The Influence of Lactic Acid Bacteria from Xinjiang Traditional Fermented Yogurt on Antioxidation of Geniposide in vitro

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Abstract. To investigate the influence of lactic acid bacteria on the antioxidant effect of geniposide in vitro, seven strains of lactic acid bacteria were isolated from natural fermented yoghurt from Xinjiang, China. Lactic acid bacteria, geniposide and their mixtures were used separately to evaluate the antioxidant effect through detecting the scavenging activity of 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH), Hydroxyl free radicals and 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS). The results showed that, compared with the group that geniposide separately used, the strains numbered x70-1, x70-2, x70-3, x70-4 and x70-5, combined with geniposide had more remarkable antioxidant effect, while the other two strains (numbered x43-2 and x43-3) combined with geniposide showed low oxidation resistance. These results may provide a potential method to promote antioxidant effect of geniposide.

1 Introduction

Traditional fermented yogurt—a popular milk product in Xinjiang, China—contains abundant lactic acid bacteria (LAB). Its nutritional value is even more than pure milk [1]. LAB as normal flora in the intestinal tract, they has many physiological effects on the health of the body, such as lowering cholesterol, inhibiting the growth of pathogenic bacteria, promoting digestion and absorption, maintaining the balance of intestinal flora, regulating the body's immunity, and improving the intestinal function [2, 3]. Some redox reactions in body can cause some harm to human. Some lactobacillus had been proved to have antioxidant effect [4, 5].

Cell’s metabolism process can produce a variety of free radicals, and they are a double-edged sword. On the one hand, free radicals have high chemical activity and it’s an effective defense system, on the other hand, if we failure to maintain a certain level of free radicals, our body's vital activities will be affected [6]. In fact, the process from the generation of free radicals to death is the process of electron transfer, and it’s also an oxidation process. So scavenging free radicals should start from antioxidant [7, 8]. As a resident intestinal flora, lactobacillus can directly play an antioxidant role in a key site of oxidative damage in body. As a resident intestinal flora, lactobacillus has been proved in many in vitro and in vivo experiments [3, 4, 6, 7, 8]. Geniposide is mainly derived from gardenia, some studies have shown that it has the functions of regulating high glucose and fat and so on [9]. And we also know that geniposide eventually function in the form of genipin, and lactobacillus may contain β-D-glucosidase, which can convert geniposide into genipin and affect its antioxidant effect [10, 11].

In this paper, we studied the influence of lactic acid bacteria, isolated from yogurt collected in Xinjiang, on the antioxidant effect of geniposide in vitro. DPPH radical-scavenging rate, hydroxyl radical-scavenging rate and ABTS radical clearance rate were used as indexes to compare the antioxidant capacity of lactic acid bacteria, geniposide and their mixtures.

2 Experimental method

2.1 Isolation and identification of lactobacillus

Lactic acid bacteria were isolated from traditional fermented yak yoghurt using the serial dilution and spread plate method and cultured at 37°C for 48h in MRS both (BD, Franklin Lakes, NJ, USA); The cell morphology was observed after gram staining, and the genus was analyzed using 16s rDNA sequence. The genome DNA from isolated strains was extracted by TIANamp bacteria DNA kit (Tiangen, Beijing, China), its 16s rDNA was amplified with S1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA), and the amplification product was tested by using 1.5% agarose gel electrophoresis. The remaining amplification product was sent to sequence (BGI, Shenzhen, Guangdong, China).

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2.2 In Vitro Antioxidant Analysis

As previously described methods by Thaipong[6], Roberta [7] and Zhang [5], we prepared 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay (Solarbio Life Sciences, Beijing, China), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Solarbio Life Sciences, Beijing, China) working fluid and hydroxyl radical scavenging assay. The absorbance was measured using a multi-function micro-plate reader (Thermo Fisher Scientific, New York, USA).

2.3 Statistical Analysis

The data were statistically analyzed by SPASS 17.0 and GraphPad Prism 7 statistical software. Experimental results are expressed as the mean ± standard deviation (SD). One-way ANOVA or t-test was used for comparison between groups. P ≤ 0.05 was considered statistically significant.

3 Results and analysis

3.1 Isolation and identification of lactobacillus

As showed in Fig.1, we observed seven different colonial morphology on MRS agar plate, numbered x43-2, x43-3, x70-1, x70-2, x70-3, x70-4 and x70-5, separately.

Fig.1. Colonial morphology of seven strains

Microscopic observation showed that 5 of them are rod shaped and 2 of them are globular shaped (Fig.2).

