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Evaluation of Antioxidative Effects of *Lactobacillus plantarum* with Fuzzy Synthetic Models

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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 27 *Lactobacillus plantarum* strains isolated from fermented foods were determined in terms of 2,2-diphenyl-1-picrylhydrazyl, hydroxyl radical, and superoxide radical scavenging abilities, 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. Examination of the antioxidative abilities of these 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 the 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, antioxidation, mouse 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 probiotic administration improved oxidative stress in a D-galactose (D-gal)-induced mouse 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 randomized 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 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 the potential probiotics, *Lactobacillus plantarum* is 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*. [20] found that soymilk with *L. plantarum* A7 significantly increased SOD activities in type 2 diabetic patients. *L. plantarum* C88 administration also improved serum SOD activity, hepatic glutathione (GSH) and total antioxidant capacity (TAC), whereas the 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 and 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 best knowledge, the most common tests are as follows: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, hydroxyl radical scavenging activity, 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, and oxygen radical absorbance capacity 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 probiotic 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 set theory. The subjects are comprehensively judged using several parameters. An advantage of the entropy method is that it enables the calculation of weight using few subject factors. The analytic hierarchy process method combines quantitative and qualitative analyses. 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 present 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

The 27 strains of *L. plantarum* in used 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 activated in de Man, Rogosa, Sharpe (MRS) broth, successively transferred to MRS broth three times and incubated for 16 h, and then centrifuged at 6,000 ×*g* for 10 min. 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 (USA). Other chemicals were purchased from Sinopharm Chemical Reagent Company (China). All the biochemical kits were from Nanjing Jiancheng Institute of Biotechnology (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 of cell suspension was added to 1 ml of DPPH solution. In the blank, the cell suspension was replaced with saline solution. The hydroxyl radical and superoxide radical scavenging abilities of probiotics were measured using previously described methods [31]. The method by Lin and Yen [32] was conducted to analyze the reducing power of probiotics. The resistance of probiotics to 1 mmol/l hydrogen peroxide for 8 h was determined according to a previously described method [33]. The measurement of the ferrous chelating ability of lactic acid bacteria was performed as previously described [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.

$$U = (\boldsymbol{u}_1\ \boldsymbol{u}_2\ \cdots\ \boldsymbol{u}_n) = \begin{pmatrix} u_{11} & u_{12} & \cdots & u_{1n} \\ u_{21} & u_{22} & \cdots & u_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ u_{m1} & u_{m2} & \cdots & u_{mn} \end{pmatrix} (i = 1, 2, \cdots m; j = 1, 2, \cdots n) \quad (1)$$

where m is the number of samples, n is the number of indices, and 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 the reducing activity, u_4 is the superoxide radical scavenging ability, u_5 is the 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 $\boldsymbol{V} = (v_1, v_2, v_3, v_4, v_5, v_6)$, where v_1, v_2, v_3, v_4, and v_5 represent five levels of antioxidative indices from the lowest to the highest, respectively. The data of antioxidative indices were first normalized, to which the bigger the better [34],

$$x_{ij} = (u_{ij} - \min_j \{u_{ij}\}) / (\max_j \{u_{ij}\} - \min_j \{u_{ij}\})$$
$$(i = 1, 2, \cdots m; j = 1, 2, \cdots n; 0 \le x_{ij} \le 1) \quad (2)$$

then the normalized matrix X was obtained:

$$X = \begin{pmatrix} x_{11} & x_{12} & \cdots & x_{1n} \\ x_{21} & x_{22} & \cdots & x_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ x_{m1} & x_{m2} & \cdots & x_{mn} \end{pmatrix} (i = 1, 2, \cdots m; j = 1, 2, \cdots n) \quad (3)$$

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.

