

TWO DIMENSIONAL ELECTROPHORESIS OF PROTEINS FROM ROOT-KNOT NEMATODES

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Polyacrylamide gel electrophoresis (PAGE) has been employed since long to study variations in enzyme-protein profile of phytoparasitic nematodes (see review by Hussey, 1979). However, the high resolution two-dimensional electrophoretic ISODALT-system (O'Farrell, 1975) and the ultra-sensitive silver stain which replaced the conventional Coomassie Brilliant Blue R-250 are rather recent introductions to nematology (Poehling, Wyss & Neuhoff, 1980 ; Bakker & Gommers, 1982). We give here the method used in our laboratory to study the proteins of *Meloidogyne* spp.

Females of three species of *Meloidogyne* viz. *M. incognita*, *M. javanica* and *M. arenaria*, extracted from infected tomato roots by the method of Hussey (1972), were homogenised with Tris-HCl buffer (0.01M, pH 7.4) in a Potter-Elvehjem homogenizer. After centrifugation at 40 000 g for 30 minutes, supernatants were tested for protein content by the folin-phenol method (Lowry *et al.*, 1951) using bovine serum albumin as standard. Solid urea was added to make a final concentration of 9 M. Then an equal volume of lysis buffer (O'Farrell, 1975) was added to the above solution. The solution was

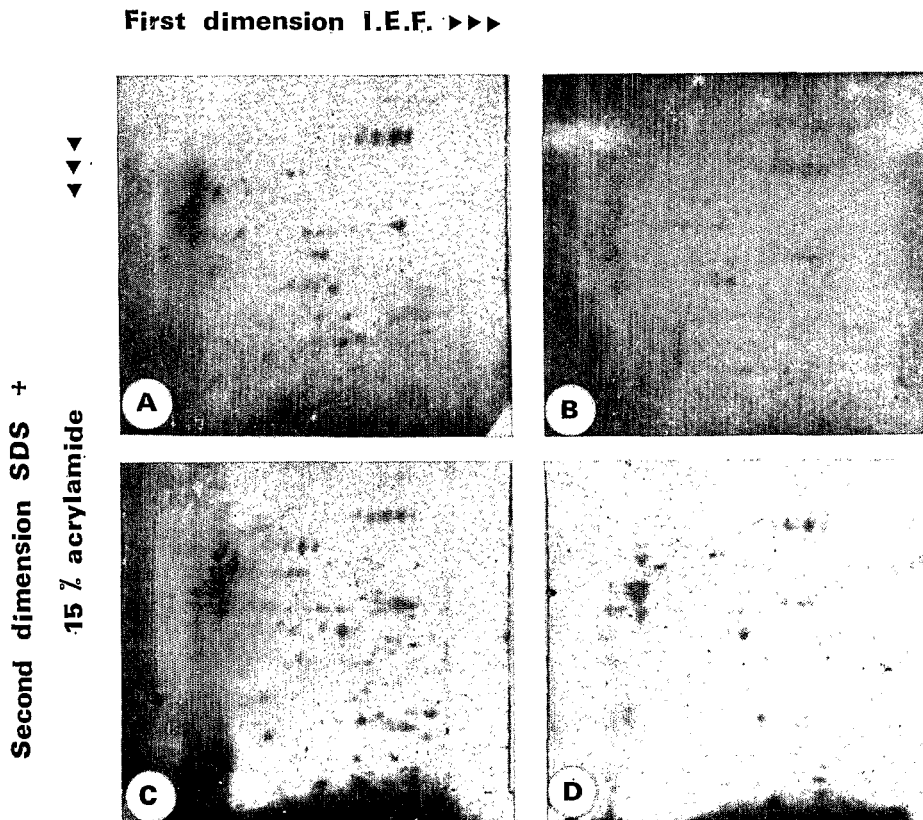


Fig. 1. Comparison of two-dimensional electrophoretic analysis of *Meloidogyne* proteins. A : *M. incognita*, population Valbonne ; B : *M. javanica*, population Abu Dhabi ; C : *M. arenaria*, population Le Grau ; D : *M. arenaria*, population Ain-Toujdate.

