Title: Evaluation of antioxidative effects of Lactobacillus plantarum with fuzzy synthetic models

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Evaluation of antoxidative effects of *Lactobacillus plantarum* with fuzzy synthetic models

Running title: Study of antioxidat abilities of probiotics

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Abstract

Numerous studies suggest that the effects of lactic acid bacteria (LAB) on oxidative stress in vivo are correlated with their antioxidative activities in vitro, however the relationship is still unclear and contradictory. The antioxidative activities of Lactobacillus plantarum strains isolated from fermented foods were determined with 2, 2-diphenyl-1-picrylhydrazyl, hydroxyl radicals and superoxide radical scavenging ability, reducing activity, resistance to hydrogen peroxide and ferrous chelating ability in vitro. Two fuzzy synthetic evaluation models, one with an analytic hierarchy process and one using entropy weight, were then used to evaluate the overall antioxidative abilities of these L. plantarum strains. Although there was some difference between the two models, the highest scoring strain (CCFM10), the middle scoring strain (CCFM242) and the lowest scoring strain (RS15-3) were obtained with both models. The examination of the antioxidative abilities of three strains in D-galactose-induced oxidative stress mice demonstrated that their overall antioxidative abilities in vitro could reveal the abilities to alleviate oxidative stress in vivo. The current study suggests that assessment of overall antioxidative abilities with fuzzy synthetic models can guide evaluation of probiotic antioxidants. It might be a more quick and effective method to evaluate the overall antioxidative abilities of LAB.

Keywords

Lactobacillus plantarum, fuzzy synthetic model, antioxidative, mice model
Introduction

Oxidative stress is believed to result when cellular antioxidative systems are overwhelmed by reactive oxygen species (ROS), which are caused by environmental factors and ageing [1]. Many antioxidative diet supplements such as vitamins, alpha-lipoic acid, fruits and herbs have been investigated as ways to reduce oxidative stress in various pathological conditions [2-6]. In recent years, the antioxidative abilities of probiotics have also attracted increasing interest [7-9]. Our previous meta-analysis demonstrated that probiotics administration improved oxidative stress in D-galactose (D-gal) induced mice model through inducing a significant increase in serum superoxide dismutase (SOD) activity and glutathione peroxidase (GSH-PX) activity and a significant decrease in malondialdehyde (MDA) content [10]. In one double-blind randomised controlled trial, administration of yoghurt with Bifidobacterium lactis Bb12 and Lactobacillus acidophilus La5 was associated with significant increases in the level of plasma SOD, GSH-PX and total antioxidant status (TAS) in type 2 diabetic subjects [11]. Nevertheless, the administration of probiotic capsules did not improve oxidative stress indexes in the same pathological subjects [12], perhaps because the probiotic antioxidative activities vary across different species.

As one of potential probiotics, Lactobacillus plantarum are mainly isolated from traditional fermented foods such as vegetables, dairy products, soybeans and meat products [13-16]. The antioxidative activities of L. plantarum have been discussed in...
numerous studies [17-19]. Hariri et al. found that soymilk with *L. plantarum* A7 significantly increased SOD activities in type 2 diabetic patients [20]. *L. plantarum* C88 administration also improved serum SOD activity, hepatic glutathione (GSH) and total antioxidant capacity (TAC), while MDA content was significantly decreased in D-gal-induced mice. Different strains of *L. plantarum* had different protective effects against oxidative stress, suggesting that the antioxidative abilities of probiotics are also strain-specific [7].

Therefore, it is necessary to hunt for an effective method to mine the probiotics with high antioxidative abilities and compare the antioxidative activities of different strains. Obviously, human clinical trials and animal studies can provide convincing evidence for the antioxidative activities of probiotics [21, 22]. However, both methods are high cost, time-consuming and must comply with relevant laws and animal welfare regulations.

Numerous studies suggest that the effects of probiotics on oxidative stress *in vivo* are correlated with antioxidative activities *in vitro* [23-25]. Various methods have been used to evaluate the antioxidative abilities of lactic acid bacteria *in vitro*. To our knowledge, the most common tests are as follows: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging ability, hydroxyl radicals scavenging, reducing activity, superoxide radical scavenging ability, resistance to hydrogen peroxide, metal ion (Fe$^{2+}$ and Cu$^{2+}$) chelating ability, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC), and oxygen radical absorbance...
capacity (ORAC) assays.