$$A = (a_{ij})_{6\times6},\ a_{ij} = 1/a_{ji},\ i, j = 1,2,3,4,5,6,\ a_{ii} = 1, \quad (4)$$

where 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 normalizing the matrix A. The largest eigenvalue was calculated as follows:

$$\lambda_{\max} = \sum_{i=1}^{n} \frac{(AW)_i}{n\omega_i} \quad (5)$$

If the coincidence indicator $C = \frac{\lambda_{\max} - n}{n-1}$, the random coincidence rate $C_R = C/R < 0.1$, which meant that the evaluation matrix A was effective. The weight vector w was the normalization of the eigenvector. Because 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 the five levels was as follows:

$$\boldsymbol{A}_1(\chi) = \begin{cases} 1 & 0 \le \chi \le 0.15 \\ (0.25 - \chi)/(0.25 - 0.15) & 0.15 < \chi \le 0.25 \\ 0 & 0.25 < \chi \le 1 \end{cases} \quad (6)$$

$$\boldsymbol{A}_2(\chi) = \begin{cases} 0 & 0 \le \chi \le 0.15 \\ (\chi - 0.15)/(0.25 - 0.15) & 0.15 < \chi \le 0.25 \\ 1 & 0.25 < \chi \le 0.35 \\ (0.45 - \chi)/(0.45 - 0.35) & 0.35 < \chi \le 0.45 \\ 0 & 0.45 < \chi \le 1 \end{cases} \quad (7)$$

$$\boldsymbol{A}_3(\chi) = \begin{cases} 0 & 0 \le \chi \le 0.35 \\ (\chi - 0.35)/(0.45 - 0.35) & 0.35 < \chi \le 0.45 \\ 1 & 0.45 < \chi \le 0.55 \\ (0.65 - \chi)/(0.65 - 0.55) & 0.55 < \chi \le 0.65 \\ 0 & 0.65 < \chi \le 1 \end{cases} \quad (8)$$

$$\boldsymbol{A}_4(\chi) = \begin{cases} 0 & 0 \le \chi \le 0.55 \\ (\chi - 0.55)/(0.65 - 0.55) & 0.55 < \chi \le 0.65 \\ 1 & 0.65 < \chi \le 0.75 \\ (0.85 - \chi)/(0.85 - 0.75) & 0.75 < \chi \le 0.85 \\ 0 & 0.85 < \chi \le 1 \end{cases} \quad (9)$$

$$\boldsymbol{A}_5(\chi) = \begin{cases} 0 & 0 < \chi \le 0.75 \\ (\chi - 0.75)/(0.85 - 0.75) & 0.75 < \chi \le 0.85 \\ 1 & 0.85 < \chi \le 1 \end{cases} \quad (10)$$

$A_1(\chi)$, $A_2(\chi)$, $A_3(\chi)$, $A_4(\chi)$, and $A_5(\chi)$ represent the membership function for five levels of the overall antioxidative abilities of *L. plantarum*. The figure for the membership function is shown in Fig. 1. The values of six antioxidative indices were brought into the membership function, and the fuzzy evaluation matrix R_k was then gained. The overall antioxidative abilities of a certain strain

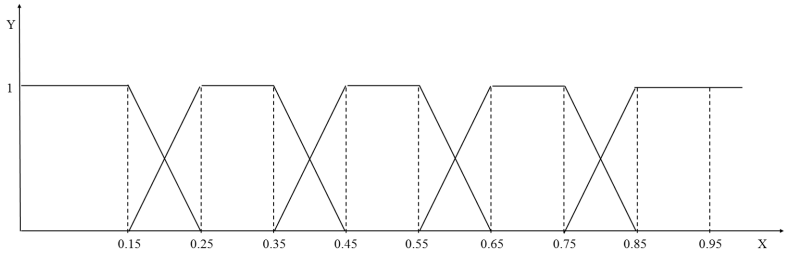

Fig. 1. Membership function for five levels.

was evaluated by the weight average model $M(\cdot, +)$: $B = \omega \cdot R_k$, (k = 1, 2, …, 27).

Fuzzy Synthetic Evaluation with Entropy Weight Theory

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

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

$$\text{where } r_{ij} = x_{ij} / \sum_{i=1}^{m} x_{ij} \; (i = 1,2\cdots,27; j = 1,2,3,4,5,6) \quad (12)$$

The entropy weight was calculated using

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

The weight vector was gained from the entropy weight. Other calculation processes were the same as those described in the previous section.

