Curr Microbiol (2011) 62:1097–1103
DOI 10.1007/s00284-010-9827-7

In Vitro and In Vivo Antioxidant Activity of *Bifidobacterium animalis* 01 Isolated from Centenarians

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Received: 22 September 2010 / Accepted: 18 November 2010 / Published online: 4 December 2010

Abstract Several studies reported the antioxidant activity of bifidobacteria using assays in vitro. In present study, the in vitro and in vivo antioxidant activity of *Bifidobacterium animalis* 01 was investigated. Culture supernatant, intact cells, and intracellular cell-free extracts of *B. animalis* 01 were involved in this study. The antioxidant assays in vitro included lipid peroxidation assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, hydroxyl radical ($^{\bullet}OH$) assay and superoxide anion (O_2^-) assay. The antioxidant assays in vivo were conducted using mice model. Activities of antioxidative enzymes, malondialdehyde (MDA) content in serums and livers of aging mice were evaluated. Monoamine oxidase (MAO) activity and lipofuscin level in brains of aging mice were also characterized. Results showed that culture supernatant, intact cells and intracellular cell-free extracts of *B. animalis* 01 could effectively scavenge free radicals, significantly enhance mice's activities of antioxidative enzymes and reduce mice's MDA content, lipofuscin level and MAO activity. Our results indicated that *B. animalis* 01 has the potential to be developed into a dietary antioxidant supplements.

Introduction

Increasing evidence indicates that excessive reactive oxygen species (ROS) plays an important role in damaging cellular components, causing cell membrane injury, protein denaturation and wrong DNA replications, and inducing many diseases including aging, cancer, diabetes, and rheumatoid arthritis [12, 22]. Although antioxidant and repair systems such as GSH-Px, CAT, and SOD exist in human bodies, sometimes they are not sufficient to protect human bodies from oxidative damage. Hence consuming antioxidant supplements would help to prevent human bodies from oxidative damage.

Bifidobacteria is a member of dominant microbiota in human intestinal tract. It plays an important role in promoting human health. For example, in the presence of β-galactosidase producing bifidobacteria, people suffering from lactose malabsorption could improve their lactose digestibility and tolerance [14]. Some *Bifidobacterium* species can produce antimicrobial compounds (bacteriocin) to control gastrointestinal infections [7, 13, 28]. In addition, it also contributes to stimulation of the immune system, anti-ulcer activity, anti-tumor activity, and gastrointestinal disorder prevention or treatment [16, 17, 27]. Various health promoting functions of bifidobacteria has enabled it to be the ideal dietary supplement.

Several studies have been conducted on the antioxidant activity of bifidobacteria. Lin and Yen [11] evaluated the inhibitory effect of *Bifidobacterium longum* on lipid peroxidation. Based on DPPH-free radical scavenging activity assay and oxygen tolerance assay, Kim et al. [8] isolated antioxidative *Bifidobacterium* species from infant feces. However, the studies mentioned above were performed without involving animal experiments which failed to reveal the antioxidative potential of bifidobacteria in vivo completely. In addition, it has been reported that fermentation products by bifidobacteria possessed strong antioxidant activity [25]. And in our previous study, we demonstrated that proteins extracted from *Bifidobacterium*

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animalis 01 cells showed evident antioxidant activity in vitro [20]. Therefore, in present study, the antioxidant activities of culture supernatant, intact cells and intracellular extracts of *B. animalis* 01 in vitro and in vivo were characterized, trying to explore the application potential of *B. animalis* 01 as dietary antioxidant supplements.

Materials and Methods

Microbial Strains

B. animalis 01 (China General Microbiological Collection Center 1353), identified by the method based on 16S rRNA sequences analysis, was isolated from the fecal samples of the healthy centenarian volunteers living in Bama of Guangxi province in China, which was listed as the fifth officially certificated longevity village by the International Society of Natural Medicine in 1991. *B. animalis* 01 was cultured and in MRS broth at 37°C for 24 h under anaerobic conditions.