However, almost all extracorporeal methods mainly focus on one aspect of antioxidative abilities of probiotics, and it is difficult to determine the true overall antioxidative abilities of probiotics on the basis of *in vitro* results. Therefore it is significant to search for a high-efficiency and simple strategy to evaluate the antioxidative abilities of probiotics strains.

Fuzzy synthetic evaluation is used in decision-making about the levels of subjects or to group subjects into categories on the basis of fuzzy sets theory. The subjects are comprehensively judged using several parameters. An advantage of entropy method is that it enables the calculation of weight using few subject factors. Analytic hierarchy process method combines quantitative and qualitative analysis. The entropy method and the analytic hierarchy process are often combined with fuzzy synthetic evaluation in decision-making. Fuzzy synthetic evaluation models have been used for water quality assessment, air quality forecasting and portfolio selection [26-28]. The sensory scores of black pigmented rice wine fortified with probiotics were evaluated using fuzzy logic with classification as ‘not satisfactory’, ‘fair’, ‘medium’, ‘good’ and ‘excellent’[29].

In the presenting study, six antioxidative indices of 27 *L. plantarum* strains were measured according to previous protocols. A fuzzy synthetic evaluation model with an analytic hierarchy process and a fuzzy synthetic evaluation model with entropy weight were then performed to calculate the scores and ranks of the 27 probiotic strains. Finally,
three strains with high, middle and low antioxidative levels were selected and further examined in mice with D-gal-induced oxidative stress.

Materials and methods

Incubation of bacteria

27 strains of *L. plantarum* in this study were obtained from the Culture Collections of Food Microbiology (CCFM), Jiangnan University (Wuxi, China). These strains are presented as follows: CCFM10, CCFM11, CCFM232, CCFM242, CCFM362, CCFM382, CCFM595, CCFM634, CCFM173, CCFM639, CCFM308, CCFM309, CCFM411 HY6-2, JXJ6-12, RS14-4, RS44-1, QS1-2, DL4-2, DL8-2, RS32-1, RS16-1, RS15-3, QS6-1, RS70-1, RS35-1 and RS15-3. These strains were incubated in MRS broth, transferred three times, and then centrifuged at $6000 \times g$ for 10 minutes. After the cell pellets were washed three times with saline solution (0.85%, w/v), the cell pellets were resuspended in saline solution and adjusted to $10^9$ CFU/mL.

Chemicals and kits

DPPH, ethanol, 1, 10-Phenanthroline, L-cysteine hydrochloride and sodium ascorbate were obtained from Sigma-Aldrich (St. Louis, USA). Other chemicals were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). All the biochemical kits were from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China).
Measurement of antioxidative abilities

The method of analysis of DPPH scavenged by lactic acid bacteria was based on a previous study [30]. The only change was that 1 mL cell resuspension was added to 1 mL DPPH solution. In the blank, the cell resuspension was replaced with saline solution. The hydroxyl radical and superoxide radical scavenging ability of probiotics was measured using the methods [31]. The method by Li et al. was conducted to analyse the reducing power of probiotics [32]. The resistance of probiotics to 1 mmol/L hydrogen peroxide for eight hours was determined according to the method [33]. The measurement of the ferrous chelating ability of lactic acid bacteria was performed as per the study [32].

Fuzzy synthetic evaluation model

The overall antioxidative ability of the probiotic strains was scored and ranked using a fuzzy synthetic model. First, membership matrix $U$ was established.

