PROTEIN ANALYSIS OF ROOT-LESION NEMATODES USING SDS-PAGE

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ABSTRACT

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Pratylenchus spp. are an economically important group of nematodes that are often morphologically similar. Comparisons of total protein patterns analyzed by SDS-PAGE with silver staining resulted in at least one to three distinct protein bands for differentiating the following species: *P. vulnus* (27.5 kDa), *P. scribneri* (38.4 kDa), *P. goodeyi* (63.1 and 31.6 kDa), *P. coffeae* (61.6 kDa), *P. thornei* (100 kDa and absence of a 77 kDa band present in all populations), *Radopholus similis* (105 kDa), and a protein of 24.5 kDa shared by *R. similis* and *P. goodeyi*, but missing in the other populations. Results show that this biochemical method can be useful for diagnosis at the interspecific level, at least for *Pratylenchus* species of major economic importance. The main limitation is to provide an abundant and pure source of the nematode species.

Key words: Diagnosis, interspecific variability, *Pratylenchus* spp., protein, *Radopholus similis*, SDS-PAGE.

RESUMEN

Jaumot, M., J. Pinochet y C. Fernández. 1997. Análisis de proteínas de nematodos lesionadores mediante SDS-PAGE. Nematrópica 27:33-39.

Pratylenchus spp. son un grupo de nematodos fitoparásitos económicamente importante que muestran una elevada semejanza morfológica entre las diferentes especies que componen el género. La comparación de los patrones de proteínas totales analizadas por SDS-PAGE, teñidas con plata, resultaron en por lo menos una a tres bandas de proteínas definidas para la diferenciación de las siguientes especies: *P. vulnus* (27.5 kDa), *P. scribneri* (38.4 kDa), *P. goodeyi* (63.1 y 31.6 kDa), *P. coffeae* (61.6 kDa), *P. thornei* (100 kDa y ausencia banda proteica de 77 kDa presente en todas las poblaciones), *R. similis* (105 kDa), y una proteína de 24.5 kDa compartida por *R. similis* y *P. goodeyi*, pero ausente en el resto de las poblaciones. Los resultados muestran que este método bioquímico puede ser útil para el diagnóstico a nivel de especie, al menos para las especies de *Pratylenchus* de mayor importancia económica. La principal limitación es poder contar con una fuente abundante y pura de la especie del nematodo.

Palabras clave: Diagnóstico, electroforesis en gel de poliacrilamida SDS, *Pratylenchus* spp., proteína, *Radopholus similis*, variablidad interespecífica.

INTRODUCTION

After root-knot nematodes (*Meloidogyne* spp.), root-lesion nematodes of the genus *Pratylenchus* are considered the most important plant parasitic nematodes in relation to yield losses (Agrios, 1969). Sixty eight species of *Pratylenchus* have been described (Siddiqi, 1986), although not more than 15 species account for most of the damage inflicted on agricultural crops. Among these, a few can be readily identified, while the majority are taxonomically difficult to identify due to their overlap-

ping morphologic and morphometric characters (Thorne, 1961). This problem is compounded by their broad host ranges and the occurrence of mixed populations of different *Pratylenchus* species in agricultural habitats. The correct diagnosis of lesion nematode species is important for implementing adequate management strategies, because the host range and pathogenicity of *Pratylenchus* spp. vary.

Biochemical techniques have been widely used to characterize nematode species at the inter and intraspecific level, and to establish their relationships (Ferris, 1994; Hussey, 1979). Most studies, however, deal with root-knot and cyst nematode species (Esbenshade and Triantaphyllou, 1990; Ibrahim and Perry, 1993; Nobbs *et al.*, 1992; Pozdol and Noel, 1984; Radice *et al.*, 1988; Subbotin *et al.*, 1996). Information for root-lesion nematodes is more limited. Cytogenetic (Román and Triantaphyllou, 1969), molecular (Pinochet *et al.*, 1994) and biochemical (Ibrahim *et al.*, 1995; Payan and Dickson, 1990) methods have been developed to identify several *Pratylenchus* species. Although these methods may be more or less reliable for species differentiation, their application appears to be too complex for diagnostic purposes, which requires skilled personnel and well equipped laboratories.