Reagents and Materials

Xanthine, xanthine oxidase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), linoleic acid, Tween 20, and 5,5-dimethyl-pyrroline-*N*-oxide (DMPO) were purchased from Sigma Company. Diethylenetriaminepentaacetic acid (DETAPAC), *N*,*N*,*N*,*N*-Tetramethyl ethylenediamine (TEMED), thiobarbituric acid (TBA), trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), ascorbic acid, copper sulfate, and ferrous sulfate were purchased Beijing Chemicals Co. Ltd. Assay kits for malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (TAOC), glutathione-*S*-transferase (GST), and monoamine oxidase (MAO) were the products of Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The male Kunming mice were purchased from the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences (Beijing, China). All other reagents were of analytical grade.

Preparation of Culture Supernatant, Intact Cells, and Intracellular Cell-Free Extracts

The preparation process was conducted basically according to the method of Lin and Yen [11]. In the preparation process, total cell numbers were adjusted to about 10^9 CFU/ml. Through centrifugation at 4,400 rpm for 10 min, culture supernatant was separated from intact cells. After being washed three times with deionized water, intact cells were obtained and resuspended in deionized water. For the preparation of intracellular cell-free extracts, cells were quickly washed twice with deionized water and resuspended in deionized water followed by ultrasonic disruption. Sonication was performed in an ice-water bath for 60 cycles of 5 s, with 5 s intervals until no cells could be checked under microscope. Cell debris was removed by centrifugation at 7,800 rpm for 10 min and the supernatants were collected as intracellular cell-free extracts.

Inhibition of Linoleic Acid Peroxidation

The anti-lipid peroxidation activity of *B. animalis* 01 was carried out by the thiobarbituric acid (TBA) method, according to the method of Grossman and Zakut [6] with some modifications. Linoleic acid emulsion (20 ml) was composed of 0.1 ml linoleic acid, 0.2 ml Tween 20, and 19.7 ml deionized water. 0.2 ml phosphate buffer solution (0.02 M, pH 7.4), 1 ml of linoleic acid emulsion, 0.2 ml $FeSO_4$ (0.01%, w/v), 0.2 ml H_2O_2 (0.02%, v/v), and 0.4 ml sample (MRS broth, culture supernatant, intact cells or intracellular cell-free extracts) were mixed and then incubated at 37°C for 12 h. Afterwards 2.0 ml reaction solution was mixed with 0.2 ml TCA (4%, w/v), 2 ml TBA (0.8%, w/v), and 0.2 ml BHT (0.4%, w/v). The mixture was incubated at 100°C for 30 min and then allowed to cool. The absorbance was measured at 532 nm. In the blank control, samples were substituted with deionized water. The inhibition rate was calculated as follows:

$$\text{Inhibition effect } (\%) = \left[1 - A_{532(\text{sample})}/A_{532(\text{blank})}\right] \times 100$$

DPPH-free Radical Scavenging Activity

Scavenging capacity of DPPH-free radical was evaluated by the method of Blois [3]. The assay mixture was composed of 2.2 ml DPPH-free radical (0.1 mM) and 0.8 ml sample. The mixture was shook up vigorously and allowed to stand in dark room temperature for 30 min. The absorbance was measured at 517 nm. In the blank control, the sample was substituted with deionized water. The scavenging effect was calculated using the following equation:

$$\text{Scavenging effect } (\%) = \left[1 - A_{517(\text{sample})}/A_{517(\text{blank})}\right] \times 100$$

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was performed at room temperature on an ER200D-SRC spectrometer (Bruker, Germany). The Fenton reaction system containing ferrous ion and H_2O_2 was used as hydroxyl radical generating system. DMPO was used as a spin trap reagent [5]. The reaction mixture consisted of 5 µl $FeSO_4$ (0.1 mM), 5 µl of DETAPAC (4 mM), 5 µl DMPO (0.25 M), and 5 µl sample. The reaction was started in the presence of

