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AN IMPROVED SILVER STAINING PROCEDURE FOR SCHIZODEME ANALYSIS IN POLYACRYLAMIDE GRADIENT GELS

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A simple protocol is described for the silver staining of polyacrylamide gradient gels used for the separation of restriction fragments of kinetoplast DNA [schizodeme analysis of trypanosomatids (Morel et al., 1980)]. The method overcomes the problems of non-uniform staining and strong background color which are frequently encountered when conventional protocols for silver staining of linear gels are applied to gradient gels. The method described has proven to be of general applicability for DNA, RNA and protein separations in gradient gels.

Key words: silver staining – schizodeme analysis – DNA – RNA – protein – trypanosomatids

Schizodeme analysis (schizo = cleave, demos = population), a technique for the genotypic characterization of trypanosomatids, was introduced by Morel et al. (1980) and modified by Gonçalves et al. (1984). It is based on the electrophoretic separation of restriction-endonuclease generated fragments of kinetoplast DNA (kDNA), the abundant and unique mitochondrial DNA that defines the order Kinetoplastida.

In this paper we describe a methodology for silver staining of DNA fragments in polyacrylamide gradient gels and its application in schizodeme analysis.

MATERIALS AND METHODS

Schizodeme analysis – The technique is based on the protocols described by Morel et al. (1980) and Gonçalves et al. (1984). Parasites are lysed using a strong detergent (sarkosyl) and digested with pronase at 60 °C. The kDNA networks are collected by centrifugation, extracted with phenol-chloroform and precipitated with ethanol.

The kDNA obtained is digested with restriction enzymes and uncubated with proteinase K. The electrophoretic analysis of the restriction fragments is carried out overnight at room temperature in a high resolution 5-10% linear polyacrylamide gradient gel, 130 x 135 x 1 mm (W x H x T) in a vertical apparatus, using 1X TBE as buffer and a constant current of 8 mA.

Protocol for silver staining – The following steps were followed:

Step 1: after the electrophoretic run, wash the gel with 250 ml of 1X wash buffer for 30 min (1X wash buffer is 78.4 mM phosphate, 21.6 mM citrate, pH 3.0). Stock 10X wash buffer is made by mixing 200 ml 1M sodium phosphate monobasic with 55 ml 1 M citric acid.

Step 2: fix the gel (unstained or stained with ethidium bromide) with 200 ml of 50% methanol – 10% trichloroacetic acid for 30 min.

Step 3: wash two times with 250 ml of 5% acetic acid – 10% ethanol for 20 min.

Step 4: wash two times with 250 ml for 10% ethanol for 10 min.

Step 5: soak the gel in 200 ml of silver staining solution. This solution is made by mixing 40 ml of 0.1 M sodium hydroxide, 3 ml of concentrated ammonium hydroxide and 1 g of silver nitrate dissolved in 10 ml of water. Mix until the black precipitate is dissolved and adjust final volume to 200 ml with water. Stain the gel in this solution for 40 min.

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Step 6: wash with 300 ml of water for 5 min.

Step 7: develop the gel with 300 ml of fresh solution of 0.01% (30 mg) citric acid, containing 450 µl of 35% formaldehyde. When development is complete, the DNA bands will appear dark brown or black against a yellowish background.

Step 8: stop development by soaking the gel in 20% ethanol – 5% acetic acid. Leave the gel in this solution until the background turns light yellow in colour.

Step 9: wash the gel three times (first wash for 30 min and the other two for 60 min) with 250 ml of 20% ethanol. If necessary the gel can be kept in this solution for several days without damage at room temperature.

Note: all the above steps are carried out with mild agitation. Wear rubber or polyvinil gloves to hold the gel in order to avoid the appearance of spots. The quality of the water used seems to be critical for the silver staining protocol. Use double distilled or deionized water.

Drying the gel – Soak the gel in 200 ml of 20% ethanol – 1% glycerol for three hours, with mild agitation, at room temperature. Dry between two sheets of permeable cellophane paper.

With apparatus: we use a slab gel dryer under vacuum, without mylar sheet on the gel, at 80 °C for 60 min.

Without apparatus: wrap a glass plate with a plastic film (Saranwrap). Place on the plate a sheet of wet cellophane paper longer than the plate and remove all air bubbles. Place the gel on the covered plate and place another sheet of cellophane paper, treated as above on top. Remove air bubbles, fold the cellophane paper behind the plate and finally cover with another glass plate of the same size. Hold the whole system with the aid of bulldog clips. With a needle, pierce the cellophane paper all around the gel to remove air bubbles and excess water. This assembled gel should be stored at 37 °C for twelve hours.