Fig.2. Gram staining of 7 strains of lactic acid bacteria

As showed in Fig.3, the electrophoresis result of 16s rDNA amplification products from seven strains were satisfactory with clear band, correct size, and non-specific amplification, separately.

Fig.3. Electrophoretogram of 16s rDNA PCR amplification products of 7 strains

The analyze results of 16s rDNA sequences showed that seven isolates were considered to be Lactobacillus Bulgarian or Lactobacillus Swiss separately, as described in Table 1.

Table 1. Identification of strains by 16s rDNA sequencing

| Strain | Genus             |
|--------|-------------------|
| x43-2  | Lactobacillus Bulgarian |
| x43-3  | Lactobacillus Swiss     |
| x70-1  | Lactobacillus Bulgarian |
| x70-2  | Lactobacillus Bulgarian |
| x70-3  | Lactobacillus Bulgarian |
| x70-4  | Lactobacillus Swiss     |
| x70-5  | Lactobacillus Swiss     |
### 3.2 In Vitro Antioxidant Analysis

#### 3.2.1 DPPH radical-scavenging assay

As depicted in Table 2, seven strains of lactic acid bacteria all had DPPH radical-scavenging ability. Compared with geniposide, except for strain x70-5, mixtures with other six strains improved the DPPH radical-scavenging rate, especially the mixture with strain x70-1, x70-2 and x70-3.

| Strain | Scavenging rate of lactobacillus (%) | Scavenging rate of mixtures (%) | Scavenging rate of geniposide (%) |
|--------|-------------------------------------|--------------------------------|----------------------------------|
| x43-2  | 37.00±0.0052b                      | 10.70±0.012b                   |                                  |
| x43-3  | 31.27±0.019c                       | 15.33±0.017bc                  |                                  |
| x70-1  | 55.03±0.067c                       | 19.63±0.043c                   |                                  |
| x70-2  | 19.93±0.010a                       | 24.01±0.015c                   | 3.83                             |
| x70-3  | 42.43±0.0058b                      | 38.60±0.018c                   |                                  |
| x70-4  | 40.03±0.040c                       | 13.10±0.018c                   |                                  |
| x70-5  | 7.50±0.018a                        | 4.63±0.0099a                   |                                  |

(Note: Different uppercase letters in the upper right corner of the same column of data indicate significant differences (p<0.05))

#### 3.2.2 Hydroxyl radical-scavenging assay

Seven strains of lactic acid bacteria had different effects on the hydroxyl radical scavenging capacity of geniposide. Among them, the strains numbered x43-3, x70-1, x70-2, x70-4 and x70-5 showed the obvious promoting effect, while the strain x43-2 and x70-3 showed inhibitory effects. The results were shown in the table 3.

| Strain | Scavenging rate of lactobacillus (%) | Scavenging rate of mixtures (%) | Scavenging rate of geniposide (%) |
|--------|-------------------------------------|--------------------------------|----------------------------------|
| x43-2  | 15.97±0.0032a                      | 4.59±0.0043a                   |                                  |
| x43-3  | 11.73±0.0038c                      | 6.38±0.00056c                  |                                  |
| x70-1  | 5.52±0.0020a                       | 6.71±0.0015c                   |                                  |
| x70-2  | 9.07±0.0012a                       | 6.90±0.0096c                   | 5.00                             |
| x70-3  | 19.83±0.0055c                      | 3.69±0.0048a                   |                                  |
| x70-4  | 15.50±0.0063c                      | 8.85±0.0026a                   |                                  |
| x70-5  | 12.27±0.0024a                      | 6.15±0.0036a                   |                                  |

#### 3.2.3 Resistance of each group to ABTS

Seven strains of lactobacillus have different effects on geniposide’s ABTS radical scavenging abilities (Table 4). The x70-2 and x70-3 strains show the inhibition influence, while the x43-2, 3, x70 43-1, x70-4 and x70-5 strains were play the promote role.

| Number | Lactobacillus (%) | Mixture (%) | Geniposide (%) |
|--------|-------------------|-------------|----------------|
| x43-2  | 10±0.040c         | 10.67±0.031c|                |
| x43-3  | 13.33±0.042c      | 8.67±0.12c  | 7.33           |
| x70-1  | 17.33±0.012c      | 9.33±0.058a |                |

### 4 Conclusion

In conclusion, seven strains of lactic acid bacteria were isolated and identified, revealed that there are abundant lactobacillus resources in Xinjiang natural fermented yoghurt, especially Lactobacillus Swiss and Lactobacillus Bulgarian. Meanwhile, five of the seven strains could promote the antioxidant effect of geniposide in vitro. These results may provide a potential method for promoting antioxidant effect of geniposide.

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