744 | 7.56±1.83$^{d}$ |
| *L. acidophilus* LA5 | 7.80±9.41$^{c}$ |
| *L. bulgaricus* LB 207 | 9.71±3.60$^{b}$ |

The cell lysate was adjusted to 1 mg protein/ml.
$^{a-c}$ Values in the same column with different superscripts letters are significantly different ($p<0.05$).

gastrointestinal tract and to release their intracellular antioxidants. A recent study hypothesized that non-absorbable antioxidants are effective against colonic oxidative stress (Nishino et al., 2000). There are few reports on the antioxidant action of lactic acid bacteria on the gut; further *in vivo* experiments are needed.

**Hydroxy radical scavenging activity**

The scavenging activity of yogurt starter culture for the hydroxy radical was evaluated. As shown in Table 2, all strains tested showed varying degrees of scavenging activity. The highest scavenging activity was measured in *L. bulgaricus* LB207. The percentage of hydroxy radical scavenging was 75.0%. A lower activity was observed in *L. rhamnosus* GG744, for which the radical scavenging activity was 51.85%. Scavenging of different types of ROS was thought to be one of the main antioxidant mechanisms of the antioxidant action exhibited by lactic acid bacteria (Namiki, 1990). The hydroxy radicals are extremely reactive free radicals, which can react rapidly with almost every type of molecule in living cell, such as polyunsaturated fatty acid, proteins, DNA base and organic acid (Halliwell, 1994). At the same time, it is known that hydrogen peroxide is an important component of oxidative damage. It will react with iron to generate toxic hydroxy radical. In our study, no strains showed hydrogen peroxide scavenging activity (Data not shown).

**Chelating activity on ferrous iron**

Control of iron is important because iron participates in hydroxy radical generation. Iron, especially ferrous iron causes tissue damage by catalyzing the formation of ROS and stimulate lipid peroxidation (Halliwell and Gutteridge, 1990). Therefore, metal iron chelating is very effective antioxidant action for the prevention of lipid peroxidation (Halliwell and Gutteridge, 1999). Although *L. bulgaricus* LB207 showed remarkable antioxidant activity, the activity of chelating iron was low (Table 2). The highest ferrous iron chelating activity was observed for *L. casei* 01, reaching 72.06% of chelating activity. The chelating activity of these strains could be due to the chelators, which can capture metal irons and prohibit metal irons from catalyzing oxidation, so the higher chelating effects of lactic acid bacteria would be beneficial (Gutteridge et al., 1979). Although our strains contained strong chelating activity, this result would not be sufficient to explain the difference in antioxidant activity.

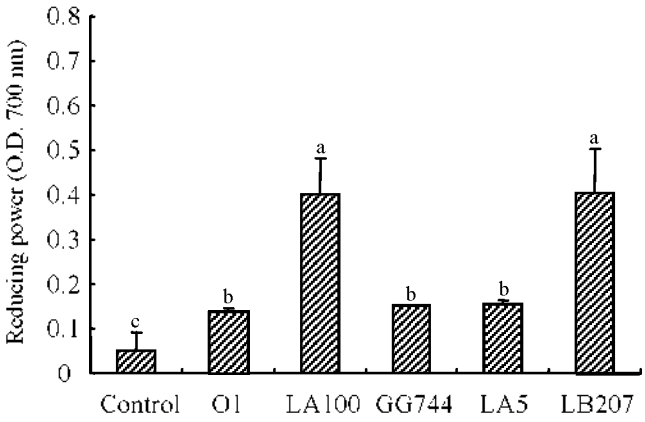


**Figure 1.** Reducing power of yogurt starter cultures. $^{a-c}$ Values in the same column with different superscripts letters are significantly different ($p<0.05$).

**Reducing power**

Reducing power refers to all enzymatic (catalase, SOD, peroxidase) or non-enzymatic compounds (ascorbate, α-tocopherol, glutathione) with ability to reduce oxygen radicals or iron, therefore make it unavailable for oxidative reactions (Warriner and Morris, 1995). Reducing power was observed for all yogurt starter cultures and is shown in Figure 1. *L. bulgaricus* LB207 and *L. acidophilus* LA100 exhibited the strongest reducing power. The present reducing power in the strains might contain higher amount of reductone, which can react with free radicals to stabilize and terminate the radical reactions (Yen and Chen, 1995).

**SOD activity**

When microorganisms were exposed to ROS, to protect themselves against oxidative damage, cells evolve a mechanism of enzymatic antioxidants, which may directly scavenge ROS, or produce the non-enzymatic antioxidant

(Farr and Kogoma, 1991). SOD eliminates direct toxicity of superoxide anions and prevents the superoxide anions mediating reduction of iron followed by hydroxy radical generation (Fridovich, 1997). In this study, all strains showed very low SOD activity, and *L. acidophilus* LA100 among the strains showed the highest SOD activity as 10.66 U/g protein. We suggest that SOD would have a minor role in inhibition of lipid peroxidation.

In any case, the antioxidant mechanism of lactic acid bacteria is still unclear. Ahotupa et al. (1996) reported that the protection of lipid peroxidation of *Lactobacillus* GG could be due to iron chelation and superoxide scavenging ability. In this study, *L. bulgaricus* LB207 inhibited lipid peroxidation probably due to its high hydroxy radical scavenging activity and reducing power. Thus, it is a preventive antioxidant.

## CONCLUSION

The results of this study indicate that yogurt starter cultures have good antioxidant activity. *L. bulgaricus* LB207 among the strains showed the highest antioxidant activity on the inhibition of lipid peroxidation systems. Antioxidant activity of *L. bulgaricus* LB207 may be due to its hydroxy radical scavenging activity and reducing power. Yogurt starter cultures with good antioxidant activity would be useful for a natural antioxidant by reducing oxidative damages in humans. To explain the antioxidant action of lactic starter culture on gut, further investigation *in vivo* is needed.

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