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Use of Cellulose Acetate Electrophoresis in the Taxonomy of Steinernematids (Rhabditida, Nematoda)[1]

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Abstract: A steinernematid nematode was isolated from soil samples collected near St. John's, Newfoundland, Canada. On the basis of its morphometry and RFLPs in ribosomal DNA spacer, it was designated as a new strain, NF, of *Steinernema feltiae.* Cellulose acetate electrophoresis was used to separate isozymes of eight enzymes in infective juveniles of *S. feltiae* NF as well as four other isolates: *S. feltiae* Umeå strain, *S. feltiae* L1C strain, *Steinernema carpocapsae* All strain, and *Steinernema riobravis* TX strain. Based on comparisons of the relative electrophoretic mobilities (μ) of the isozymes, one of the eight enzymes (arginine kinase) yielded zymograms that were distinctive for each of the isolates, except for the Umeå and NF strains of *S. feltiae,* which had identical banding patterns. Four enzymes (fumarate hydratase, phosphoglucoisomerase, phosphoglucomutase, and 6-phosphogluconate dehydrogenase) yielded isozyme banding patterns that were characteristic for all isolates, except for the L1C and NF strains of *S. feltiae,* which were identical. Two enzymes (aspartate amino transferase and glycerol-3-phosphate dehydrogenase) yielded zymograms that permitted *S. carpocapsae* All strain to be discriminated from the other four isolates, while the remaining enzyme (mannose-6-phosphate isomerase) was discriminatory for *S. riobravis* TX strain. Except for one enzyme, the isozyme banding pattern of the NF isolate of *S. feltiae* was the same as in the L1C strain, isolated 13 years previously from Newfoundland. Cellulose acetate electrophoresis could prove invaluable for taxonomic identification of isolates of steinernematids, provided that a combination of enzymes is used.

Key words: cellulose acetate electrophoresis, entomopathogenic nematode, isozyme, ribosomal DNA, *Steinernema carpocapsae, Steinernema feltiae, Steinernema riobravis,* steinernematid, taxonomy.

Nematodes of the genus *Steinernema* have attracted considerable attention for development as novel biopesticides. Infective juveniles (IJ) of these nematodes gain access to the hemocoel of soil inhabiting insect hosts via natural openings (19), then release a mutualistic bacterium that kills the host and creates a nutritional milieu conducive for nematode development.

There are currently 18 recognized species of *Steinernema* (2), several of which exist as geographically distinct strains (19). Strain identification is an important aspect of the overall research and development of this family of entomopathogenic nematodes. Curran (4) concluded that DNA sequence analysis constitutes the preferred molecular method for discriminating between categories of entomopathogenic nematodes below the species level. To facilitate this, DNA amplification techniques by polymerase chain reaction (PCR) have been developed for entomopathogenic nematodes (16).

Protein electrophoresis also could be a useful taxonomic tool, enabling nematologists to more rapidly and cost effectively discriminate between species (5,24) and, in some instances, strains (12). With respect to entomopathogenic nematodes, starch gel electrophoresis revealed an array of esterase and alkaline phosphatase isozymes in *Steinernema glaseri* that were qualitatively and quantitatively different from those in *S. carpocapsae* (24). This same procedure, adapted to separate isozymes of several enzymes, was used by Akhurst (1) to distinguish between 22 strains representing 3 to 8 species of *Heterorhabditis.* Polyacrylamide gel electrophoresis was used to discriminate between five isolates (= four species) of *Steinernema* on the basis of their esterase isozyme patterns (23) and 10 isolates (= four species) of this genus according to their total protein, nonspecific esterase,

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and alkaline phosphatase patterns (14). Poinar and Kozodoi (20) showed that two morphologically similar steinernematids, *S. glaseri* and *Steinernema anomali,* possessed dissimilar and distinctive enzyme patterns, as revealed by polyacrylamide gel electrophoresis (PAGE).

Cellulose acetate is a simpler, more rapid type of electrophoresis, regarded by some authorities as being more sensitive and providing superior resolution to starch gel or PAGE (6). It has not been used in the taxonomy of entomopathogenic nematodes.