Note: after drying, the gel can be plasticized or simply kept protected against humidity. Contact prints can be made using the dried gel as a photographic negative.

Destaining the gel – If the gel becomes overstained, it can be destained as follows: after stoping development wash the gel in distilled water with agitation for 10 min. Soak the gel in 100 ml of a solution containing 0.012% potassium permanganate (prepare 0.4% stock solution and use 3 ml) and 1% glacial acetic acid, keeping it under agitation. When the gel reaches the desired colour, stop the reaction by soaking it in a 4% sodium, sulphite solution. Wash the gel for 30 min (three times) in water and afterwards in 20% ethanol.

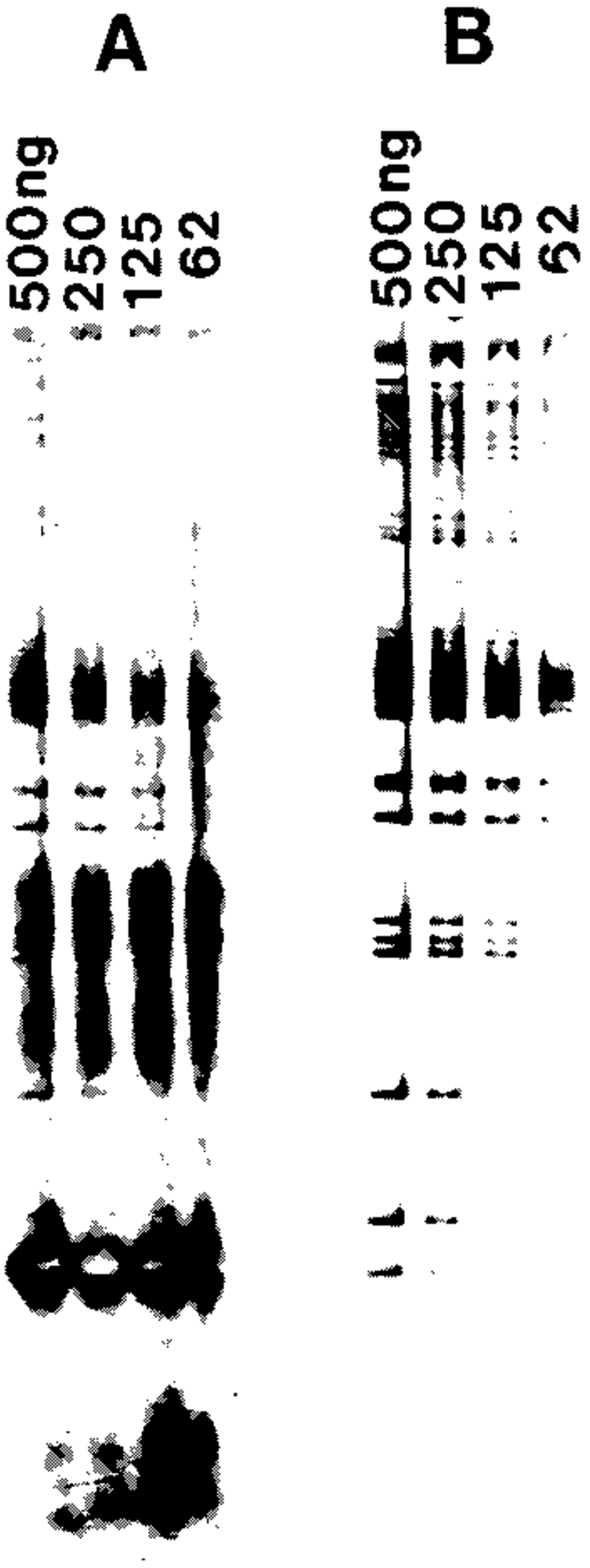

Fig. 1: 5-10% polyacrylamide gradient gel stained with silver, as described in the text, containing kDNA of *Trypanosoma cruzi* digested with EcoRI. A) Without treatment with proteinase K. B) Treated with proteinase K (2 µg/µg kDNA/1-2 u restriction enzyme)