5 μl H_2O_2 (10 mM). In the blank control, the sample was substituted with deionized water.

$$\text{Scavenging effect } (\%) = [(H_o - H_s)/H_o] \times 100$$

where H_o was the amplitude of the second peak in the ERS spectrum for the control, H_s was the amplitude of the second peak in the ERS spectrum for the sample. The ESR instrument conditions were as follows: freq, 9.438350 GHz; field center, 336.00 mT; Mod, 100 Hz; width 0.2 × 1 mT; time constant, 0.1 s; power, 10 mw; sweep time, 2 min; amplitude, 50.

Superoxide Anion Assay

The xanthine/xanthine oxidase system was used to generate superoxide anion [29]. The reaction mixture was composed of 5 μl xanthine (0.75 mM), 5 μl DETAPAC (4 mM), 5 μl DMPO (1 M), and 5 μl sample. The reaction was started by the addition of 5 μl xanthine oxidase (0.71 U/ml). The ESR signal obtained for the DMPO-OH adduct was measured in 50 s. In the blank control, the sample was substituted with deionized water.

$$\text{Scavenging effect } (\%) = [(H_o - H_s)/H_o] \times 100$$

where H_o was the amplitude of the first peak in the ERS spectrum for the control, H_s was the amplitude of the first peak in the ERS spectrum for the sample. The ESR instrument conditions were as follows: freq, 9.4386 GHz; field center, 336.0 mT; Mod, 100 Hz; width, 0.79 × 0.1 mT; time constant, 0.1 s; power, 10 mw; sweep time, 2 min; amplitude, 200.

Animal and Experimental Design

Male Kunming mice (8 weeks old, 25 ± 2 g) were kept in plastic cages under a controlled atmosphere (Temperature 22 ± 2°C; humidity 50 ± 5%). They were kept on a 12 h light/dark cycles and were free to access to food and water during the experiments. After adaptation for 1 week, the mice were randomly divided into seven groups (6 mice per group): normal control group (NCG), D-galactose model control group (MCG), ascorbic acid positive control group (PCG), MRS broth treatment group, culture supernatant treatment group, intact cell treatment group, and intracellular cell-free extracts treatment group. Mice in NCG were given 0.5 ml physiological saline solution (0.85%, w/v) per day via gastric gavage. Mice in MCG were fed with 0.5 ml physiological saline solution (0.9%, w/v) via gastric gavage and 0.2 ml (20 mg per mouse) D-galactose through subcutaneous injection per day. Mice in PCG were given 0.5 ml (5 mg per mouse) ascorbic acid via gastric gavage and 0.2 ml D-galactose (20 mg per mouse) through subcutaneous injection per day. The mice in sample treatment groups were given 0.5 ml MRS broth, culture supernatant, intact cell suspension or intracellular cell-free extracts via gastric gavage and 0.2 ml D-galactose (20 mg per mouse) through subcutaneous injection per day. All groups were performed once daily for 30 consecutive days.

Assays of SOD, CAT, GSH-Px, TAOC, and MDA in Mice Serums

Twenty-four hours after the last drug administration, mice were killed. Blood samples were collected and centrifuged at 4,000 rpm for 10 min at 4°C to afford the serums. The activities of SOD, CAT, and GSH-Px and levels of MDA and TAOC in serum were measured according to the instructions on the kits.

Assays of MDA and GST in Mice Livers

The liver was removed, washed, and homogenized in ice-cold physiological saline to prepare 10% (w/v) homogenate. Then the homogenate was centrifuged at 4,000 rpm for 10 min at 4°C to remove cellular residue. Its supernatant was collected to measure the GST activity and MDA level. Both assays were conducted according to the instructions on the kits.