The purpose of this investigation was to determine the reliability and usefulness of total protein patterns for diagnosis of several *Pratylenchus* species that are common in tropical and sub-tropical environments using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Nematode isolates: A total of 14 isolates of migratory endoparasitic nematodes, comprising 5 *Pratylenchus* species and *Radopholus similis* (Cobb) Thorne, were assembled for the study. Populations originating from different geographical locations (mostly tropical and subtropical regions) and diverse hosts were obtained from several sources (Table 1). *Pratylenchus vulnus* Allen & Jensen and *P. goodeyi* Sher & Allen were the species that included the largest number of populations. The other lesion nematode species included in the study were *P. scribneri* Steiner, Sherbakoff & Stanley, *P. coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven, and *P. thornei* Sher & Allen. A population of the burrowing nematode, *R. similis* was included as an outgroup for comparison. Most of the nematode populations were extracted from infected root tissues and reared monoxenically on carrot (*Daucus carota* L.) disk cultures (Moody *et al.*, 1974) and incubated at 21°C for several generations. All cultures were generated from a few female specimens (approximately 10-15). A few populations were supplied from other live nematode collections. All *Pratylenchus* populations were verified by morphologic and morphometric analysis to assure the correct species identity of the nematode isolates. With populations that were difficult to identify, identification was confirmed by the Commonwealth Institute of Parasitology, St. Albans, United Kingdom.

Inoculum was obtained by adding sterile water to the carrot disk cultures and collecting the nematodes with a pipette. Suspensions were filtered through paper towels, placed on Baermann funnels and rinsed on a 20 μm pore-screen (600 mesh) with sterile-distilled water. Nematode material formed mainly by juvenile and adult stages was gradually accumulated in 1.5 ml sterile Eppendorf tubes and stored at -80°C until protein extraction.

Protein extraction and determination: Fifty μl of pelleted nematodes containing 5 000 to 10 000 juveniles and adults was resuspended in 400 μl of buffer A (50 mM Tris-

Table 1. Geographic and host origin of the different populations of *Pratylenchus* spp. and *Radopholus similis* used in this study.

Populations[y]	Geographic origin	Host	Source[z]
1 Pv RO-S	Barcelona, Spain	Rose (*Rosa multiflora*)	IRTA
2 Pv AT-F	Antibes, France	Apricot (*Prunus armeniaca*)	INRA
3 Pv WA-A	Córdoba, Argentina	Walnut (*Juglans nigra*)	UNC
4 Pv AP-US	Idaho, USA	Apple (*Malus domestica*)	UI
5 Pv OL-I	Taranto, Italy	Olive (*Olea europea*)	INA
6 Pv PL-S	Alicante, Spain	Plum (*Prunus insititia*)	IRTA
7 Ps CO-US	South Carolina, USA	Corn (*Zea mays*)	USDA
8 Pc CF-GU	Retalhuleu, Guatemala	Coffee (*Coffea arabica*)	CIRAD
9 Pt GA-S1	Cañete, Córdoba, Spain	Chickpea (*Cicer arietinum*)	CSIC
10 Pg BA-K	Oyugis, Kisi, Kenya	Banana (*Musa* AAB)	IITA
11 Pg BA-GC	Arucas, G. Canaria, Spain	Banana (*Musa* AAA)	ICIA
12 Pg BA-LP	Barlovento, La Palma, Spain	Banana (*Musa* AAA)	ICIA
13 Pg BA-TE	Galletas, Tenerife, Spain	Banana (*Musa* AAA)	ICIA
14 Rs BA-P	Changuinola, Panama	Banana (*Musa* AAA)	UB

[y]Pv = *Pratylenchus vulnus*; Ps = *P. scribneri*; Pc = *P. coffeae*; Pt = *P. thornei*; Pg = *P. goodeyi*; Rs = *Radopholus similis*.
[z]IRTA = Institut de Recerca i Tecnologia Agroalimentàries; INRA = Institut National de la Recherche Agronomique; UCN = Universidad Nacional de Córdoba; UI = University of Idaho; INA = Instituto de Nematologia Agraria; USDA = United Sates Dept. of Agriculture; CIRAD = Centre de Coopération Internationale en Recherche Agronomique pour le Développement; CSIC = Consejo Superior de Investigaciones Científicas; IITA = International Institute of Tropical Agriculture; ICIA = Instituto Canario de Investigaciones Agrarias; UB = United Brands Company.