$$
U = \begin{pmatrix}
  u_{11} & u_{12} & \cdots & u_{1n} \\
  u_{21} & u_{22} & \cdots & u_{2n} \\
  \vdots & \vdots & \ddots & \vdots \\
  u_{m1} & u_{m2} & \cdots & u_{mn}
\end{pmatrix} (i = 1, 2, \cdots m; j = 1, 2, \cdots n) \tag{1}
$$

where $m$ is the number of samples, and $n$ is the number of indices, $u_{ij}$ represents the value of indice $j$ of the sample $i$. $u_1$ is the scavenging ability of DPPH, $u_2$ is hydroxyl radical scavenging ability, $u_3$ is reducing activity, $u_4$ is superoxide radical scavenging ability, $u_5$ is resistance to hydrogen peroxide, and $u_6$ is the ferrous chelating ability.
Considering different antioxidative indices have different effects on the evaluation results, the corresponding weight vectors were set as \( \omega = (\omega_1, \omega_2, \omega_3, \omega_4, \omega_5, \omega_6) \). The Collection of evaluation was set as \( V = (v_1, v_2, v_3, v_4, v_5, v_6) \), and \( v_1, v_2, v_3, v_4 \) and \( v_5 \) represented five levels of antioxidative indices from the lowest to the highest, respectively. The data of antioxidative indices were firstly normalised, to which the bigger the better [34],

\[
x_y = \frac{u_y - \min_j \{u_y\}}{\max_j \{u_y\} - \min_j \{u_y\}}(i = 1, 2, \ldots m; j = 1, 2, \ldots n; 0 \leq x_y \leq 1) \tag{2}
\]

then the normalised matrix \( X \) was obtained

\[
X = \begin{pmatrix}
x_{11} & x_{12} & \cdots & x_{1n} \\
x_{21} & x_{22} & \cdots & x_{2n} \\
\vdots & \vdots & \ddots & \vdots \\
x_{m1} & x_{m2} & \cdots & x_{mn}
\end{pmatrix}
\tag{3}
\]

**Fuzzy synthetic model with analytic hierarchy process**

The steps were as follows. The evaluation matrix \( A \) was built by comparing the relative importance of any two antioxidative indices.

\[
A = (a_{ij})_{6 \times 6}, a_{ij} = 1/a_{ji}, i, j = 1, 2, 3, 4, 5, 6, a_{ii} = 1, \tag{4}
\]

And \( a_{ij} \) and \( a_{ji} \) were determined by comparing the importance of the index \( i \) and the index \( j \). The eigenvector corresponding to the largest eigenvalue was calculated by normalising the matrix \( A \). The largest eigenvalue was calculated as follows:

\[
\lambda_{\text{max}} = \sum_{i=1}^{n} \frac{(AW)_{ii}}{n\omega_i} \tag{5}
\]

If coincidence indicator \( C = \frac{\lambda_{\text{max}} - n}{n - 1} \), random coincidence rate \( C_R = C/R < 0.1 \), it meant that the evaluation matrix \( A \) was effective. The weight vector \( \omega \) was the
normalisation of the eigenvector. Due to \( v_1, v_2, v_3, v_4 \) and \( v_5 \) represented five levels of antioxidative evaluation from the lowest to the highest, the corresponding membership function for five levels was as follows:

\[
A_1(\chi) = \begin{cases} 
1 & 0 \leq \chi \leq 0.15 \\
0 & 0.15 < \chi \leq 0.25 \\
(0.25 - \chi)/(0.25 - 0.15) & 0.25 < \chi \leq 1 
\end{cases}
\]

\( (6) \)

\[
A_2(\chi) = \begin{cases} 
0 & 0 \leq \chi \leq 0.15 \\
(\chi - 0.15)/(0.25 - 0.15) & 0.15 < \chi \leq 0.25 \\
1 & 0.25 < \chi \leq 0.35 \\
(0.45 - \chi)/(0.45 - 0.35) & 0.35 < \chi \leq 0.45 \\
0 & 0.45 < \chi \leq 1 
\end{cases}
\]

\( (7) \)

\[
A_3(\chi) = \begin{cases} 
0 & 0 \leq \chi \leq 0.35 \\
(\chi - 0.35)/(0.45 - 0.35) & 0.35 < \chi \leq 0.45 \\
1 & 0.45 < \chi \leq 0.55 \\
(0.65 - \chi)/(0.65 - 0.55) & 0.55 < \chi \leq 0.65 \\
0 & 0.65 < \chi \leq 1 
\end{cases}
\]