Assays of MAO and Lipofuscin in Mice Brains

The brain was removed, washed, and homogenized in ice-cold physiological saline to prepare 10% (w/v) homogenate. The homogenate was centrifuged at 4,000 rpm for 10 min at 4°C. Its supernatant was collected to measure MAO activity and lipofuscin level.

The MAO activity was measured according to the instructions on the kits. The lipofuscin level was determined by previous extraction method with slight modifications [15, 23]. Briefly, 2 ml 10% (w/v) brain tissue homogenate was mixed into 4 ml chloroform–methanol solution (2:1, v/v). After centrifugation at 2,000 rpm for 10 min, the lipofuscin levels were evaluated in the chloroform phase using a RF-510 fluorescence spectrophotometer (Shimadzu, Japan). The luminescence of the sample was determined at an emission maximum of 450 nm. The concentrations of lipofuscin were determined using quinine (0.1 μg/ml) as a standard.

Statistical Analysis

All experiments were performed at least three times. Results were expressed as the mean ± standard deviation. The Student–Newman–Keuls test and one-way analysis of variance (ANOVA) were used for multiple comparisons by the SPSS 11.5 software package. Difference was considered to be statistically significant if $P < 0.05$.

Results

Inhibition of Linoleic Acid Peroxidation

Culture supernatant, intact cells and intracellular cell-free extracts of *B. animalis* 01 all exhibited evident inhibitory effect on the inhibition of linoleic acid peroxidation (Table 1). The inhibitory effect was 30.48, 41.12, and 71.02% for culture supernatant, intact cells, and intracellular cell-free extracts, respectively. MRS broth showed certain inhibitory effect, but the inhibitory effect of culture supernatant was significantly ($P < 0.05$) higher than that of MRS broth.

DPPH-Free Radical Scavenging Activity

DPPH-free radical scavenging effects of culture supernatant, intact cells and intracellular cell-free extracts of *B. animalis* 01 were presented in Table 1. In this assay, the culture supernatant of *B. animalis* 01 exhibited the highest scavenging effect (73.11%), which was significantly ($P < 0.05$) higher than that of MRS broth. The scavenging effect of intact cells on DPPH-free radicals was the lowest among the groups.

Scavenging Effect on Hydroxyl Radical and Superoxide Anion

Culture supernatant, intact cells and intracellular cell-free extracts of *B. animalis* 01 exhibited excellent scavenging activity on both hydroxyl radical and superoxide anion (Table 2). In both assays, the culture supernatant of *B. animalis* 01 showed the strongest scavenging ability, which was significantly ($P < 0.05$) higher than that of MRS broth, whereas intact cells exhibited weak scavenging effect.

Activities of Antioxidant Enzymes in Mice Serums

As shown in Table 3, compared with NCG, significant ($P < 0.05$) increase in MDA level, decrease of antioxidative enzymes activities (SOD, GSH-Px, and CAT) and TAOC level were observed in serums of MCG. Culture supernatant, intact cells, intracellular cell-free extracts, and ascorbic acid significantly ($P < 0.05$) enhanced the activities of antioxidant enzymes (SOD, CAT, and GSH-Px) and the TAOC level in mice serums, reduced the MDA content in mice serums. In addition, results showed that culture supernatant, was significantly ($P < 0.05$) more effective than MRS broth on enhancing activities of antioxidant enzymes and reducing MDA content in mice serums.

