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Development of colloidal gold-based immunochromatographic assay for rapid detection of *Alicyclobacillus acidoterrestris* in apple juice

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**ABSTRACT**

The colloidal gold quick test card which we developed was used for rapid detection of *Alicyclobacillus acidoterrestris (A. acidoterrestris)* in apple juice concentrate. Two antibodies against *A. acidoterrestris*, obtained from Sprague Dawley (SD) rats and Japanese White Rabbits, were adopted to construct this quick test card. The results showed that the colloidal gold quick test card had better specificity, and was more rapid and convenient which deserved notice especially (the test could be completed within 5–10 min) and concorded with ELISA method (we previously reported) and K medium methods (traditional classical methods). This new developed colloidal gold quick test card (we named it TAB quick test card) could be more convenient for apple juice safety testing.

1. Introduction

*Alicyclobacillus acidoterrestris* is a thermophilic, acidophilic, and spore-forming bacterium (TAB) in many fruit juices (Cerny, Hennlich, & Poralla, 1984), such as apple, pear, orange, citrus, and so on. It can survive through pasteurization and cause juice spoilage (Breveglieri, Masiero, Spisani, & De Taddeo, 2011; Huang, Yuan, Guo, Gekas, & Yue, 2014). The traditional method for the detection of *A. acidoterrestris* is plate cultivation, which requires 4–5 days to identify the target bacteria (Pinhatti, Variane, Eguchi, & Manfio, 1997). In order to monitor and control these spoilage organisms timely and effectively, a rapid detection technology is needed. Numerous studies for rapid detection of *A. acidoterrestris* have been reported previously. For example, the reverse-transcription polymerase chain reaction (RT-PCR) detection by Yamazaki, Teduka, Inoue, and Shinano (1996), a real-time PCR rapid detection method by Luo, Yousef, and Wang (2004), a qualitatively competitive PCR (QC-PCR) method by Li, Feng, and Qiu (2006), and a Fourier transform infrared (FT-IR) spectroscopy detection by Al-Qadiri, Lin, Cavinato, and Rasco (2006). However, those methods are difficult to use in factory because it requires skilled personnel and relatively expensive equipments (Li, Huang, Xia, & Liu, 2014).

We reported a novel method of indirect enzyme-linked immunosorbent assay (ID-ELISA) and staphylococal protein A enzyme-linked immunosorbent assay (SPA-ELISA) to rapidly detect *A. acidoterrestris* in apple juice concentrate (AJC) (Li, Wang, & Xia, 2011; Li, Xia, & Yu, 2013) for the first time. And recently an improved double-antibody sandwich ELISA (DAS-ELISA) (Li et al., 2014) has been proved to be convenient and suitable for application.

In this study, a new colloidal gold quick card (TAB quick test card) was developed with the aid of colloidal gold immunochromatographic assay. This test card showed more rapidness and convenience for rapid detection of *A. acidoterrestris* in apple juice.

2. Materials and methods

2.1. Bacterial strains and materials

*Alicyclobacillus acidoterrestris* DSM 3922 was purchased from DSMZ (Brunswick, Germany). *Escherichia coli* ATCC...
8739, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 6538 and *Shigella* ATCC 12022 were obtained from the Laboratory of Microbiology, College of LifeScience, Shaanxi Normal University. Japanese White Rabbits and Sprague Dawley (SD) rats were obtained from Laboratory Animal Research Center, College of Medicine, Xi’an Jiaotong University. Juice concentrate (pH 3.2–4.6) was obtained from Shaanxi Hengxing Fruit Juice Co., Ltd. Freund’s complete and incomplete adjuvant was purchased from Sigma Chemical (St. Louis, MO, USA). ELISA Well Strips and Plate Frames were bought from JET BIOFIL (Canada). Colloidal gold and Goat anti-rabbit were purchased from Shanghai Jiening Biotechnology Co., Ltd (Shanghai, CN). Nitrocellulose membranes, sample pads, absorbent pads and conjugate pads were purchased from Millipore Corporation. Polyvinylpyrrolidone (PVP) was purchased from JingBo, Ltd (Shaanxi, CN). All inorganic chemicals and organic solvents used in this study were of reagent grade or chemically pure, which were purchased from DingGuo Biotech (Beijing, CN) or Sigma (USA).