Antioxidative Abilities In Vivo

A D-gal-induced oxidative stress mouse model was established to examine the antioxidative abilities of probiotics according to previous studies [35, 36]. Eight-week-old male BALB/c mice were purchased from Shanghai Laboratory Animal Centre (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 1-week adaptation, 60 mice were randomly divided into six groups of 10 mice. Mice in the model, intervention, and positive groups were treated with a 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 were given skimmed milk orally. The positive group was given sodium ascorbate (50 mg/kg body weight) orally, whereas mice in the three probiotic 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 anesthesia. 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 (China).

Results

Antioxidative Abilities In Vitro

The antioxidative abilities of 27 strains of *L. plantarum* in vitro are shown in Table 2. The DPPH radical 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%), whereas 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 H_2O_2 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 Fe^{2+} 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 normalized antioxidative data are shown in Table S1. We estimated the relative significance of the antioxidative indices (Table S2), which were composed of matrix A.

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

Table 1. Animal experimental protocol.

Group	Treatment (8 weeks)
Control (n = 10)	Saline s.i. + skimmed milk i.g.[a]
D-Galactose (n = 10)	1.2 g/kg D-gal s.i. + skimmed milk i.g.
LAB with high antioxidative activity (n = 10)	1.2 g/kg D-gal s.i. + *L. plantarum* CCFM10 (10^9 CFU) i.g.
LAB with middle antioxidative activity (n = 10)	1.2 g/kg D-gal s.i.+ *L. plantarum* CCFM242 (10^9 CFU) i.g.
LAB with low antioxidative activity (n = 10)	1.2 g/kg D-gal s.i.+ *L. plantarum* RS15-3 (10^9 CFU) i.g.
Sodium ascorbate (n = 10)	1.2 g/kg D-gal s.i.+ sodium ascorbate (50 mg/kg) i.g.

[a]s.i., subcutaneous injection; i.g., intragastric gavage.

Table 2. Antioxidative abilities of 27 LAB strains[a].