In this paper, we report on the identification of a steinernematid, *S. feltiae* NF strain, isolated from soil in Newfoundland. As part of this identification process, we evaluated the taxonomic value of cellulose acetate electrophoresis by separating isozymes of eight metabolic enzymes in five isolates (= three species) of *Steinernema: S. carpocapsae* All strain, *S. riobravis* TX strain, *S. feltiae* Umeå strain (= *S. carpocapsae* Umeå strain), *S. feltiae* L1C strain, and *S. feltiae* NF strain.

Materials and Methods

Sources of nematodes: Steinernema carpocapsae All strain was provided by Plant Products Ltd., Ontario, Canada, and *S. riobravis* TX strain by H. E. Cabanillas, USDA ARS, Crop Insects Research Unit, Weslaco, Texas. *Steinernema feltiae* Umeå strain was provided by R. West, Canadian Forest Service (CFS), St. John's, Newfoundland, Canada, from a stock colony that had been initially obtained from Biologic Biocontrol Products, Willow Hill, Pennsylvania. *Steinernema feltiae* L1C strain was obtained from a colony being maintained at CFS, St. John's, Newfoundland. This nematode was isolated in 1983 (7) from a location close to St. John's Newfoundland, and has been maintained in laboratory culture at CFS since then. The original collection site has been paved over. *Steinernema feltiae* NF strain is a new strain that we isolated in Summer 1994 from soil on a farm site close to St. John's, Newfoundland, using *Galleria* bait traps (26). All nematode isolates were maintained at 25°C by propagation through *G. mellonella* larvae (26).

I. *Identification of the Newfoundland (NF) isolate:* This was accomplished by measurements of the infective third-stage juveniles (IJ) and by restriction fragment length polymorphisms (RFLPs) of amplified rDNA. Infective juveniles of *S. feltiae* NF strain were collected from the White traps (26) immediately after emergence from the *Galleria* cadavers, i.e. 7 to 10 days after infection. Nematodes were heat-killed by placing them in a small beaker containing water (room temperature), which was then plunged into another beaker filled with water (100°C) for 2 to 3 minutes or until the specimens assumed the almost straight-form characteristic of heat death (11). The killed nematodes were fixed and stained for 24 hours in Chang's nerve stain (9), differentiated repeatedly in warm lactophenol (40 to 50°C) until no more superfluous stain was evident, and then mounted in glycerin (11). Measurements of 25 randomly selected IJ were made under a light microscope (×1,000) equipped with a micrometer eyepiece. The morphological parameters and ratios measured were those used by Poinar (19).

Restriction fragment length polymorphisms of amplified rDNA internal transcribed spacer (ITS) fragments were used to separate the NF strain from other commercially used strains of *Steinernema* (*S. carpocapsae* All strain, or *S. feltiae* Umeå and L1C strains). Other strains of *S. feltiae* type A (Sf76, Sf509a, Sf519) or *S. feltiae* type B (B1 Nashes and B2 216) were provided by Alex Reid (Imperial College of Science, Technology and Medicine, Department of Biology, Berks, UK). The strains of *S. carpocapsae* (DD136, 42, Breton, Kapow) were provided by M. Hubbes (Forestry Department, University of Toronto, Ontario, Canada). Nematodes were stored at −20°C until use. For each population, five nematodes were placed in a 5-µl drop of lysis buffer (1 ml of lysis buffer contained 100 µl 10% SDS [sodium dodecyl sulfate], 100 µl 1.0 M Tris pH 8.7,