HCl pH 7.4, 5 mM EDTA, 250 mM NaCl, 50 mM NaF, 0.1% Triton X-100, 1mM phenylmethylsulphonyl fluoride and 10 µg/ml aprotinin). The nematodes were sonicated with four pulses of 20 sec each at setting 2 using a Branson sonifier. Total protein contents in the homogenates were measured according to the method described by Bradford (1976) using bovine serum albumin as a standard.

Polyacrylamide gel electrophoresis: Samples of the 14 nematode isolates containing 1 µg of protein each, were separated according to molecular weight on Laemmli type (Laemmli, 1970) sodium dodecyl sulfate 10% polyacrylamide mini slab gels (0.75 mm thick). Electrophoreses were carried out in a Bio-Rad Protein II Multi Cell at 150 V constant voltage for 45 min. Low molecular weight standards from BioRad (BioRad Laboratories, Hercules, CA, USA) were utilized. For protein staining, the gels were fixed with 50% ethanol/12% acetic acid and stained with silver nitrate following the procedure of Blum *et al.* (1987).

Data analysis: Total protein bands of different molecular weights were recorded for each isolate as present or absent. A low protein SDS-PAGE standard was used for monitoring electrophoresis banding patterns. Only bands unambiguously present as stained bands were considered as valid. A minimum of three electrophoresis runs were performed in