Strains	DPPH radicals (%)	Hydroxyl radical scavenging (%)	Reducing activity (μmol/l)[b]	Superoxide radical scavenging (%)	Resistant to hydrogen peroxide (%)	Fe^{2+} chelating ability (mg/kg)
CCFM10	27.54 ± 1.72	59.47 ± 10.38	91.83 ± 1.74	51.26 ± 4.24	92.18 ± 2.34	4.33 ± 0.24
CCFM11	22.31 ± 2.12	36.03 ± 9.74	45.55 ± 0.56	48.86 ± 4.06	77.78 ± 5.12	1.88 ± 0.18
CCFM232	19.46 ± 2.58	18.08 ± 3.02	63.31 ± 2.18	45.86 ± 3.61	55.42 ± 1.33	1.98 ± 0.41
CCFM242	15.17 ± 2.04	39.18 ± 11.04	41.42 ± 2.35	45.76 ± 3.76	34.56 ± 2.19	1.79 ± 0.29
CCFM362	15.81 ± 1.78	61.74 ± 3.88	40.98 ± 1.48	49.94 ± 2.68	74.58 ± 5.18	4.34 ± 0.73
CCFM382	11.17 ± 0.99	46.53 ± 7.39	54.02 ± 1.29	37.69 ± 4.82	22.84 ± 2.26	2.34 ± 0.46
CCFM595	10.15 ± 2.85	35.04 ± 7.60	16.43 ± 1.20	39.50 ± 5.71	46.93 ± 1.89	1.13 ± 0.09
CCFM634	21.55 ± 1.47	51.77 ± 7.10	50.50 ± 1.29	46.09 ± 3.74	1.91 ± 0.02	1.53 ± 0.03
CCFM173	15.97 ± 0.86	13.30 ± 1.38	31.90 ± 0.62	34.11 ± 6.06	71.83 ± 6.33	1.58 ± 0.46
HY 6-2	13.54 ± 0.67	24.36 ± 7.00	114.37 ± 0.35	44.87 ± 6.74	7.92 ± 0.25	2.27 ± 0.12
JXJ6-12	28.76 ± 1.05	54.25 ± 2.69	34.63 ± 3.18	64.84 ± 1.63	0.76 ± 0.01	5.08 ± 1.99
RS14-4	28.15 ± 2.80	20.52 ± 5.35	24.43 ± 4.90	55.79 ± 1.48	7.13 ± 0.51	2.33 ± 0.12
RS44-1	28.37 ± 1.43	25.82 ± 3.96	31.14 ± 1.36	55.31 ± 0.30	0.21 ± 0.00	2.14 ± 0.34
QS1-2	23.89 ± 2.77	43.73 ± 3.66	30.27 ± 1.42	45.30 ± 2.44	0.00 ± 0.00	3.54 ± 0.26
DL4-2	25.16 ± 2.83	52.06 ± 5.97	30.63 ± 3.55	54.84 ± 1.97	0.00 ± 0.00	2.81 ± 0.21
DL8-2	27.04 ± 1.86	49.77 ± 6.19	44.51 ± 2.96	49.77 ± 1.30	14.88 ± 0.91	3.15 ± 0.09
RS32-1	29.40 ± 2.36	41.78 ± 6.27	33.38 ± 3.75	67.70 ± 1.30	0.21 ± 0.01	3.12 ± 0.22
RS16-1	27.30 ± 1.60	19.35 ± 1.10	22.59 ± 5.21	43.47 ± 1.13	41.49 ± 1.42	3.66 ± 0.27
RS15-3	26.76 ± 2.12	15.55 ± 1.96	19.33 ± 3.00	49.25 ± 3.63	0.20 ± 0.00	2.27 ± 0.06
QS6-1	27.00 ± 1.37	29.36 ± 1.41	16.52 ± 2.37	57.26 ± 0.74	0.00 ± 0.00	2.37 ± 0.10
CCFM639	40.13 ± 3.34	13.49 ± 1.13	56.35 ± 4.67	46.52 ± 0.70	51.51 ± 3.10	1.82 ± 0.41
CCFM308	23.16 ± 1.17	12.39 ± 1.30	32.01 ± 3.20	39.41 ± 1.20	67.44 ± 3.51	0.60 ± 0.08
CCFM309	23.80 ± 1.66	16.94 ± 2.13	38.85 ± 1.86	37.10 ± 1.73	1.10 ± 0.00	0.96 ± 0.12
CCFM411	22.81 ± 1.93	19.80 ± 2.74	42.75 ± 3.97	48.00 ± 1.64	77.56 ± 4.27	0.65 ± 0.08
RS70-1	23.44 ± 2.60	49.57 ± 2.47	44.18 ± 4.69	50.21 ± 2.07	72.89 ± 3.25	3.10 ± 0.07
RS35-10	19.64 ± 2.16	12.02 ± 1.92	18.31 ± 4.48	41.30 ± 1.94	53.77 ± 2.13	0.70 ± 0.10
RS41-7	23.15 ± 1.52	17.55 ± 3.39	40.43 ± 3.27	42.11 ± 1.53	0.00 ± 0.00	1.35 ± 0.14

[a]Values are the mean ± SEM for at least three replicates per group.

[b]Reducing activity was expressed as equivalent cysteine (μmol/l).

The eigenvector ω of matrix A was $(0.104, 0.266, 0.153, 0.069, 0.034, 0.374)^T$. The largest eigenvalue λ_{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 an example, the membership of DPPH radical scavenging ability was $r_{1j} = (0, 0, 0.7, 0.3, 0)$, the membership of hydroxyl radical 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 resistance to H_2O_2 was $r_{5j} = (0, 0, 0, 0, 1)$, and the membership of Fe^{2+} chelating abilities was $r_{6j} = (0, 0, 0, 0.2, 0.8)$. The membership matrix of CCFM10 was as follows:

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

The synthetic evaluation was conducted by the formula $B = \omega \cdot R_k$. 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

Table 3. Scores and ranks of three fuzzy synthetic evaluation models.