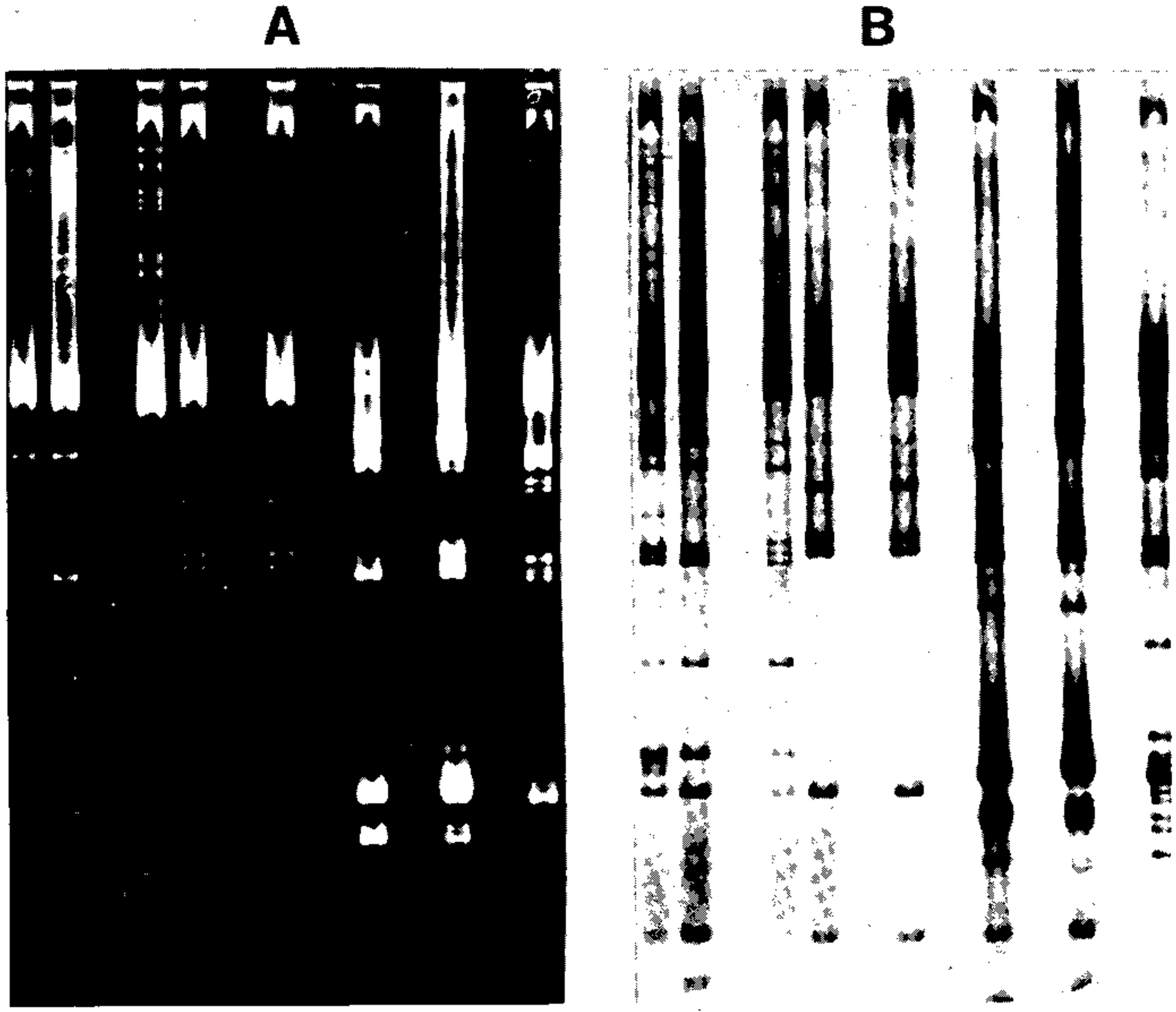

Fig. 2: 5-10% polyacrylamide gradient gel containing kDNA of different strains of *Trypanosoma cruzi* digested with EcoRI. A) Stained with ethidium bromide. B) Stained with silver, as described in the text.

RESULTS AND DISCUSSION

Several methods have been described for silver staining in regular polyacrylamide gels of *proteins* (Oakley et al., 1980; Merril et al., 1981; Schleider & Watterson, 1983) or *nucleic acids* (Boulikas & Hancock, 1981; Herring et al., 1982; Beidler et al., 1982; Guillemette & Lewis, 1983). Some of these authors have already shown that silver staining is more sensitive than Coomassie blue for the detection of proteins or ethidum bromide for nucleic acids.

When these techniques are applied to *gradient* gels (which are required for schizodeme analysis), the staining obtained is not uniform. A strong, dark-yellow background colour is allways present in the regions of high acrylamide concentration making difficult the viasualization and analysis of the bands of lower molecular weight. The technique we describe here overcomes these problems and has the additional advantage that the same protocol can be used without modifications for protein and/or nucleic acid staining.

For the silver staining of nucleic acids it is important that the material be free of proteins. Fig. 1 shows the importance of the use of protease for eliminating residues of proteins not removed during DNA extraction or added during restriction endonuclease digestions. Samples treated with protease are free of proteins, displaying only kDNA bands.