40 µl 5 M NaCl, 10 µl 0.5 M EDTA, 750 µl deionized water) on a silated glass cover slip and cut open. The resulting suspension was pipetted into a 1.5-ml microfuge tube, 15 µl of lysis buffer with 40 µg of proteinase K (Sigma Chemical Co. St. Louis, MO) added, and incubated at 60°C for 1 hour. DNA was recovered from the suspension by binding to powdered glass (geneclean kit, Bio/Can Scientific, Mississauga, ON, Canada) and eluted into 10 µl deionized water. Amplification of ribosomal ITS sequences using the PCR was as described in Vrain et al. (25). Two primers, 21 base pairs long, one with sequence at the 3′ end of the 18 S gene and the other at the 5′ end of the 26 S gene, allowed for the amplification of a rDNA fragment composed of the 3′ end of the 18 S gene, the ITS 1, the 5.8 S gene, the ITS 2, and the 5′ end of the 26 S gene. Negative controls containing all necessary chemicals but without added DNA as well as positive controls containing *Caenorhabditis elegans* DNA were included in all experiments. The amplified ribosomal ITS fragments were digested using five restriction enzymes–Eco RI, Hae III, Hinf I, Mbo I, and Rsa I—in a 10-µl volume according to manufacturers' instructions (Pharmacia, Uppsala, Sweden). Fragments of each restriction digest were separated by electrophoresis and visualized in the agarose gels (25).

II. Cellulose acetate electrophoresis studies:

Enzyme extraction: Infective juveniles of each of the five isolates were transferred from the dilute formalin in the White traps (26) to separate 250-ml beakers containing distilled water and then allowed to settle (about 10 minutes). Using an Eppendorf micropipeter, IJ were transferred from the bottom of the beakers to Whatman no. 4 filter papers so that they formed confined blobs on the papers. Samples of each isolate (10 mg wet weight) were transferred into separate polypropylene microcentrifuge tubes (1.5 ml), then macerated in the tubes with a pellet pestle mixture motor (Baxter Diagnostic Corporation, Canlab Division, Mississauga, ON, Canada). After maceration, 30 µl of distilled water containing bromophenol blue tracking dye (pH 6.0) was added to each tube, the homogenates centrifuged at 3,200*g* for 2 minutes and then held on ice. Aliquots (10 µl) of the supernatants were transferred from the tubes to separate wells in the sample holder and kept on ice until the sample loading was performed on the cellulose acetate plates (Helena Laboratories, Beaumont, TX).

Cellulose acetate electrophoresis procedure: Aliquots (0.6 µl) of the supernatants were transferred from the sample holder to Titan III Zip Zone Cellulose Acetate Plates using a Super Z Applicator (Helena Laboratories). Plates had been pre-soaked (20 minutes) in Tris-Glycine buffer (3.0 g Tris, 14.4 g glycine, 1 liter distilled water, pH 8.5) prior to spotting. Electrophoresis was carried out (2 mA/plate; 20–30 minutes) in Tris-Glycine buffer in a horizontal electrophoresis chamber at room temperature (20 to 25°C).

Enzyme staining: Specific stains were used to visualise the following enzymes: arginine kinase (EC 2.7.3.3), aspartate amino transferase (EC 2.6.1.1), fumarate hydratase (EC 4.2.1.2), glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), mannose-6-phosphate isomerase (EC 5.3.1.8), phosphoglucoisomerase (EC 5.3.1.1), phosphoglucomutase (EC 2.7.5.1), and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) (10).

Plates were removed from the electrophoresis chamber and placed on a levelled glass surface. Melted agar (60°C) was added to the stain mixture and then the resultant solution was poured immediately over the plates, which were then incubated in the dark until isoenzyme bands became prominent (about 10 to 15 minutes). Plates were then washed (2 to 3 times) under tap water and the bands fixed by immersion in acetic acid:methanol:distilled water (1:4:10) for 10 minutes. Fixed plates were dried overnight, and then photographed and isozyme bands were measured. Three replicate plates were prepared for each nematode isolate, each replicate representing a

separate IJ homogenate. The relative electrophoretic mobility (μ) for each isoenzyme was measured to compare the migration rates (15), and isozyme bands among isolates were considered the same if their μ values were within 10% of one another. This margin of error was selected because the highest and lowest μ values among three replicates of the same isozyme were always found to be within 10% of one another.

Results

I. *Identification of the Newfoundland (NF) isolate:* The measurements and ratios of morphometric characters of the Newfoundland isolate corresponded with those reported for *S. feltiae* (Table 1). Restriction fragment length polymorphisms separated the NF strain from *S. carpocapsae* All and Umeå strains (Table 2). The Umeå strain was clearly associated with the restriction fragment length polymorphism type A of *S. feltiae* as defined by Reid and Hominick (22), whereas the *S. feltiae* NF strain appeared to belong to the restriction fragment length polymorphism type B (22). No polymorphisms were identified between *S. feltiae* L1C and NF strains using five restriction enzymes to digest the rDNA ITS amplified fragment. Thus, on the basis of morphometry and RFLPs in ribosomal DNA ITS, we have designated the isolate as *S. feltiae* NF strain.