Strains	Model 1[a]		Model 2[b]	
	Scores	Ranks	Scores	Ranks
CCFM10	0.7976	1	0.8581	1
CCFM11	0.3978	11	0.6711	3
CCFM232	0.2876	19	0.4739	7
CCFM242	0.338	15	0.3420	14
CCFM362	0.6882	3	0.7454	2
CCFM382	0.411	9	0.3560	13
CCFM595	0.2214	21	0.3839	11
CCFM634	0.4038	10	0.2378	21
CCFM173	0.1862	24	0.5088	6
HY 6-2	0.3792	13	0.2497	20
JXJ6-12	0.7356	2	0.3383	15
RS14-4	0.309	18	0.1667	24
RS44-1	0.3208	17	0.1770	23
QS1-2	0.534	5	0.2639	17
DL4-2	0.5198	7	0.2611	18
DL8-2	0.5318	6	0.2875	16
RS32-1	0.5118	8	0.2513	19
RS16-1	0.3976	12	0.4276	10
RS15-3	0.259	20	0.1503	25
QS6-1	0.3504	14	0.1859	22
CCFM639	0.3376	16	0.4417	9
CCFM308	0.1622	26	0.4676	8
CCFM309	0.1954	23	0.1445	26
CCFM411	0.2204	22	0.5994	5
RS70-1	0.5432	4	0.6696	4
RS35-10	0.1446	27	0.3785	12
RS41-7	0.166	25	0.1342	27

[a]Model 1 is fuzzy synthetic evaluation with the analytic hierarchy process.

[b]Mode 2 is fuzzy synthetic evaluation with entropy weight theory.

score of CCFM10 was (0, 0, 0.142, 0.228, 0.630) $(0.1, 0.3, 0.5, 0.7, 0.9)^T = 0.798$. The scores and ranks of the 27 strains are shown in Table 3.

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 the 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 3.

Antioxidative Abilities In Vivo

Mice were given *L. plantarum* CCFM10, CCFM242, and RS15-3, the highest, middle and lowest scorers, respectively, in the fuzzy evaluation models, to examine their antioxidative abilities in vivo. The 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 ($p < 0.05$) (Fig. 2). D-Gal administration also caused a decrease in plasma TAC, although it was not significant. Compared with 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 ($p < 0.05$), whereas RS15-3 nearly had no effects on these serum parameters. Only strain CCFM10 induced an increase in the level of TAC. Moreover, the antioxidative ability of CCFM10 was higher than that of CCFM242. In fact, mice in the CCFM10 group had significantly higher levels of CAT and GSH compared with 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 a physicochemical reaction in vitro, including scavenging ROS, inhibiting oxidation of lipids or ascorbic acid, chelating metal ion, and measuring reduction activity. Besides these, cellular models are also used to determine the 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 the reaction system and internal environment. For instance, as an artificial ROS, DPPH does not actually exist in living cells. 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 H_2O_2 among these probiotics, whereas the smallest was in the hydroxyl radical scavenging activity. Obviously, these antioxidant data are inconstant among the six indices. It is necessary to establish a