GST Activity and MDA Level in Mice Livers

Each group's GST activity and MDA level in mice livers were presented in Table 4. The MDA level and GST activity of MCG were significantly ($P < 0.05$) higher than that of NCG. Compared with MCG group, culture supernatant, intact cells, intracellular cell-free extracts, and ascorbic acid significantly ($P < 0.05$) reduced GST activity and MDA level in mice livers. In addition, the GST activity

Table 1 Anti-lipid peroxidation effect and DPPH-free radical scavenging effect of *B. animalis* 01

Group	Anti-lipid peroxidation assay		DPPH-free radical scavenging assay	
	A_{532}	Inhibition rate (%)	A_{517}	Scavenging effect (%)
Blank control	0.398 ± 0.011	N/A	0.401 ± 0.014	N/A
MRS broth	0.318 ± 0.007	20.17 ± 1.37a	0.374 ± 0.006	6.59 ± 2.23a
Culture supernatant	0.277 ± 0.007	30.48 ± 0.66b	0.108 ± 0.005	73.11 ± 1.67b
Intact cells	0.234 ± 0.010	41.12 ± 1.59c	0.356 ± 0.006	11.10 ± 0.63a
Intracellular cell-free extracts	0.115 ± 0.003	71.02 ± 0.51d	0.289 ± 0.012	27.72 ± 2.12c

Values are the means ± standard deviations ($n = 3$), values followed by *different letters* in same column are significantly different ($P < 0.05$)

Table 2 Scavenging effect of *B. animalis* 01 on oxygen-free radicals

Group	EPR amplitude for DMPO-OH	·OH scavenging effect (%)	EPR amplitude for DMPO-OOH	O_2^- scavenging effect (%)
Blank control	626 ± 8	N/A	397 ± 5	N/A
MRS broth	308 ± 9	50.71 ± 1.88a	284 ± 4	28.49 ± 0.52a
Culture supernatant	135 ± 3	78.32 ± 0.46b	54 ± 2	86.39 ± 0.56b
Intact cells	514 ± 6	17.74 ± 0.21c	363 ± 1	8.39 ± 1.35c
Intracellular cell-free extracts	415 ± 6	33.61 ± 1.41d	302 ± 2	23.78 ± 0.27d

Values are the means ± standard deviations ($n = 3$), values followed by *different letters* in same column are significantly different ($P < 0.05$)

Table 3 Effect of *B. animalis* 01 on the activities of SOD (U/ml), CAT (U/ml), GSH-Px (U/ml), TAOC (U/ml), and levels of MDA (nmol/ml) in mice serums

Group	SOD	CAT	GSH-Px	TAOC	MDA
NCG	164.01 ± 13.06a	5.67 ± 0.32d	1307.13 ± 46.71a	27.01 ± 1.80bc	6.20 ± 0.39ab
MCG	136.88 ± 11.20b	3.39 ± 0.41a	1122.50 ± 38.99d	22.30 ± 2.05a	8.86 ± 0.83d
PCG	192.15 ± 13.50c	5.36 ± 0.46 cd	1209.07 ± 23.95b	30.52 ± 2.41d	5.45 ± 0.87a
MRS broth	145.60 ± 7.47b	4.36 ± 0.47b	1196.24 ± 33.67b	25.95 ± 1.91b	7.54 ± 1.22c
Culture supernatant	205.87 ± 9.64d	4.98 ± 0.36c	1433.27 ± 63.27c	29.35 ± 1.71cd	5.79 ± 0.64ab
Intact cells	182.45 ± 7.14c	4.69 ± 0.70bc	1272.69 ± 53.85a	26.39 ± 1.49bc	7.05 ± 0.88bc
Intracellular cell-free extracts	217.62 ± 11.95d	5.06 ± 0.15cd	1290.13 ± 41.17a	27.28 ± 2.21bc	5.92 ± 1.21ab

Values are the means ± standard deviations ($n = 6$), values followed by *different letters* in same column are significantly different ($P < 0.05$)

Table 4 Effect of *B. animalis* 01 on GST activity (U/mg protein) and MDA level (nmol/mg protein) in mice livers, lipofusion level (μg/g) and MAO activity (U/h/mg protein) in mice brains