The preparation of the immunochromatographic strips

The immunochromatographic strip (ICS) included four parts (named as TAB quick test card): a sample pad, a conjugate pad, an immobilized nitrocellulose membrane, and an absorbent pad. The sample pads were treated with the treatment liquid (50 mM pH 8.1Tris-HCl, 0.3% tween) for 30 min. The conjugate pads were sprayed with colloidal gold-labeled rabbit antibody against *A. acidoterrestris*. The T line was coated with rat antibody and the C line was coated with goat anti-rabbit IgG. When the samples were slowly added to the sample pads, it was moved through immobilized nitrocellulose membrane under capillarity field effect. After 5–10 min, the positive effect displayed two red lines, while the negative effect displayed one red line in the control lines. The immunochromatographic strips lost effectiveness if there was nothing in the control lines.

2.5. Preparation of the immunochromatographic strips

The immunochromatographic strip (ICS) included four parts (named as TAB quick test card): a sample pad, a conjugate pad, an immobilized nitrocellulose membrane, and an absorbent pad. The sample pads were treated with the treatment liquid (50 mM pH 8.1Tris-HCl, 0.3% tween) for 30 min. The conjugate pads were sprayed with colloidal gold-labeled rabbit antibody against *A. acidoterrestris*. The T line was coated with rat antibody and the C line was coated with goat anti-rabbit IgG. When the samples were slowly added to the sample pads, it was moved through immobilized nitrocellulose membrane under capillarity field effect. After 5–10 min, the positive effect displayed two red lines, while the negative effect displayed one red line in the control lines. The immunochromatographic strips lost effectiveness if there was nothing in the control lines.

2.6. Specificity and sensitivity of the TAB quick test card

In order to determine the specificity of the TAB quick test card, we used the test card to test *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 6538 and *Shigella* ATCC 12022.

The five representative food-borne bacteria were cultured on medium at 37 C for 12 h, then they were harvested with sterile saline (0.9% NaCl), and the cells were washed three times by centrifugation (3500 g, 10 min), and kept the resuspension in sterile saline. Bacterial density of the final suspension was 2 × 10⁸ CFU/mL, which was determined by spectrophotometer (OD/600 nm) (Wang, Li, Liu, & Jiang, 2009; Xia, Li, & Yu, 2012).

2.7. AJC sample preparation for the TAB quick test card assay

AJC sample preparation was performed using procedures based on the methods of Li et al. (2011). About 10 mL AJC samples was heat-treated at 80 C for 10 min and then deionized water was added. The diluted sample was filter sterilized by discmembrane filters (0.45 μm pore size; 50 mm in diameter). After filtering, the membranes were transported into an enrichment broth of 402 medium (DingGuo Biotech, pH 4.0, 200 mL) and incubated at 45 C for 12 h with shaking for pre-enrichment. After incubation, the broth was centrifuged at 10,000 g for
10 min to collect precipitation and then diluted the precipitation with pH 7.4 PBS buffer (0.2 g KH$_2$PO$_4$, 2.9 g Na$_2$HPO$_4$, 0.2 g KCl, 8.0 g NaCl, 1000 mL distilled water) (Li et al., 2014) that achieved concentration above the sensitivity of the TAB quick test card.

2.8. Analysis of consistency among quick test card, ELISA kit and K medium method

Adding 150 µL of samples on the TAB quick test cards, after 5–10 min, we observed the phenomena and compared with the results of DAS-ELISA kit (Li et al., 2014) and K medium method (The bacteria were cultured on K medium(plate) at pH 3.7 at 43°C for 12 h) that achieved concentration above the sensitivity of the TAB quick test card.

3. Results and discussion

3.1. Polyclonal antibodies

The titers of the Japanese White Rabbits polyclonal antibodies against A. acidoterrestris were reached at 1/25,600 and the titers of the SD rats polyclonal antibodies against A. acidoterrestris were reached at 1/12,800.

3.2. Specificity of the TAB quick test card

Escherichia coli ATCC 8739, Bacillus subtilis ATCC 6633, Bacillus cereus ATCC 11778, Staphylococcus aureus ATCC 6538 and Shigella ATCC 12022 were cultured on beef-protein medium at 37°C for 12 h. A. acidoterrestris was cultured on K medium at pH 3.7 at 43°C for 12 h. Different suspensions were collected to evaluate the specificity of the strip. Only A. acidoterrestris showed positive effect and other samples showed negative effect. As shown in Figures 1 and 2, the TAB quick test card exhibited better specificity.

