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*Short Communication*

# Pre-staining thin layer chromatography method for amino acid detection

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**Thin layer chromatography (TLC) is an analytical method that is used commonly. It usually includes five steps: spotting, separating, drying, spraying/immersing and color development. In this paper, TLC was modified with pre-staining method, which only consisted of 3 steps: spotting, separating and color development. The modified thin layer chromatography can be used for the analysis of amino acids. When compared to the classical thin layer chromatography, the improved method was more rapid and inexpensive and the results obtained were clean and reproducible. However, it is suitable for the high throughput screening of amino acid-producing strains.**

**Key words:** Thin layer chromatography, pre-staining, amino acid detection.

## INTRODUCTION

Several analytical techniques have been often used for amino acid analysis, such as high performance liquid chromatography (HPLC) (Schwarz et al., 2005), amino acid analyzer (Kaspar et al., 2009), gas chromatography (GC) (Paik et al., 2008), capillary electrophoresis (CE) (Zahou et al., 2000) and thin layer chromatography (TLC) (Bhushan et al., 2007; Kazmierczak et al., 2004; Mohammad et al., 2007 ). Among these methods, HPLC, amino acid analyzer and GC are automated and accurate for the analysis of amino acids. However, a large number of samples are always involved and need to be pre-treated and analyzed at the right time in the screening of amino acid-producing microbes as well as amino acid fermentation processes. These methods do not meet the demands of high throughput detection of amino acids, because the expensive equipments and tedious sample preparations have to be involved in the experiment. Thin layer chromatography can also be employed to detect amino acid, which needs no expensive outfits and tedious sample pretreatments, and allows a parallel separation of many samples. As a convenient and economical method in detection, TLC has been successfully applied in the detection of amino acids from numbers of samples.

Traditional TLC method includes five experimental steps: spotting, separating, drying, spraying/immersing and color development (Komatsuzake et al., 2005; Mohammad et al., 2007). To make it more simple and suitable for the detection of amino acids, the traditional thin layer chromatography was modified in this study. The improved method only consisted of spotting, separating and color development. Compared to the conventional thin layer chromatography, the improved method is clean, rapid, inexpensive and reproducible. In our previous work, we have improved the paper chromatography with the same method (Li et al., 2009), however, the improved TLC is much more efficient for the detection of amino acid.

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**Abbreviations: TLC,** Thin layer chromatography; **HPLC,** high performance liquid chromatography; **GABA,** γ-aminobutyric acid.

## MATERIALS AND METHODS

### Materials

Microcrystalline cellulose glass plates were purchased from Taizhou Si-Jia Biochemical Plastic Company (Taizhou, China). Amino acids were of ultra pure grade. γ-aminobutyric acid was purchased from Aldrich (Milwaukee, WI, USA). All other reagents used were of analytical grade. A new developing solvent *N*-butanol–

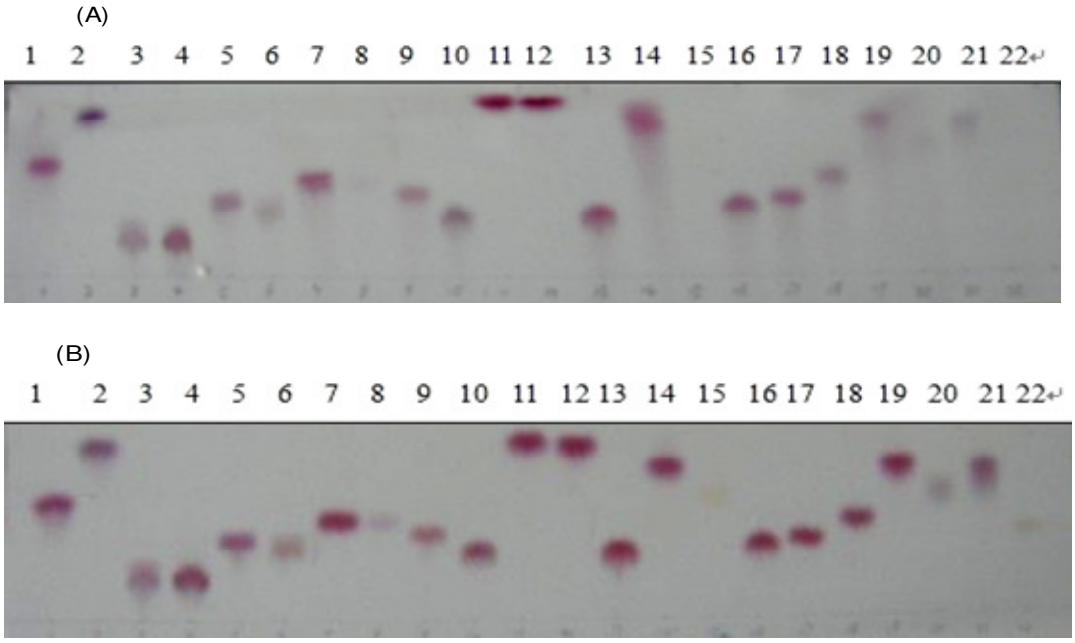


**Figure1. (A)** The traditional TLC chromatogram of 22 amino acids. **(B)** The pre-staining TLC chromatogram of 22 amino acids. The two TLC methods were performed simultaneously under the same conditions. Line1: Alanine, 2: Phenylalanine, 3: Cysteine, 4: Cystine, 5: Aspartic Acid, 6: Asparagine, 7: Glutamic Acid, 8: Glutamine, 9: Glycine, 10: Histidine, 11: Leucine, 12: Isoleucine, 13: Lysine, 14: Methionine, 15: Proline, 16: Arginine, 17: Serine, 18: Threonine, 19: Valine, 20: Tyrosine, 21: Tryptophan, 22: Hydroxyroline.

acetic acid–water (5:3:2) containing 0.4% (w/v) of ninhydrin was prepared.