Figure 2 shows the comparison, in the same gel, of restriction profiles of *Trypanosoma cruzi* kDNA stained simultaneously with ethidium bromide and with silver. The silver staining is more sensitive, displaying bands which had not been detected by ethidium bromide.

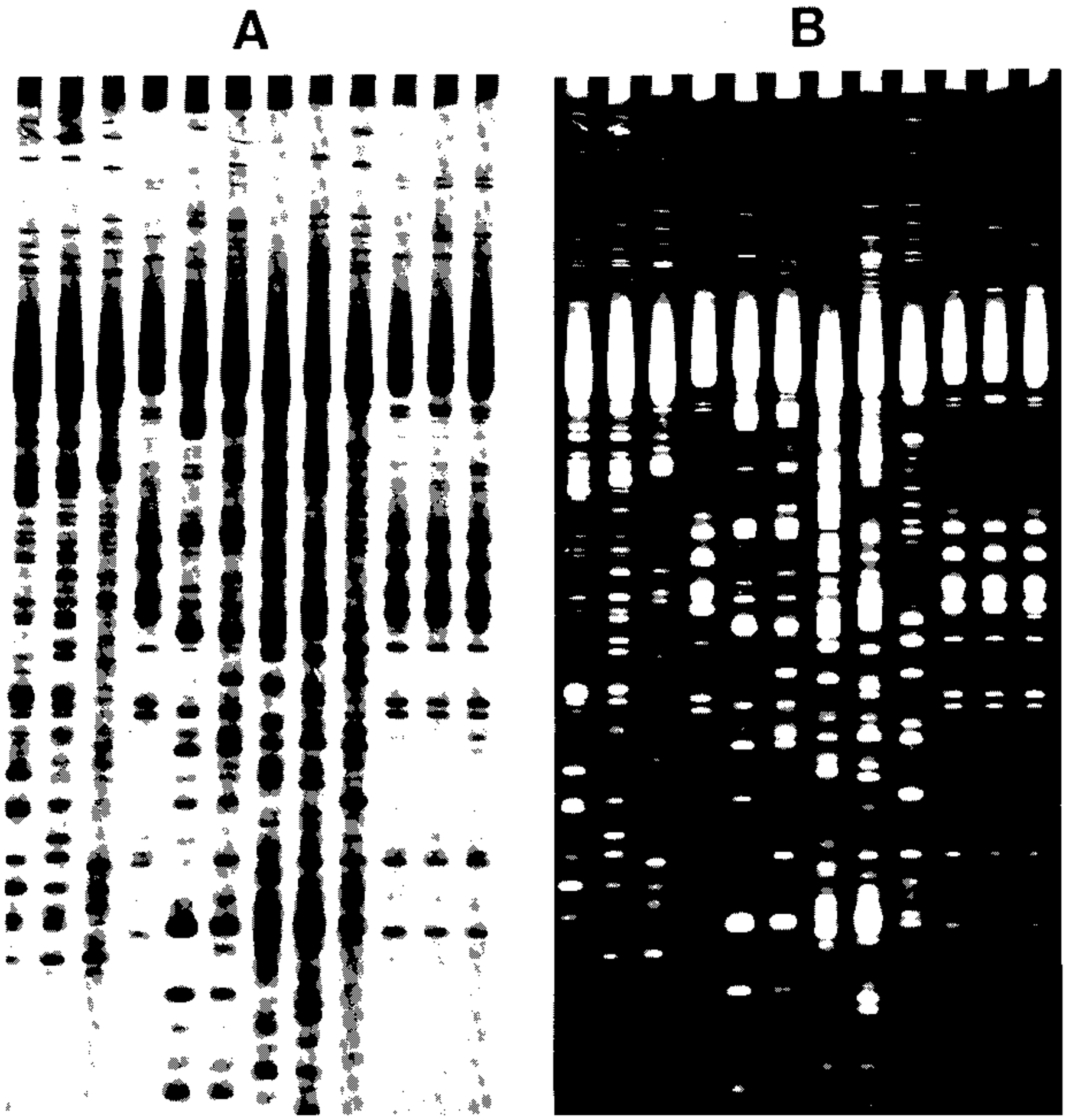

Fig. 3: 5-10% polyacrylamide gradient gel containing kDNA of different strains of *Trypanosoma cruzi* digested with HinfI. A) Photo of the gel stained with silver, as described in the text. B) Photo obtained by contact print using the dried gel as negative.

Photographs can be taken using the dried gel as a negative to make contact prints. In Fig. 3 we show this possibility where one can observe the photograph of the gel stained with silver and its respective contact photograph using the dried gel as a negative. The picture obtained is similar to a gel stained with ethidium bromide.