3.3. Sensitivity of the TAB quick test card

Different concentrations of A. acidoterrestris were detected by the TAB quick test card. When the concentration was $1 \times 10^6$ CFU/mL, just the control line showed red, while the concentration exceeded $1 \times 10^7$ CFU/mL (45°C for 12 h with shaking for pre-enrichment), the test line and control line both displayed red. The phenomena are shown in Figures 3 and 4, and the sensitivity of the TAB quick test cards is $1 \times 10^7$ CFU/mL.

3.4. Analysis of consistency among quick test card, ELISA kit and K medium method

Twelve samples of AJC were assayed, respectively, by TAB quick test card, ELISA kit and K medium method. For the quick test card assay, samples with the test line and control line both displayed red, which indicated a positive effect. For the DAS-ELISA assay, samples with OD 450 nm more than 0.038 were considered as a positive effect. In the terms of the K medium, 10 mL samples with more than 1 CFU were
considered as a positive effect. The results are shown in Table 1. The TAB quick test card assay was well in concordance with ELISA kit and K medium method.

The traditional method and the main current way for the detection of *A. acidoterrestris* is plate cultivation (K medium), but it took long times (Chang & Kang, 2004). In our previous study, we reported an ID-ELISA, SPA-ELISA and DAS-ELISA method to detect the *A. acidoterrestris*, which is suitable and time-saving in ordinary laboratory (Li et al., 2014, 2011, 2013). The ICS assay method (TAB quick test card) proved to be more rapid and convenient. It took 5–10 min, excluding sample pretreatment and enrichment time. The TAB quick test card (sealed package) could be stored at room temperature in dry condition for 1 year.

4. Conclusion
A high sensitivity and more convenience TAB quick test card based on colloidal gold-based immunochromatographic assay was developed to detect *A. acidoterrestris* in juice. The results
Detección de Alicyclobacillus inferior a 0,038 fueron consideradas negativas. La unidad de (Método K-medias) (Método de placa de cultivo) detección de resultados fue 0.024 ± 0.011.

Detection of Alicyclobacillus by test card, ELISA kit and K medium.

| Sample | 1 | 2 | 3 | 4 | 5 | 6 |
|--------|---|---|---|---|---|---|
| Quick test card | – | – | – | – | – | + |
| ELISA kit | 0.034 ± 0.011 | – | – | – | – | – |
| K medium method | <1 | <1 | <1 | <1 | <1 | 2 |
| (plate culture method) | – | – | – | – | – | + |

| Sample | 7 | 8 | 9 | 10 | 11 | 12 |
|--------|---|---|---|----|----|----|
| Quick test card | – | – | – | – | – | + |
| ELISA kit | 0.024 ± 0.011 | 0.098 ± 0.014 | 0.038 ± 0.011 | 0.034 ± 0.016 | 0.036 ± 0.012 | 0.102 ± 0.013 |
| K medium method | <1 | 2 | <1 | <1 | <1 | 3 |
| (plate culture method) | – | – | – | – | – | + |

Notes: The ELISA detection results were the average values of 10 times detection plus or minus coefficient of variation values and samples with OD<sub>90</sub> less than 0.038 were considered negative. The unit of K medium method (plate culture method) detection results was CFU per 10 mL (CFU/10 mL) and samples with KFL results less than 1CFU/10 mL were considered as negative.

Notas: los resultados de detección de ELISA fueron los valores promedio de 10 veces más o menos los valores de coeficiente de variación y las muestras con OD<sub>90</sub> inferior a 0.038 fueron consideradas negativas. La unidad de (Método K-medias) (Método de placa de cultivo) detección de resultados fue CFU para 10 mL (CFU/10 mL) y las muestras con resultados de KFL inferiores a 1CFU/10 mL fueron consideradas negativas.

can be obtained within 5–10 minutes excluding pretreatment and enrichment (see Section 2.7 for the details). Our results showed that the quick test card had no cross-reaction with the five representative food-borne bacteria and showed high sensitivity and excellent agreement with K medium method and DAS-ELISA kit. TAB quick test card showed a great potential in fruit juice detection.

Disclosure statement
No potential conflict of interest was reported by the authors.

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