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mixed once again and centrifuged at 100 000 g in an airfuge for 75 minutes. The clear supernatant was used for electrophoresis. Iso-electrofocusing was carried out in glass tubes (10 cm × 0,25 cm i.d.). Gels were prepared according to the method of O'Farrell (1975). Proteins (375-400 µg) were loaded on tube and focused for about 6 000-7 000 volt-hours. After focusing, gels were equilibrated for twenty minutes in Tris-HCl (0.0625 M, pH 6.8) containing 10% (w/v) glycerol, 2.3% SDS and 0.001% bromophenol blue. Separation in the second dimension was carried out on 15% acrylamide slab gels (1 mm thickness) following the method of Laemmli (1970). A stacking gel of 4.75% acrylamide in Tris HCl buffer (0.125 M, pH 6.8) was layered above the running gel. I.E.F. gels were maintained on the second dimension gels by a 1% solution of agarose in the equilibration buffer. Electrophoresis at 40 V constant per gel was carried out as per the method of O'Farrell (1975) until the bromophenol dye had migrated to the bottom end of the gel.

After electrophoresis, gels were stained either with Coomassie Brilliant Blue R-250 (0.1% in a solution of 40% methanol and 7% acetic acid) or

by the modified method of Guevara *et al.* (1982).

Fig. 1 shows that some major "constellations" were invariably present in *M. incognita*, *M. arenaria* and *M. javanica*. However, we observed variations in the number and position of some spots. The main difference observed is that for the same quantity of proteins there are many more spots in *M. incognita* compared to other species.

Fig. 2 shows that Coomassie Brilliant Blue revealed only about twenty protein spots while more than 140 spots could be detected by silver staining. In conclusion, silver-stain in combination with ISO-DALT system resulted in much better resolution of *Meloidogyne* proteins than the conventional methods employed hitherto. Due to the presence of quite a large number of spots, comparison between these ISO-DALT gels was not easy. The present technique may be a valuable aid in identifying peptides while comparing clones of close genetic affiliation and differing only slightly in biological characters. The lack of an easily accessible system to analyse 2-D gels at present and the relative ease in genetic interpretation of isoenzyme patterns makes the latter preferable for taxonomic purposes.

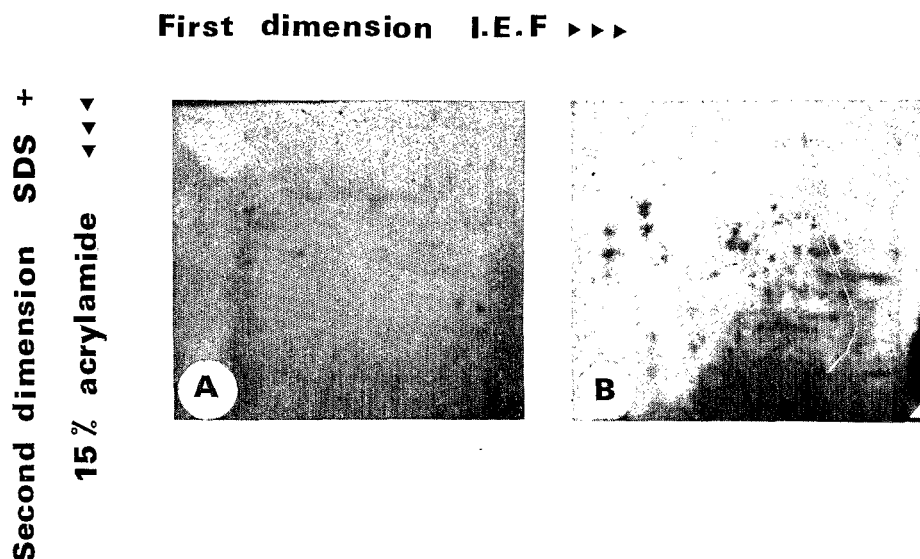


Fig. 2. Comparison of two methods of staining of proteins from *Meloidogyne* spp. A : Staining by Coomassie Brilliant Blue R-250 ; B : Silver staining. Increased number of spots for the same quantity of protein loaded on the gel may be noted.

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