\( (8) \)

\[
A_4(\chi) = \begin{cases} 
0 & 0 \leq \chi \leq 0.55 \\
(\chi - 0.55)/(0.65 - 0.55) & 0.55 < \chi \leq 0.65 \\
1 & 0.65 < \chi \leq 0.75 \\
(0.85 - \chi)/(0.85 - 0.75) & 0.75 < \chi \leq 0.85 \\
0 & 0.85 < \chi \leq 1 
\end{cases}
\]

\( (9) \)

\[
A_5(\chi) = \begin{cases} 
0 & 0 \leq \chi \leq 0.75 \\
(\chi - 0.75)/(0.85 - 0.75) & 0.75 < \chi \leq 0.85 \\
1 & 0.85 < \chi \leq 1 
\end{cases}
\]

\( (10) \)

\( A_1(\chi), A_2(\chi), A_3(\chi), A_4(\chi) \) and \( A_5(\chi) \) represented membership function for five levels of the overall antioxidative abilities of \( L. \text{plantarum} \). The figure for membership function was shown in Figure 1. The values of six antioxidative indices were brought into membership function, and fuzzy evaluation matrix \( R_k \) was then gained. The overall antioxidative abilities of certain strain was evaluated by weight average model \( M(\bullet, \star, \otimes, \oplus, \ominus, \Delta) \).
\[ B = \omega \cdot R_k, \quad (k = 1, 2, \ldots, 27). \]

**Fuzzy synthetic evaluation with entropy weight theory**

For the normalised membership matrix (3), the definition of entropy was as per the formula

\[ S_j = -k \sum_{i=1}^{m} r_{ij} \ln r_{ij}, (j = 1, 2, \cdots, n), \quad k = \frac{1}{\ln m} \quad (11) \]

In which

\[ r_{ij} = \frac{x_{ij}}{\sum_{j=1}^{m} x_{ij}} (i = 1, 2, \cdots, 27; j = 1, 2, 3, 4, 5, 6) \quad (12) \]

The entropy weight was calculated using

\[ \omega_j = \left(1 - S_j\right) / \left(n - \sum_{j=1}^{n} S_j\right), \quad \sum_{j=1}^{n} \omega_j = 1 \quad (13) \]

The weight vector was gained from the entropy weight. Other calculation processes were the same as those in part 2.4.2.

**Antioxidative abilities in vivo**

A D-gal-induced oxidative stress mice model was established to examine the antioxidative abilities of probiotics according to previous studies [35, 36]. Eight-week-old male BLAB/c mice were purchased from Shanghai Laboratory Animal Centre (Shanghai, China). These mice were housed in a standard room at 22±1°C and 50% humidity, and were given free access to food and water during the trial period. All protocols for animal trials were approved by the Ethics Committee of Jiangnan University (JN No. 20161011-20161211-70) and complied with EU guidelines (Directive 2010/63/EU).

After one-week adaptation, 60 mice were randomly divided into six groups of 10
mice. Mice in the model, intervention and positive groups were treated with subcutaneous injection of 1.2 g/kg body weight D-gal, and mice in the control group were subcutaneously injected with the same volume of saline. At the same time, the control group and D-gal group mice were given skimmed milk orally. The positive group were given sodium ascorbate (50 mg/kg body weight) orally, whereas mice in the three probiotics groups were given *L. plantarum* CCFM10, CCFM242 and RS15-3 daily via intragastric gavage ($10^9$ CFU/mice). The antioxidative abilities of *L. plantarum* CCFM10, CCFM242 and RS15-3 were high, middle and low respectively (*Table 1*). After eight weeks, all the mice were sacrificed following carbon dioxide anaesthesia. The blood of the mice were obtained for biochemical experiments.

Levels of serum SOD, catalase (CAT), GSH, GSH-PX and TAC were measured using corresponding kits from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China).

