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A COMPARISON OF SILVER STAINING PROTOCOLS FOR DETECTING DNA IN POLYESTER-BACKED POLYACRYLAMIDE GEL

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ABSTRACT

Eight silver-staining protocols were applied to detect DNA in polyester-backed gels to select the optimal. Results showed important differences in staining quality and that four methods were well-suited for TGGE gels due to high sensitivity and low background, including the Bassam *et al.* methods, the manufacturer method and our improved method.

Key words: Backed gel, DNA, Silver staining

In the past decade, temperature-gradient gel electrophoresis (TGGE) technique has been employed as a powerful tool for investigating microbial diversity in various environments (7, 10, 11, 13, 14). Due to high sensitivity and low toxicity with the use of very simple and cheap equipments and chemicals (4), the silver staining methods are widely used for DNA visualization in polyacrylamide gels and fall into two categories based on the reagent used for silver impregnation. Alkaline methods use diamine or ammoniacal silver solutions for gel impregnation and dilute acid solutions of formaldehyde for image development. In contrast, acidic methods impregnate with silver nitrate in a weakly acidic milieu and use formaldehyde to reduce silver under alkaline conditions (3). The silver staining methods are too many for the investigator to choose one suited for the research goals, thus, numerous comparisons of silver staining methods have been reported for polyacrylamide gels unbound to any backing surface (1, 5, 6, 8, 15), such as glass plate or plastic, but to our knowledge relatively few have been published for backed gels (3, 9). Moreover, the silver staining methods suited for unbacked gel may be not available for backed gel since the backing film serves as a surface for silver deposition and restricts diffusion of reagents in and out of the gel to one face only (3). In the present study, eight protocols, including alkaline and acidic silver staining, were applied to DNA visualization in TGGE gels bound to a polyester support film (9×9 cm). The objective of this study was to select an optimal silver staining method for TGGE gels.

The 16S rDNA V3 fragments from five soil samples of different sites (P1, P2, P3, P4, P5) in a paddy field were amplified using the fD1/rD1 (16) and F341GC/R534 (12) primers with nested PCR. The PCR products were loaded on 0.45mm thick 8% denaturing gels (8% polyacrylamide gel (Acr/Bis=37.5:1), 1×TAE (40 mM Tris-Cl, 1 mM EDTA), 2% glycerol, and 8 M urea). The loading orders of the samples in each gel were identical. TGGE was performed using a TGGE

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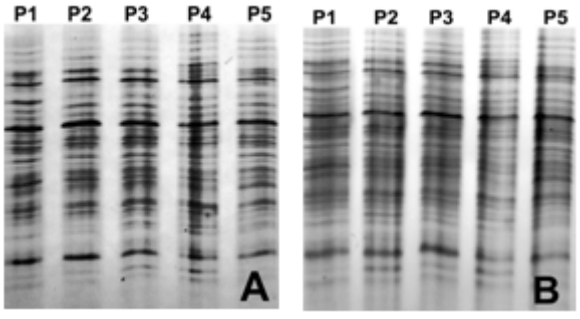
system (Whatman-Biometra). All gels were run at 130 V for 2 h with 1×TAE buffer. The temperature gradient was optimized at 56-69℃. All chemicals used for preparation of gels and buffers were analytical grade from Amresco (Cleveland, OH, USA) and all solutions were prepared in ultrapure distilled water. After electrophoresis, gels were silver stained using eight protocols according to the steps described in Table 1. The protocols included the procedures devised by Bassam *et al.* (2, 3), Creste *et al.* (9), Benbouza *et al.* (5), An *et al.* (1), Cong *et al.* (8), the protocol described by the manufacturer of TGGE system (method 3) and its simplified procedure (method 4). All chemicals used for staining were analytical grade from Sinopharm (Shanghai, China).