Group	GST	MDA	Lipofuscin	MAO
NCG	52.11 ± 4.67b	1.23 ± 0.07bc	0.153 ± 0.008b	1.29 ± 0.11b
MCG	75.52 ± 4.56d	2.53 ± 0.38e	0.184 ± 0.016a	1.73 ± 0.16c
PCG	42.68 ± 2.45a	1.01 ± 0.20ab	0.147 ± 0.010b	1.25 ± 0.10b
MRS broth	58.81 ± 7.52c	1.76 ± 0.48d	0.173 ± 0.008a	1.31 ± 0.13b
Culture supernatant	43.15 ± 5.37a	1.51 ± 0.15cd	0.149 ± 0.010b	0.98 ± 0.13a
Intact cells	50.12 ± 3.16b	1.29 ± 0.15bc	0.147 ± 0.008b	1.13 ± 0.22ab
Intracellular cell-free extracts	46.79 ± 2.72ab	0.82 ± 0.20a	0.145 ± 0.009b	1.29 ± 0.10b

Values are the means ± standard deviations ($n = 6$), values followed by *different letters* in same column are significantly different ($P < 0.05$)

of MRS broth treatment group was significantly ($P < 0.05$) higher than that of culture supernatant treatment group.

MAO Activity and Lipofuscin Level in Mice Brains

Compared with NCG group, there was a significant ($P < 0.05$) increase of MAO activity and lipofuscin level in MCG group (Table 4). The results showed that culture supernatant, intact cells, and intracellular cell-free extracts significantly ($P < 0.05$) reduced MAO activity and lipofuscin level in mice brains. Moreover, MAO activity and lipofuscin level of MRS broth treatment group were significantly ($P < 0.05$) higher than that of culture supernatant treatment group.

Discussion

The antioxidative potential of bifidobacteria has been reported by several studies. Most of them involved antioxidant assays in vitro such as DPPH-free radical scavenging assay and inhibition of lipid peroxidation assays. In our study, the results indicated that intact cells and intracellular cell-free extracts of *B. animalis* 01 showed strong antioxidant activity in vitro, which was similar to the antioxidant activity of *Bifidobacterium longum* ATCC 15708 described by Lin and Chang [10]. Moreover, intracellular cell-free extracts exhibited stronger antioxidant activity than intact cells in vitro. This was probably because most antioxidative compounds of bifidobacteria existed in vivo and the reaction time in vitro was too short for intact cells to exhibit antioxidant activity. This result also can be supported by our previous research which revealed that protein extracted from *B. animalis* 01 cells possessed evident antioxidant activity in vitro [20]. Culture supernatant exhibited stronger scavenging effect than MRS broth, which may result from hydrolyzed medium composition. It has been reported that the antioxidant activity of fermented milk by lactic acid bacteria may came from hydrolysed components [24]. On the other hand, MRS broth also exhibited certain antioxidant activity in vitro. It could be attributed to the components of MRS medium such as glucose, peptone, and yeast extracts.

In the present study, the antioxidant activities of culture supernatant, intact cells, and intracellular cell-free extracts of *B. animalis* 01 in vivo were also characterized. SOD, GSH-Px, and CAT are regarded as major antioxidant enzymes in vivo to eliminate ROS. SOD transformed superoxide radicals to form hydrogen peroxide, which then was degraded into water and oxygen, thus preventing

organisms from oxidative damage [26]. MDA could induce cell damage in various ways and its level in mice body reflects the content of free radicals produced by lipid peroxidation. Our results indicated that culture supernatant, intact cells, and intracellular cell-free extracts enhanced activities of antioxidative enzymes in serums, and reduced MDA content both in serums and livers significantly ($P < 0.05$) (Tables 3, 4). In addition, the TAOC level of aging mice's serums was enhanced, which might come from the increase of non-enzymatic antioxidants, such as glutathione, thiols, and some vitamins [18]. The GST activities of all sample treatment groups were significantly ($P < 0.05$) lower than that of MCG group, which was in consistent with recent Tanas et al.'s [21] results, suggesting that GST activity of paw tissues decreased by administration of lichen extracts which was characterized to be an effective antioxidant. MAO activity and lipofuscin content increases with the increase of body age, thus they are regarded as important index of body aging [1, 19]. We found that administration of culture supernatant, intact cells, and intracellular cell-free extracts reduced MAO activity and lipofuscin content significantly ($P < 0.05$) in aging mice brains (Table 4). Furthermore, results showed that intact cells and intracellular cell-free extracts possessed similar antioxidant activities in vivo, which was in contrast to the results in vitro. The reason could be that intact cells were broken in mice body and antioxidative compounds in vivo were released, or intact cells produced bioactive metabolites in mice body.