### Apparatus

A convection oven (Sanyo, Osaka, Japan) was used to control the color temperature.

### Strains, medium and fermentation conditions for GABA

*Lactobacillus brevis* NCL912 was used for γ-aminobutyric acid (GABA) fermentation in MRSS medium (Li et al., 2008). MRSS medium contains (w/v) 2.5% glucose, 0.625% yeast extract, 0.625% soya peptone, 0.02% $MgSO_4 \cdot 7H_2O$, 0.005% $MnSO_4 \cdot 4H_2O$, 1% sodium L-glutamate and 2 ml $L^{-1}$ Tween 80. The strain was stationarily cultivated at 34°C for 1 day in 250 mL flasks containing 100 ml medium.

### Methods

### Sample preparation

Amino acid was dissolved in 0.01 M sodium hydroxide solution to a final concentration of 0.01M. The fermentation broth was directly used for thin layer chromatography (TLC) spotting.

### TLC analysis

The classical thin layer chromatography was modified in the present study. The main alteration was that ninhydrin was directly added into the developing solvent for the development of chromatography plate, and then the plate was directly dried in the convection oven for color yield. The improved method was conducted as follows: 0.5 µl of samples were spotted onto the TLC plate. Then the thin layer chromatography was developed at 30°C with the new developing solvent (ascending technique). After development, the plate was directly dried for color yield in the convection oven at 90°C for 5 min.

The traditional TLC was performed simultaneously under the same conditions except that the drying and immersing operations were conducted after it was developed in the *N*-butanol-acetic acid-water (5:3:2, v/v/v) solvent.

## RESULTS AND DISCUSSION

### Efficiency improvements on the traditional TLC

The chromatograms obtained from the modified thin layer chromatography (TLC) and the conventional thin layer chromatography is shown in Figure 1. The profiles of 22 amino acids from the two methods were almost identical and the $R_f$ values obtained (data not shown) are not influenced by the modification. Furthermore, the modified method was more rapid, convenient and inexpensive, and the spots of amino acids in the chromatogram from improved thin layer chromatography were clearer and more condensed than those from traditional thin layer chromatography.

In the traditional thin layer chromatography, the spraying and the immersing methods usually result in a

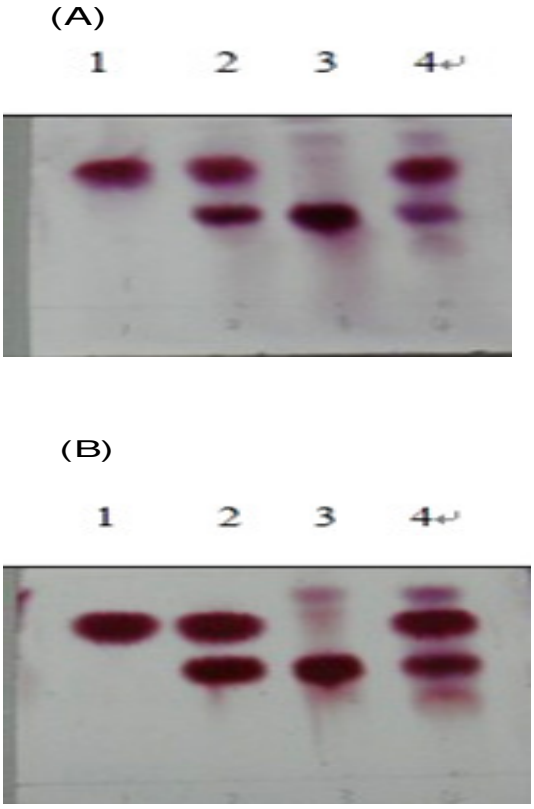


**Figure 2.** (A) The traditional TLC chromatogram and (B) the pre-staining TLC chromatogram of strain NCL912 culture broth and the controls. The two TLC methods were performed simultaneously under the same conditions. Lane 1: standard GABA (10 $gl^{-1}$), 2: the mixture of the standard GABA (10 $gl^{-1}$) and sodium L-glutamate (10 $gl^{-1}$), 3: fresh MRSS broth, and 4: fermentation broth of strain NCL912.

heterogeneous color development due to an uneven spraying and the loss of amino acids when the glass plate is immersed in the ninhydrin reagent. In addition, the diffusion of amino acid spots would increase in the color yield because of the complicated experimental steps. In the modified thin layer chromatography, ninhydrin was evenly distributed on the plate during amino acids separation, the diffusion of amino acids would be reduced when the drying and spraying/immersing steps were avoided. On the other hand, the modified method had a lower solvent consumption and was beneficial to environment protection.

The present improved method was proposed as pre-staining TLC because the ninhydrin was directly added to the developing solvent.

## An example for application

The pre-staining thin layer chromatography method was suitable for the screening of amino acid-producing microbes and monitoring of the amino acid fermentation process. To verify the validation of the proposed method, a gamma-aminobutyric acid-producing lactic acid bacterium, *L. brevis* NCL912 was selected as an example to be tested. As shown, a new spot that has an $R_f$ value similar to standard γ-aminobutyric acid (GABA) appeared in strain NCL912 culture broth (Figure 2). The results demonstrated the practicality of the proposed method.

## Conclusions

In comparison with the traditional thin layer chromategraphy, the modified thin layer chromatography is clean, rapid, inexpensive and reproducible. It can be applied in the high throughput detection of amino acids and monitoring of the amino acid fermentation process as well as in the screening of amino acid-producing strains.

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