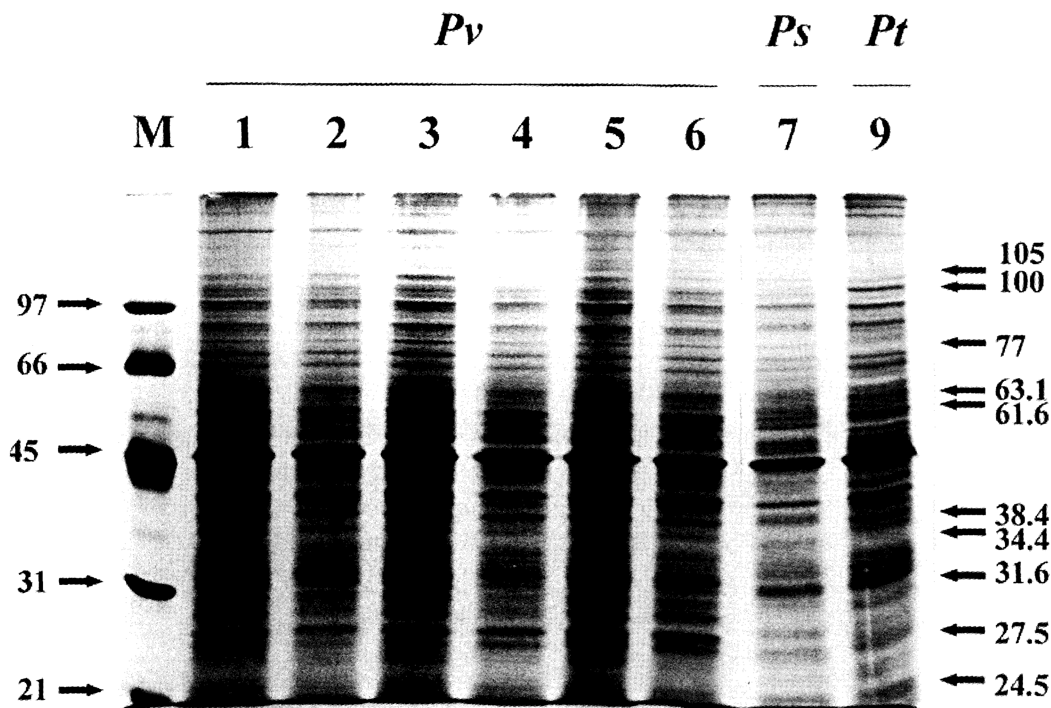


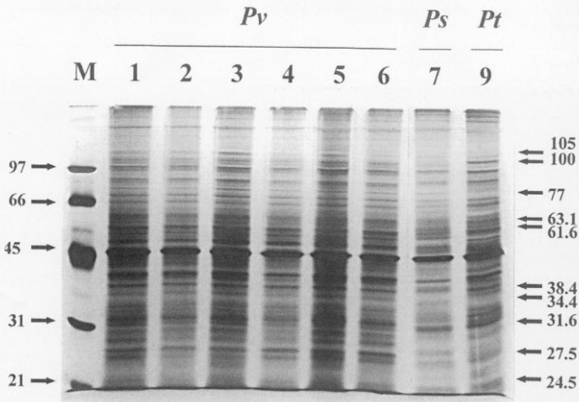
Fig. 1. Comparative protein patterns of three *Pratylenchus* species (8 populations). In successive lanes from left to right: lane M (marker size in kDa); lanes 1 to 6, *P. vulnus* (Pv); lane 7, *P. scribneri* (Ps); lane 9, *P. thornei* (Pt). Arrows at the right indicate molecular weights (kDa) that allow species differentiation.

order to confirm the reproducibility of protein bands.

RESULTS

Protein patterns of 13 populations of five *Pratylenchus* species and one of *R. similis* were reproduced on SDS-PAGE allowing a clear discrimination of the six species (Figs. 1 and 2). Total proteins from combined larval and adult stages separated into 32 to 41 bands that were clearly stained and readily detected. The analysis revealed that a general protein pattern involving most of the bands were shared by all populations. However, some specific protein bands allowed discrimination at the interspecific level.

The six *P. vulnus* populations shared a specific 27.5 kDa protein band not present in the other populations studied. The four *P. goodeyi* populations showed two dense protein bands at 63.1 kDa and 31.6 kDa, and the absence of a band at 41.8 kDa present in all the other populations (Fig. 2). The other species studied also presented at least one specific protein, although based on only one population of each. *Pratylenchus scribneri* yielded a differential 38.4 kDa protein (Fig. 1). A band at 34.4 kDa present in all populations was absent in this species. A single strongly stained band near 100 kDa (unidentified molecular weight) allowed differentiation of *P. thornei* from all other populations except *P. goodeyi.* A band at 77 kDa present in all populations was lacking in *P. thornei.* A 61.6 kDa protein was observed only in the *P. coffeae* population. The outgroup species,