The purpose of this study was to explore the potential of application of *B. animalis* 01 as dietary antioxidant supplements. Thus, antioxidant activity assays in vivo were conducted, which was different from previous studies that only involved assays in vitro [8, 10, 11]. Some investigation suggested that milk or soymilk fermented by bifidobacteria enhanced their antioxidant activities [24, 25]. In addition, bioactive compounds such as exopolysaccharide produced by bifidobacteria may contribute to its antioxidant activity [2, 9]. Hence, culture supernatant was involved in this study to determine whether *B. animalis* 01 was able to improve the antioxidant activity of MRS broth, and MRS broth group was a control to culture supernatant. Our results revealed that the antioxidant activity of MRS broth was enhanced significantly ($P < 0.05$) after the cultivation of *B. animalis* 01. Thus, it is entirely possible that after *B. animalis* 01 is consumed by humans, it takes advantage of the substances in human intestinal tract to produce antioxidative compounds to benefit the host. Compared with intracellular cell-free extracts, although intact cells showed relatively lower antioxidant activity, it could compete with potential pathogenic gas producing and putrefactive bacteria, thereby increasing colonic bifidobacteria populations. It is supported by Bouhnik et al.'s [4] conclusion that the bifidobacteria populations increased in human body as long as exogenous bifidobacteria was consumed.

According to the results obtained, culture supernatant, intact cells, and intracellular cell-free extracts of *B. animalis* 01 exhibited excellent antioxidant activity in vitro and in vivo. It indicated that as long as *B. animalis* 01 was consumed, it will benefit the host in any forms: if cells are alive, they can improve the intestinal tract microflora and produce antioxidative compounds; if cells are broken after they are ingested by human beings, the antioxidative compounds in vivo will be released to benefit the host. Besides its health promoting functions, bifidobacteria is safe for human to ingest which stems from the long historical consumption of fermented milk. Therefore, *B. animalis* 01 is suitable to be developed into a novel antioxidant supplement for human beings.

Acknowledgments This research was funded by China National 863 Program (2008AA10Z324) and Beijing Natural Science Foundation (5072025).

References

1. Alper G, Girgin F, Ozgonol M, Mentes G, Ersoz B (1999) MAO inhibitors and oxidant stress in aging brain tissue. Eur Neuropsychopharmacol 9:247–252
2. Andaloussi SA, Talbaoui H, Marczak R, Bonaly R (1995) Isolation and characterization of exocellular polysaccharides produced by *Bifidobacterium longum*. Appl Microbiol Biotechnol 43:995–1000
3. Blois MS (2002) Antioxidant determinations by the use of a stable free radical. Nature 26:1199–1200
4. Bouhnik Y, Pochart P, Marteau P, Arlet G, Goderel I, Rambaud JC (1992) Fecal recovery in humans of viable *Bifidobacterium* sp. ingested in fermented milk. Gastroenterology 102:875–878
5. Buettner GR, Mason RP (1990) Spin-trapping methods for detecting superoxide and hydroxyl free radicals in vivo and in vitro. Methods Enzymol 186:127–133
6. Grossman S, Zakut R (1979) Determination of the activity of lipoxygenase (lipoxidase). Methods Biochem Anal 25:303–329
7. Jack RW, Tagg JR, Ray B (1995) Bacteriocins of Gram-positive bacteria. Microbiol Rev 59:171–200
8. Kim JY, Choi SI, Heo TR (2003) Screening of antioxidative activity of *Bifidobacterium* species isolated from Korean infants feces and their identification. Biotechnol Bioprocess Eng 8:199–204
9. Kohno M, Suzuki S, Kanaya T, Yoshino T, Matsuura Y, Asada M, Kitamura S (2009) Structural characterization of the extracellular polysaccharide produced by *Bifidobacterium longum* JBL05. Carbohydr Polym 77:351–357
10. Lin MY, Chang FJ (2000) Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. Dig Dis Sci 45:1617–1622
11. Lin MY, Yen CL (1999) Inhibition of lipid peroxidation by *Lactobacillus acidophilus* and *Bifidobacterium longum*. J Agric Food Chem 47:3661–3664
12. Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ (2000)