**Results**

**Antioxidative abilities in vitro**

The antioxidative abilities of 27 strains of *L. plantarum in vitro* are shown in *Table 2*. The DPPH radicals scavenging abilities of probiotics ranged from 11.17% (CCFM383) to 40.13% (CCFM639). The hydroxyl radical scavenging activity of *L. plantarum* CCFM362 was more than five times that of RS35-10 (61.74% VS 12.02%), while the difference in superoxide radical scavenging among the 27 strains was not
large (from 37.10% to 67.70%). Reducing activities were expressed with equivalent
cysteine, from 16.43 to 114.37 μmol/L. Their survival rate in 1 mmol/L H₂O₂ for 8 h
varied greatly, from 92.18% to 0.00%. The survival rate of four strains was zero,
suggesting that the number was decreased by at least four orders of magnitude. The
Fe²⁺ chelating abilities of *L. plantarum* strains ranged from 0.7 mg/kg (RS35-10) to
4.34 mg/kg (CCFM362).

**Fuzzy synthetic evaluation model**

**Fuzzy synthetic model with analytic hierarchy process**

The normalised antioxidative data are shown in Supplementary Table 1. We
estimated the relative significance of the antioxidative indices (Supplementary Table
2), which were composed of matrix A.

\[
\mathbf{A} = \begin{pmatrix}
1 & 1/3 & 1/2 & 2 & 4 & 1/4 \\
3 & 1 & 3 & 4 & 6 & 1/2 \\
2 & 1/3 & 1 & 3 & 5 & 1/3 \\
1/2 & 1/4 & 1/3 & 1 & 3 & 1/5 \\
1/4 & 1/6 & 1/5 & 1/3 & 1 & 1/7 \\
4 & 2 & 3 & 5 & 7 & 1
\end{pmatrix}
\]

(13)

The eigenvector \( \mathbf{\omega} \) of matrix A was (0.104, 0.266, 0.153, 0.069, 0.034, 0.374)\(^T\).
The largest eigenvalue \( \lambda_{\text{max}} \) was 6.223. The coincidence rate \( C_R = 0.036<0.1 \)
suggesting that the setting of relative significances was appropriate. The weight vector
was therefore (0.104, 0.266, 0.153, 0.069, 0.034, 0.374). The membership of each
index was calculated on the basis of membership function. Taking *L. plantarum*
CCFM10 as examples, the membership of DPPH radicals scavenging ability was \( r_{ij} = \)
(0, 0, 0.7, 0.3, 0), the membership of hydroxyl radicals scavenging was \( r_{2j} = (0, 0, 0, 0, 1) \), the membership of reducing activity was \( r_{3j} = (0, 0, 0, 0.8, 0.2) \), the membership of super superoxide radical scavenging was \( r_{4j} = (0, 0, 1, 0, 0) \), the membership of resistant to \( H_2O_2 \) was \( r_{5j} = (0, 0, 0, 0, 1) \) and the membership of Fe2+ chelating abilities was \( r_{6j} = (0, 0, 0, 0.2, 0.8) \). The membership matrix of CCFM10 was as follows:

\[
R1 = \begin{pmatrix}
0 & 0 & 0.7 & 0.3 & 0 \\
0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0.8 & 0.2 \\
0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0.2 & 0.8
\end{pmatrix}
\]

(14)

The synthetic evaluation was conducted by the formula \( B = \omega \times R_k \). The \( B_1 \) of CCFM10 was \((0, 0, 0.142, 0.228, 0.630)\). If the evaluation \( V \) was set with \((0.1, 0.3, 0.5, 0.7, 0.9)\), the score of CCFM10 was \((0, 0, 0.142, 0.228, 0.630) \times (0.1, 0.3, 0.5, 0.7, 0.9)^T = 0.798 \). The scores and ranks of the 27 strains are shown in Table 4.

**Fuzzy synthetic evaluation with entropy weight theory**

The weight vector was \( \omega = (0.039, 0.124, 0.116, 0.013, 0.585, 0.123) \) obtained by calculating of entropy weight. With the same membership functions, synthetic evaluation \( B_1 \) of CCFM10 was observed as follows: \( B_1 = (0, 0, 0.041, 0.129, 0.830) \).

The score of \( L. plantarum \) CCFM10 was 0.8578. Other results are shown in Table 4.