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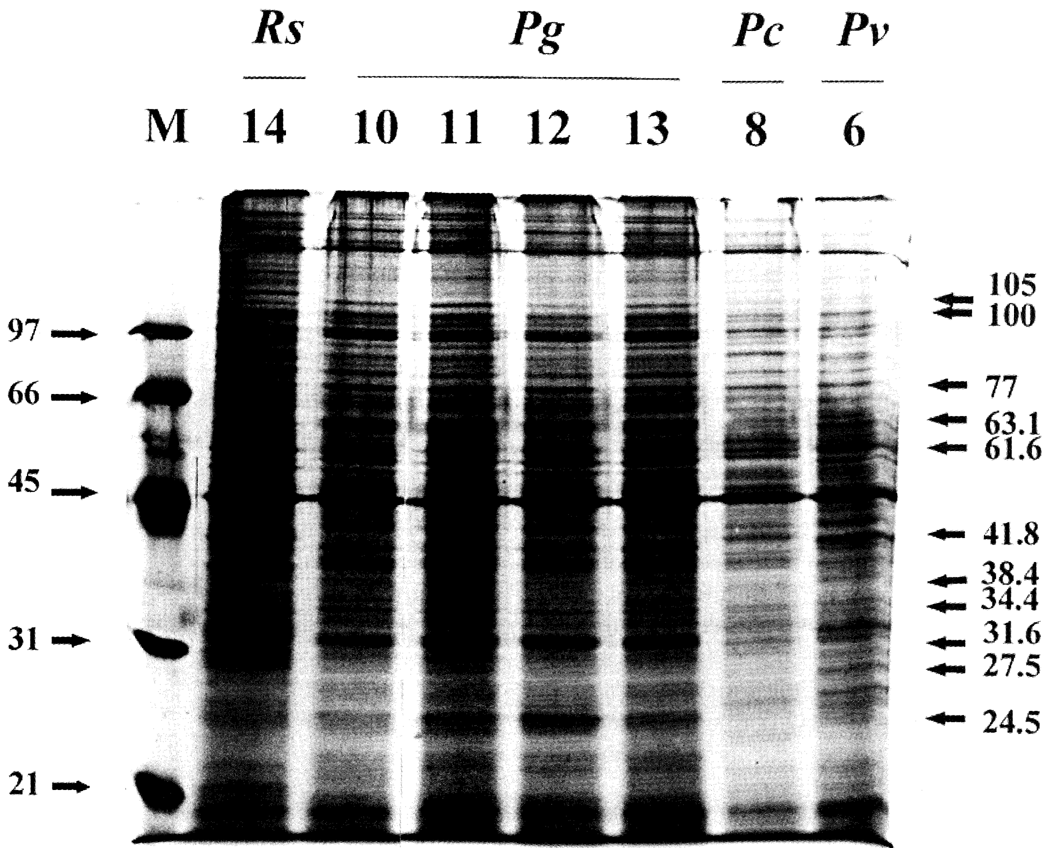
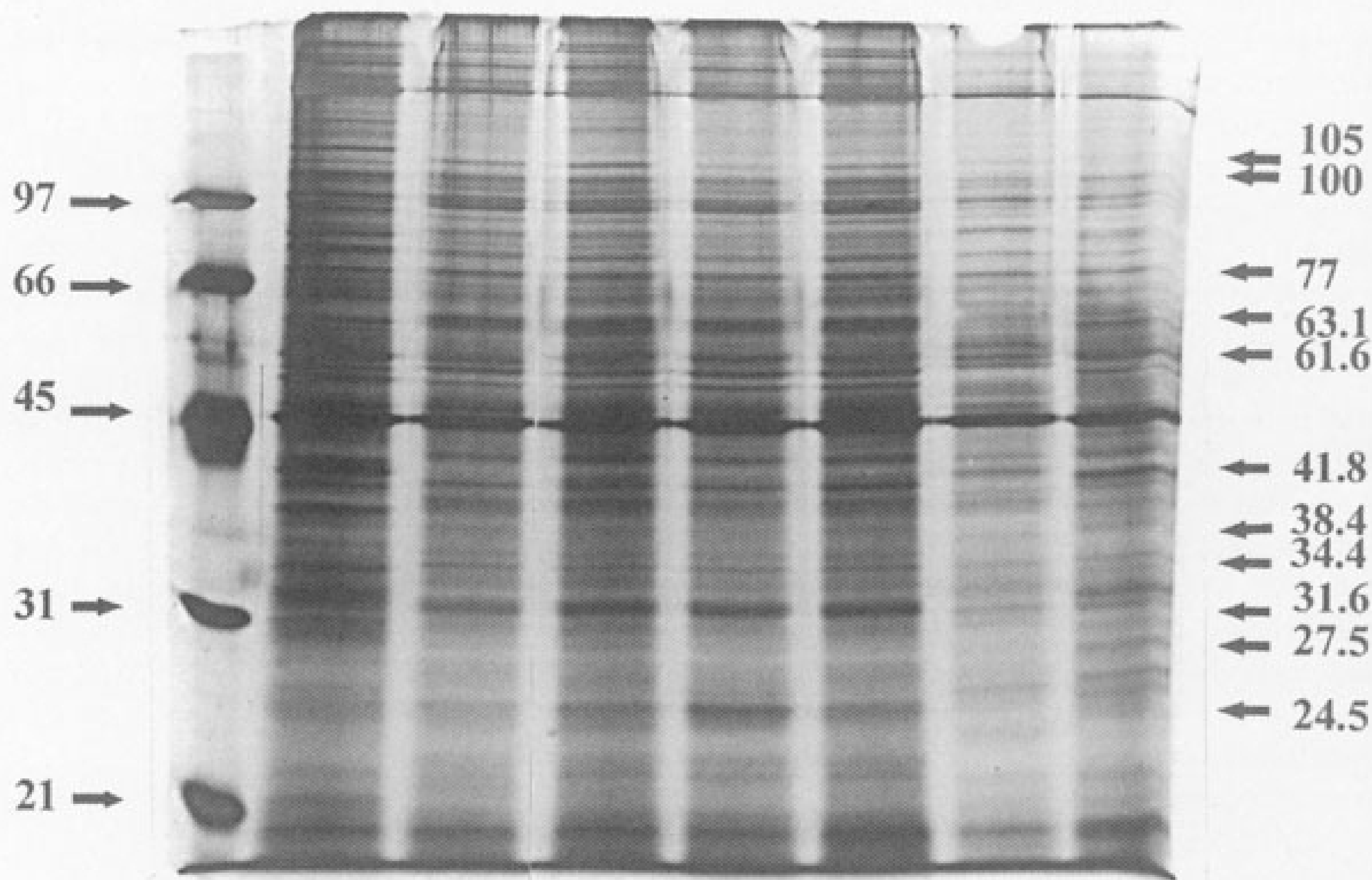