Table 1. Procedure comparison of different methods for DNA silver staining

Step	Bassam *et al.* (2) method	Bassam *et al.* (3) method	Method 3	Method 4	Creste *et al.* (9) method	Benbouza *et al.* (5) method	An *et al.*(1) method	Cong *et al.* (8) method
Rinse /Fix	10% acetic acid; 20 min	7.5% acetic acid; 10 min	30% ethanol; 10% acetic acid; 30 min	30% ethanol; 10% acetic acid; 10 min	10% ethanol; 1% acetic acid; 10 min;	10% ethanol; 0.5% acetic acid; 5 min; 10-12 ℃	-	H_2O; 10 min
Sensitize	-	-	30% ethanol; 10 min; 2 times	30% ethanol; 10 min	-	-	-	-
Wash	-	-	H_2O; 30 s	H_2O; 30 s	H_2O; 1 min	-	-	-
Pretreat	-	-	-	-	1.5% HNO_3; 3 min	-	-	-
Rinse	H_2O; 2 min; 3 times	H_2O; 2 min; 3 times	H_2O; 5 min; 5 times	H_2O; 5 min; 3 times	H_2O; 1 min	-	-	-
Stain	0.1% $AgNO_3$; 0.15% HCOH; 30 min	0.1% $AgNO_3$; 0.15% HCOH; 20 min	0.2% $AgNO_3$; 0.35% HCOH; 30 min	0.2% $AgNO_3$; 0.35% HCOH; 20 min	0.2% $AgNO_3$; 20 min	0.15% $AgNO_3$; 0.2% HCOH; 6-7 min; 22-24 ℃	0.1% $AgNO_3$; 1% HNO_3; 5% ethanol; 5 min	0.2% $AgNO_3$; 0.4% NaOH; 0.7% ammonia; 10 min
wash	H_2O; 20 s	H_2O; 5 s	H_2O; 2 min;	H_2O; 2 min;	H_2O; 30 s; 2 times	H_2O; 10-15 s	H_2O; 10 s	H_2O; 2 min
Develop	3% Na_2CO_3; 0.15% HCOH; 0.0002% $Na_2S_2O_3.5H_2O$; 2-5 min	3% Na_2CO_3; 0.3% HCOH; 0.0002% $Na_2S_2O_3.5H_2O$; 4 min; 8 ℃	2.5% Na_2CO_3; 0.35% HCOH; 0.002% $Na_2S_2O_3.5H_2O$; 2-5 min	2.5% Na_2CO_3; 0.35% HCOH; 0.002% $Na_2S_2O_3.5H_2O$; 2-5 min	3% Na_2CO_3; 0.054% HCOH; 4-7 min	1.5% NaOH; 0.3% HCOH; 3-5 min; 22-24 ℃	1.3% NaOH; 0.65% Na_2CO_3; 0.4% HCOH; 2-3 min	0.05% HCOH; 0.01% citric acid; 5-7 min
Stop	10% acetic acid; 5 min	7.5% acetic acid; 1 min; 4 ℃	10% acetic acid; 5 min	10% acetic acid; 5 min	5% acetic acid; 5min	10% ethanol; 0.5% acetic acid; 2min	5% ethanol; 1% HNO_3; 1 min	5% acetic acid; 2min

Comparisons of staining results of eight methods were shown in Figure 1. It can be clearly seen that sensitivity and contrast of the four methods were superior to the others, including the Bassam *et al.* methods (2, 3), the manufacturer method (method 3) and our simplified method (method 4). The sensitivity between A-D gels was similar. However, the protocols described by Bassam *et al.* needed relatively few steps and solutions among the four methods. The technique of Cong *et al.* gave the poorest result in terms of lowest sensitivity and a deep silvery background. The produced silver mirror made photography difficult, thus the image was not shown in Figure 1. Both the Benbouza *et al.* method and the An *et al.* technique suffered from, in our hands, low sensitivity and a strong yellow background, probably because of the developing solution containing NaOH. The method developed by Creste *et al.* displayed lower image contrast and sensitivity than the four superior methods mentioned above in our hands.



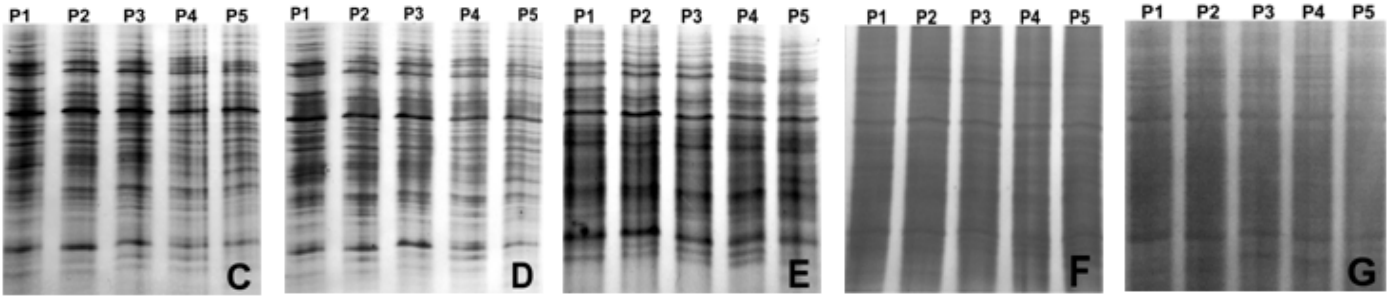


Figure 1. Sensitivity comparison of of 8 silver staining methods of 16S rDNA V3 fragment amplified using the fD1/rD1 and F341GC/R534 primers with nested PCR. (A) method of Bassam *et al.* (1991), (B) method of Bassam *et al.* (1993), (C) method 3 described by the manufacturer of TGGE system, (D) method 4 modified from method 3, (E) method of Creste *et al.* (2001), (F) method of Benbouza *et al.* (2006), (G) method of An *et al.* (2009). The image stained with method of Cong *et al.* (2010) was not shown. Each method was repeated at least three times, and representative results are shown.

In these methods, the procedure described by the manufacturer (method 3) is the most complicated and time-consuming (Table1), we simplified this procedure by shortening the time of some steps, such as fixation, sensitization, impregnation and reducing washing times. As a result, the new protocol (method 4) requires less 50 min than method 3, but offers equivalent effect of silver staining without affecting the sensitivity.

In conclusion, the four methods including the Bassam *et al.* techniques (2, 3), the manufacturer method and our updated protocol based on the manufacturer method are suited for detecting DNA in TGGE gels in terms of superior sensitivity, among which the Bassam *et al.* technique (3) is the fastest to perform. The alkaline method of Cong *et al*. and both acidic methods with developing solution containing NaOH described by Benbouza *et al.* and An *et al.* respectively are not appropriate for staining of polyester-backed gels due to poor image contrast.

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