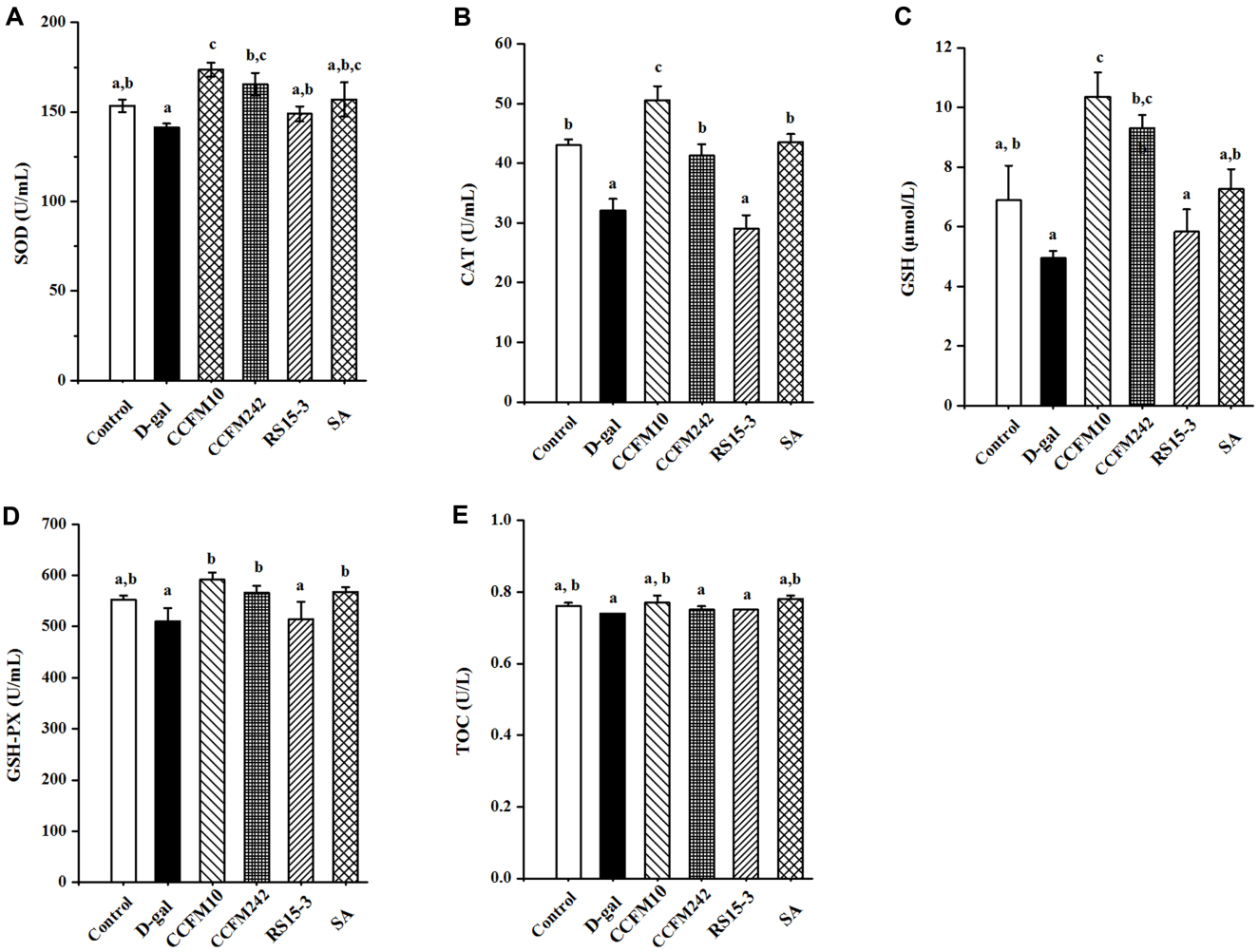

Fig. 2. Effects of *L. plantarum* CCFM10, CCFM242, and RS15-3 on serum antioxidative parameters.
(**A**) SOD activity; (**B**) CAT activity; (**C**) GSH contents; (**D**) GSH-PX activity; (**E**) total antioxidant capacity (TAC). Values are the mean ± SEM per group. Significant differences ($p < 0.05$) are indicated with subscripts a and b. D-gal, D-galactose; SA, sodium ascorbate.

comprehensive method for evaluating the overall probiotic antioxidant.

After measurement of the antioxidant abilities of the probiotics, two fuzzy synthetic models were used to evaluate the overall antioxidative activity of probiotics. Obviously, the main difference between the two fuzzy synthetic models was the calculation of weight vector ω. 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.

The D-gal-induced mimetic aging model was used to verify the results obtained from the two fuzzy synthetic models. As one of the common oxidative stress models, the 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 the D-gal model showed that the effects of the 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 resistance to H_2O_2 challenge [40]. Therefore, it is necessary to further identify antioxidant-related genes through comparative genomics in future research.

In conclusion, after the extracorporeal antioxidative abilities of 27 *L. plantarum* strains 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 (CCFM10), middle (CCFM242), and lowest (RS15-3) scoring strains were obtained by both models. The antioxidative abilities of the 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 ability of probiotics and could be further improved in future studies.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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