Extension of life-span with superoxide dismutase/catalase mimetics. Science 289:1567–1569
13. Meghrous J, Euloge P, Junelles AM, Ballongue J, Petitdemange H (1990) Screening of *Bifidobacterium* strains for bacteriocin production. Biotechnol Lett 12:575–580
14. Mitsuoka T (1990) Bifidobacteria and their role in human health. J Ind Microbiol 6:263–268
15. Nicol S (1987) Some limitations on the use of the lipofuscin ageing techniques. Mar Biol 93:609–614
16. Saikali J, Picard C, Freitas M, Holt P (2004) Fermented milks, probiotic cultures, and colon cancer. Nutr Cancer 49:14–24
17. Sartor RB (2004) Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology 126:1620–1633
18. Seifried HE, Anderson DE, Fisher EI, Milner JA (2007) A review of the interaction among dietary antioxidants and reactive oxygen species. J Nutr Biochem 18:567–579
19. Sharma D, Maurya AK, Singh R (1993) Age-related decline in multiple unit action potentials of CA3 region of rat hippocampus: correlation with lipid peroxidation and lipofuscin concentration and the effect of centrophenoxine. Neurobiol Aging 14:319–330
20. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P (2010) Antioxidant activity in vitro of the selenium-contained protein from the Se-enriched *Bifidobacterium animalis* 01. Anaerobe 16:380–386
21. Tanas S, Odabasoglu F, Halici Z, Cakir A, Aygun H, Ali A, Suleyman H (2010) Evaluation of anti-inflammatory and antioxidant activities of *Peltigera rufescens* lichen species in acute and chronic inflammation models. J Nat Med 64:42–49
22. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell B 39:44–84
23. Vernet M, Hunter JR, Vetter RD (1988) Accumulation of age-pigments 522 (lipofuscin) in two cold-water fishes. Fish Bull 86:401–407
24. Virtanen T, Pihlanto A, Akkanen S, Korhonen H (2007) Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria. J Appl Microbiol 102:106–115
25. Wang YC, Yu RC, Chou CC (2006) Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. Food Microbiol 23:128–135
26. Yao DC, Shi WB, Gou YL, Zhou XR, Tak YA, Zhou YK (2005) Fatty acid mediated intracellular iron translocation: a synergistic mechanism of oxidative injury. Free Radic Bio Med 39: 1385–1398
27. Yasui H, Kiyoshima J, Ushijima H (1995) Passive protection against rotavirus-induced diarrhea of mouse pups born to and nursed by dams fed *Bifidobacterium breve* YIT4064. J Infect Dis 172:403–409
28. Yildirim Z, Johnson MG (1998) Characterization and antimicrobial spectrum of bifidocin B, a bacteriocin produced by *Bifidobacterium bifidum* NCFB 1454. J Food Protect 61:47–51
29. Zang LY, Cosma G, Gardner H, Vallyathan V (1998) Scavenging of reactive oxygen species by melatonin. Biochim Biophys Acta 1425:469–477