**Antioxidative abilities in vivo**

Mice were given \( L. plantarum \) CCFM10, CCFM242 and RS15-3, the highest, the middle and lowest scorers respectively in the fuzzy evaluation models, to examine their antioxidative abilities in vivo. D-gal-induced mice experienced a decrease in
antioxidative enzyme and non-enzyme levels including SOD, CAT, GSH-PX and GSH, although only the alteration of CAT activity was significant \( (P < 0.05) \) (Figure 2). D-gal administration also caused a decrease in plasma TAC, although it was not significant. Compared to the D-gal group, *L. plantarum* CCFM10 and CCFM242 intervention significantly improved the contents of serum GSH, and the levels of CAT, SOD and GSH-PX \( (P < 0.05) \), while RS15-3 nearly had no effects on these serum parameters. Only the strain CCFM10 induced an increase in the level of TAC. Moreover, the antioxidative ability of CCFM10 was higher than that of CCFM242. Actually, mice in CCFM10 group had significant higher levels of CAT and GSH compared to the control group \( (P<0.05) \). The positive sodium ascorbate group was comprehensively protected against D-gal-induced oxidative stress, whose effects were similar to those of *L. plantarum* CCFM242.

**Discussion**

The traditional evaluation of antioxidative ability of probiotics usually involves physicochemical reaction *in vitro*, including scavenging ROS, inhibiting oxidation of lipids or ascorbic acid, chelating metal ion and measuring reduction activity. Besides, cellular models are also used to determine antioxidant activities of lactobacilli [37]. The major disadvantage of these methods *in vitro* is that they cannot reveal the effects of probiotics on oxidative stress *in vivo*. Because there is a great difference between reaction system and internal environment. For instance, as one of artificial ROS, DPPH
does not actually exist in the living. Another important reason is that each assay only reveals one aspect of probiotic antioxidative activities. A synthetic evaluation might provide more reasonable results.

In this study, the antioxidative activities of 27 *L. plantarum* strains were characterized using six of the most widespread methods *in vitro*. Their DPPH scavenging abilities ranged from about 11% to 43%, which are only lower than a few *L. plantarum* strains [23, 38]. The largest difference was observed in the resistance to \( \text{H}_2\text{O}_2 \) among these probiotics, whereas the smallest was in the hydroxyl radical scavenging activity. Obviously, these antioxidant data are inconstant among six indices.

It is necessary to establish a comprehensive method for evaluating the overall probiotic antioxidant.

After the measurement of antioxidant abilities of probiotics, two fuzzy synthetic models were used to evaluate the overall antioxidative of probiotics. Obviously, the main difference between the two fuzzy synthetic models was the calculation of weight vector \( \mathbf{w} \). For the model with the analytic hierarchy process, the weight vector was obtained on the basis of the relative importance of six antioxidative indices. This model has a limitation: the determination of relative importance was subjective. The weight vector of the entropy model was calculated using entropy and entropy weight with less subjective factors, but this method did not take into consideration the relative importance of antioxidative indices. Although there were some differences between the ranks of fuzzy synthetic evaluation with an analytic hierarchy process and with entropy
theory, the ranks of CCFM10, CCFM362 and RS70-1 were ranked in the top five by both models. CCFM242 and CCFM382 were ranked in the middle. CCFM309 and RS15-3 were ranked in the bottom five for the two models (Table 3). Both fuzzy synthetic evaluation models had the same membership functions.

D-gal-induced mimetic aging model was used to testify the results obtained from two fuzzy synthetic models. As one of common oxidative stress models, D-gal-induced model is widely applied in examining the effects of antioxidative substances on oxidative injury, especially for the evaluation of antioxidative activities of probiotics [35, 36, 39]. The examination in D-gal model showed that the effects of three probiotic strains on oxidative stress are positively associated with their antioxidative activities in vitro, suggesting that synthetic evaluation of antioxidative parameters in vitro could be better to estimate the antioxidative effects of probiotic antioxidants than sole indices.

Moreover, although all three strains belong to the species L. plantarum, the antioxidative ability of CCFM10 was higher than that of CCFM242 and RS15-3 perhaps which are related to certain genes. Three antioxidant-related genes from L. plantarum MA2 were supposed to be responsible for resistant to H2O2 challenge [40]. Therefore, it is necessary to further identify antioxidant-related genes through comparative genomics in future research.