Our laboratory is routinely using this methodology for silver staining of DNA, RNA and proteins. As an example, Fig. 4 shows a linear polyacrylamide gradient gel analysis of protein samples which was stained with Coomassie blue and by silver staining.

This methodology, when compared with others for staining polyacrylamide gradient gels, has shown more uniform staining for the whole extension of the gel leaving only a ligh yellowish background.

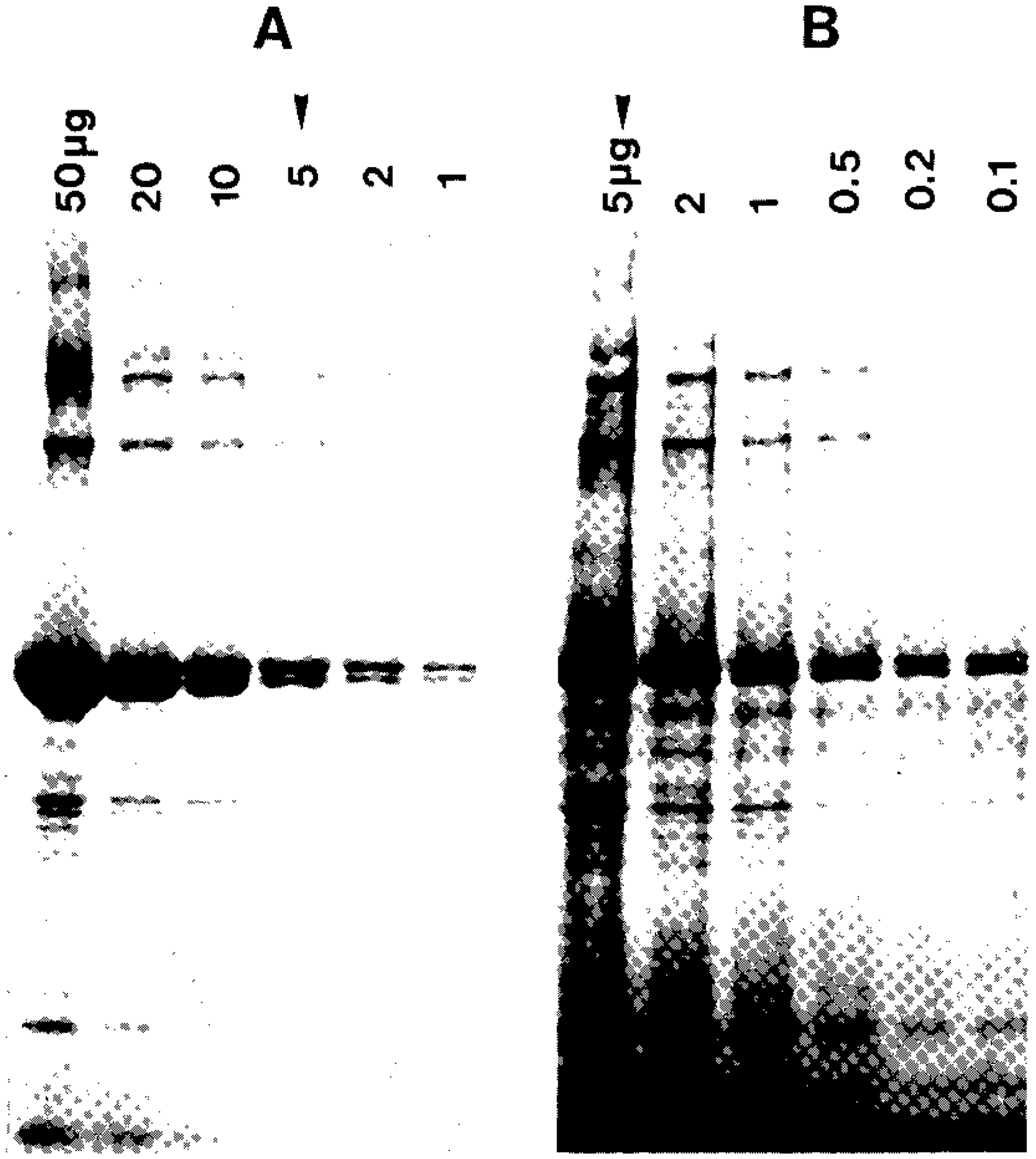

Fig. 4: 5-15% polyacrylamide gradient gel with SDS, 130 x 135 x 1 mm (W x H x T), containing hemolymph of *Rhodnius prolixus*. A) Stained with Coomassie blue. B) Stained with silver, as described in the text.

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