Free radical scavenging ability of soybean milk fermented by Lactobacillus plantarum YS4 isolated from yak yoghurt in vitro

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Abstract. The free radical scavenging ability of Lactobacillus plantarum YS4 isolated from naturally fermented yak yoghurt was tested in vitro. DPPH radical (1,1-diphenyl-2-trinitrophenylhydrazine radical), ABTS radical (2,2-diazobis (3-ethyl-benzothiazol-6-sulfonic acid) diammonium salt radical) and hydroxyl radical were detected in vitro. The results showed that the DPPH free radical, ABTS free radical and hydroxyl free radical scavenging abilities of soybean milk fermented by Lactobacillus plantarum YS4 were stronger than those fermented by Lactobacillus bulgaricus, and the free radical scavenging ability of soybean milk fermented by Lactobacillus plantarum YS4 and Lactobacillus bulgaricus was better than that of unfermented soybean milk.

1. Introduction
Yak yoghurt is a natural fermented dairy product consumed by ethnic herdsmen on the Qinghai Tibet Plateau. Because of the particularity of its region, yak milk contains more nutrients than ordinary milk [2], and microorganisms have better activity after natural screening in plateau environment [3]. Yak yoghurt obtained by natural fermentation of yak milk by these microorganisms has good quality, rich nutrients, and is more conducive to human absorption and utilization [4]. The results showed that through the isolation and identification of microorganisms in natural fermented yoghurt in Qinghai Tibet Plateau, pure strains with certain biological activity were screened, and the research on microbial resources for food processing in Qinghai Tibet Plateau was started [5]. It was found that soy milk fermented by lactic acid bacteria isolated from naturally fermented yak yoghurt could inhibit gastric ulcer, and could be used as functional food in the future [6]. In this study, a lactic acid bacteria isolated from naturally fermented yak yoghurt was used to ferment soybean milk, and its ability of scavenging free radicals in vitro was observed. At the same time, it was compared with the commonly used commercial Lactobacillus bulgaricus to judge its application prospect as a high-quality food starter.

2. Materials and methods

2.1. Materials and reagents  
Materials: Lactobacillus plantarum YS4, provided by Chongqing Collaborative Innovation Center for Functional Food, Chongqing University of Education, is deposited in China Center for Type Culture
Collection (preservation number: M2016747); *Lactobacillus bulgaricus* was purchased from China Center for Type Culture Collection; Soybeans: purchased from Mishan Gaia farm Co., Ltd.  

Reagents: DPPH, Tris HCl, Beijing solebo Technology Co., Ltd; ABTS, sigma, USA; Anhydrous ethanol, ferrous sulfate heptahydrate, salicylic acid, pyrogallol, hydrogen peroxide, Xilong Science Co., Ltd.

2.2. *Instruments and equipment*

LS-B75L autoclave, Jiangyin Binjiang Medical Equipment Co., Ltd; WPL-30 incubator, Jiangdong Precision Instrument Co., Ltd; Biomate 3S UV visible spectrophotometer, Thermo Fisher Scientific, USA.

2.3. Soybean milk fermentation

500 g soybean was soaked in 5 L distilled water at room temperature for 12 h, then beating, and then filtering with 4 layers of medical gauze to obtain unfermented soybean milk. Then, the soybean milk was sterilized by high pressure, and *Lactobacillus plantarum* YS4 and *Lactobacillus bulgaricus* were inoculated in the sterile operating platform according to the inoculation concentration of 10⁵ cells/mL. the fermented soybean milk was obtained by fermentation at 37°C for 12 h.

2.4. Detection of DPPH radical scavenging ability

Add 1 mL soybean milk to 4 mL DPPH solution (ethanol as solvent) with the concentration of 0.33 mmol/L, mix thoroughly, and then leave it at room temperature for 30 min in dark. Finally, the absorbance was measured at 517 nm and the DPPH radical scavenging rate was calculated [7].

2.5. Detection of ABTS free radical scavenging ability

Add 1 mL soybean milk to 2.5 mL ABTS detection solution, shake and mix thoroughly, and then store in dark at room temperature for 30 min. Finally, the absorbance value was measured at 734 nm and the free radical scavenging rate of ABTS was calculated [7].

2.6. Detection of hydroxyl radical scavenging ability

2 mL ethanol salicylic acid solution (9 mmol/L), 1 mL ferrous sulfate solution (9 mmol/L) and 2 mL hydrogen peroxide (8.8 mmol/L) were added to 2 ml soybean milk in order, and then the reaction was carried out in a water bath at 37°C for 30 min. Finally, the absorbance was measured at 510 nm and the hydroxyl radical scavenging rate was calculated [7].

2.7. Statistical analysis

The data of three parallel experiments were calculated, and the mean value was taken and the standard deviation was calculated. Then the SAS software was used to analyze the differences between the data of each group at the level of *P*<0.05 by one-way analysis of variance (ANOVA).

3. Results and analysis

3.1. DPPH free radical scavenging ability of soybean milk fermented by *Lactobacillus plantarum* YS4

DPPH method is a commonly used experimental method to detect the antioxidant capacity of food *in vitro* [8]. As shown in Figure 1, unfermented soybean milk, *Lactobacillus bulgaricus* fermented soybean milk and *Lactobacillus plantarum* YS4 fermented soybean milk can all play a certain scavenging effect on DPPH free radical. The DPPH free radical scavenging rates of unfermented soybean milk, fermented soybean milk by *Lactobacillus bulgaricus* and fermented soybean milk by *Lactobacillus plantarum* YS4 were 36.75%, 50.88% and 79.62%, respectively, and there were significant differences among all treatments (*P*<0.05).
3.2. **ABTS free radical scavenging ability of soybean milk fermented by Lactobacillus plantarum YS4**

After ABTS reacts with active substances with free radical scavenging ability, color reaction will occur. Antioxidant activity of substances can be determined by measuring the color change of reaction solution, and it is also widely used in food detection [9]. As shown in Figure 2, the ABTS free radical scavenging ability of soybean milk fermented by *Lactobacillus plantarum* YS4 was 72.19% significantly ($P < 0.05$) stronger than that of the other two soybean products and drinks. Meanwhile, the ABTS free radical scavenging ability of fermented *Lactobacillus bulgaricus* fermented soybean milk 40.88% was also stronger than that of unfermented soybean milk (26.59%).

3.3. **Hydroxyl radical scavenging ability of soybean milk fermented by Lactobacillus plantarum YS4**

Hydroxyl radicals have a strong damaging effect on the body, and in large quantities can damage DNA, proteins and lipids, leading to a series of diseases [10]. As shown in Figure 3, unfermented soybean milk 15.54% had the weakest hydroxyl radical scavenging ability, significantly ($P < 0.05$), which was lower than that of *Lactobacillus bulgaricus* fermented soybean milk (35.82%) and *Lactobacillus plantarum* YS4 fermented soybean milk (66.50%).
4. Conclusion

Oxygen free radicals in the body will lead to a series of imbalances, make the body abnormal, and even cause disease. The free radical scavenging activity of a strain of lactic acid bacteria isolated from naturally fermented yak yogurt was studied in vitro. The results showed that *Lactobacillus plantarum* YS4 could improve the antioxidant capacity of soybean products and beverages in vitro, and the effect was better than that of commercial *Lactobacillus bulgaricus*. It was a kind of high-quality lactobacillus worthy of further development and utilization.

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