**Conclusion**

After the extracorporeal antioxidative abilities of 27 L. plantarum were
determined with six assays, two fuzzy synthetic evaluation models were used to evaluate the overall antioxidative abilities of these strains. Although there was some difference between the two models, the highest scoring strain (CCFM10), the middle (CCFM242) and the lowest scoring strain (RS15-3) were obtained by both models. The antioxidative abilities of three strains further examined in mice demonstrated that the effects of probiotics on oxidative stress were positively correlated with their antioxidative results *in vitro*. The results also suggest that evaluation of probiotic antioxidants with fuzzy synthetic models has great advantages over previous methods. Evaluation of antioxidative activities of probiotics with fuzzy synthetic models might provide a more efficient method for measuring the antioxidative of probiotics and could be further improved in future studies.

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**Conflict of interest**

All the authors declared that they have no conflict of interest.


Figure captions

**Figure 1** Membership function for five levels

**Figure 2** Effects of *L. plantarum* CCFM10, CCFM242 and RS15-3 on serum antioxidative parameters: (A) SOD activity; (B) CAT activity; (C) GSH contents; (D) GSH-PX activity; (E) TAC. Values are mean±SEM per group. Significant differences (P<0.05) were indicated with subscripts a and b. D-gal, D-galactose; SA, sodium ascrobate.
Table 1 Animal experimental protocol

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (8 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>Saline s.i. +skimmed milk i.g.¹</td>
</tr>
<tr>
<td>D-galactose (n=10)</td>
<td>1.2 g/kg D-gal s.i. +skimmed milk i.g.</td>
</tr>
<tr>
<td>LAB with high antioxidative (n=10)</td>
<td>1.2 g/kg D-gal s.i. +<em>L. plantarum</em> CCFM10 (10⁹ CFU) i.g.</td>
</tr>
<tr>
<td>LAB with middle antioxidative (n=10)</td>
<td>1.2 g/kg D-gal s.i.+<em>L. plantarum</em> CCFM242 (10⁹ CFU) i.g.</td>
</tr>
<tr>
<td>LAB with low antioxidative (n=10)</td>
<td>1.2 g/kg D-gal s.i.+<em>L. plantarum</em> RS15-3 (10⁹ CFU) i.g.</td>
</tr>
<tr>
<td>Sodium ascorbate (n=10)</td>
<td>1.2 g/kg D-gal s.i.+ Sodium ascorbate (50 mg/kg) i.g.</td>
</tr>
</tbody>
</table>

¹ s.i., subcutaneous injection; i.g., intragastric gavage
## Table 2 Antioxidative abilities of 27 LAB strains

<table>
<thead>
<tr>
<th>Strains</th>
<th>DPPH radicals (%)</th>
<th>Hydroxyl radical scavenging (%)</th>
<th>Reducing activity (μmol/L)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Superoxide radical scavenging (%)</th>
<th>Resistant to hydrogen peroxide (%)</th>
<th>Fe&lt;sup&gt;2+&lt;/sup&gt; chelating ability (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCFM10</td>
<td>27.54±1.72</td>
<td>59.47±10.38</td>
<td>91.83±1.74</td>
<td>51.26±4.24</td>
<td>92.18±2.34</td>
<td>4.33±0.24</td>
</tr>
<tr>
<td>CCFM11</td>
<td>22.31±2.12</td>
<td>36.03±9.74</td>
<td>45.55±0.56</td>
<td>48.86±4.06</td>
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<sup>a</sup> Values are mean±SEM for at least three times per group.

<sup>b</sup> Reducing activity was expressed with equivalent cysteine (μmol/L)
### Table 3 Scores and ranks of three fuzzy synthetic evaluation models

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<tr>
<th>Strains</th>
<th>Model 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Model 2&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup> Model 1 is fuzzy synthetic evaluation with analytic hierarchy process;

<sup>b</sup> Mode 2 is fuzzy synthetic evaluation with entropy theory.
Fig. 1. Membership function for five levels
Fig. 2. Effects of L. plantarum CCFM10, CCFM242 and RS15-3 on serum antioxidative parameters: (A) SOD activity; (B) CAT activity; (C) GSH contents; (D) GSH-PX activity; (E) TAC.