Fig. 2. Comparative protein patterns of *Radopholus similis* (one population) and three *Pratylenchus* species (6 populations). In successive lanes from left to right: lane M (marker size in kDa); lane 14, *R. similis* (Rs); lanes 10 to 13, *P. goodeyi* (Pg); lane 8, *P. coffeae* (Pc); lane 6, *P. vulnus* (Pv). Arrows at the right indicate molecular weights (kDa) that allow species differentiation.

R. similis, produced an exclusive band at 105 kDa lacking in the *Pratylenchus* isolates. A 24.5 kDa protein was shared by both *R. similis* and *P. goodeyi* and was not present in the *P. thornei*, *P. scribneri*, *P. coffeae* and *P. vulnus* populations. It was not possible to discriminate protein bands at the intraspecific level among the six isolates of *P. vulnus* or among the four isolates of *P. goodeyi*.

DISCUSSION

The SDS-PAGE technique was used in this study with the aim of finding protein markers that might be useful in differentiating five major *Pratylenchus* species collected from different hosts and from a broad geographic range. This method may offer some advantages in relation to other diagnostic techniques for nematode identification, since SDS-PAGE allows a rapid and simultaneous testing of several species of migratory endoparasitic nematodes. The method is highly accurate and reliable, and does not suffer from the lack of reproducibility of PCR based methods (Pinochet *et al.*, 1994). From a practical standpoint, the method requires some



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expertise (running, staining and interpreting gels) and requires specialized (although not sophisticated) equipment. The sample used for analysis is independent of the life-stage of the nematode, unlike that needed for isozyme analysis as reported by Payan and Dickson (1990). However, sample size can be a major limitation, since large amounts of nematodes are required to amass sufficient total proteins to perform electrophoresis. An estimated 1 μg/μl extracted protein per sample is the minimum required. These quantities are difficult to obtain from soil or root samples, thus requiring culturing the nematode as an intermediate step to gather a sufficiently abundant sample. Also, this technique is not recommended for phylogenic analysis because most protein bands are shared between the different species. Interspecific differentiation is based on the lack of commonality of a very few distinct bands. This may prove to be another disadvantage for its use in systematics (Hyman, 1996).

The protein patterns among the five *P. vulnus*, and the four *P. goodeyi* populations were distinctly characteristic, and therefore, diagnostic for each species. However, for species represented by only one population (*P. thornei*, *P. coffeae*, and *P. scribneri*), a larger number of populations per species, preferably from a broad geographic range and host distribution, would have been desirable to confirm the consistency of SDS-PAGE for species differentiation. The extent of intraspecific variation based on total protein appears to be very low. Bossis (1991) working with *Heterodera carotae* and *H. cruciferae* could easily discriminate between both species using SDS-PAGE but did not find intraspecific variation within each species. Similar observations with this method have been described by Bakker *et al.* (1988) with *G. rostochiensis* and *G. pallida.* More recently, Ibrahim *et al.* (1995) using a double stain variation of this same method (Coomassie brilliant blue and silver), discriminated between six *Pratylenchus* species from Great Britain, although based on one population per species. *Pratylenchus thornei* was the only lesion nematode species used both in that and in our study.

It is noteworthy that five of the six *P. vulnus* populations analyzed by SDS-PAGE in this study had been characterized previously by PCR-RAPD (Pinochet *et al.*, 1994). These presented a high level of polymorphism to nearly all oligonucleotid DNA primers used, rendering the molecular technique less reliable, due to the difficulties of obtaining banding patterns necessary for taxonomic purposes. In contrast, the identical protein patterns exhibited by all *P. vulnus* populations, despite their broad host and geographic origins, underscores the usefulness of SDS-PAGE for higher taxonomic levels.

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