II. Cellulose acetate electrophoresis studies:

Arginine kinase (ARK): The isozyme banding pattern for this enzyme was strain specific. Each of the five isolates possessed a single band of ARK activity that migrated anodally (Fig. 1A). According to measurements of electrophoretic mobility (Table 3), the same anodal band (Band −2) was common to the three strains of *S. feltiae*. *Steinernema carpocapsae* All strain and *S. riobravis* TX strain possessed a different anodal band (Band −1). The three strains of *S. feltiae* and *S. riobravis* TX strain possessed a common cathodal band of ARK activity (Band 1) with the same electrophoretic mobility (Table 3). *Steinernema feltiae* L1C strain possessed a second cathodal band (Band 2), whereas *S. carpocapsae* All strain possessed two cathodal bands (Bands 3,4) that were electrophoretically distinct from those in any of the other four isolates.

Fumarate hydratase (FUM): Only the L1C and NF strains of *S. feltiae* had identical zymograms, consisting of three bands (Bands 1,6,7) that migrated toward the cathode (Fig. 1B). The Umeå strain of *S. feltiae* also possessed three cathodal bands, the slowest moving of which (Band 1) was common to all strains of this species. *Steinernema carpocapsae* All strain possessed two cathodal bands, including the slowest-moving band present in *S. feltiae*. There were four discernible bands in *S. riobravis* TX strain, one of which (Band 3) was

Table 1. Morphometric characters (in μm) of infective juveniles of *Steinernema feltiae* NF strain[a].

Character/Ratio	*S. feltiae* NF strain	*S. feltiae*[b]
Body length (L)	857.6 ± 65.2 (675.0–937.5)	849 (736–950)
Greatest width (W)	35.50 ± 5.72 (25.0–45.0)	26 (22–29)
Distance from head to excretory pore (EP)	66.6 ± 9.4 (55.0–87.5)	62 (53–67)
Distance from head to nerve ring (NR)	92.3 ± 11.5 (80.0–117.5)	99 (88–112)
Distance from head to pharynx base (PB)	131.0 ± 14.9 (105.0–162.5)	136 (115–150)
Tail length (T)	81.3 ± 8.5 (62.5–100)	81 (70–92)
Ratio A (L/W)	24.7 ± 4.0 (18.0–34.0)	31 (29–33)
Ratio B (L/ES)	6.6 ± 0.8 (5.3–8.0)	6 (5.3–6.4)
Ratio C (L/T)	10.6 ± 1.0 (9.0–13.2)	10.4 (9.2–12.6)
Ratio D (EP/PB)	0.5 ± 0.05 (0.42–0.61)	0.45 (0.42–0.51)
Ratio E (EP/T)	0.81 ± 0.11 (0.64–1.11)	0.78 (0.69–0.86)

[a] Mean values ± SD (n = 25). Ranges are given in parentheses.
[b] From Poinar (19).

TABLE 2. Size of DNA fragments generated by restriction of PCR amplified ribosomal sequence in strains of *Steinernema feltiae* and *Steinernema carpocapsae.*

Species	Strains	Hinf I	Mbo I	Rsa I
S. carpocapsae	All, DD136, 42, Breton, Kapow	420	590	400
		300	240	380
		280	190	320
		150		
S. feltiae type A	Sf76, Sf509a, Sf519	720	590	460
		240	240	240
		180	120	220
			100	140
S. feltiae type B	B1 Nashes, B2 216	720	340	720
		240	300	220
		160	190	140
			120	
			100	
S. feltiae	Umeå	720	590	460
		240	240	240
		180	120	220
			100	
	NF	720	340	720
		240	300	220
		160	190	140
			120	
			100	
	L1C	720	340	720
		240	300	220
		160	190	140
			120	
			100	

Size of DNA Fragments in bp. Fragments smaller than 100 bp could not be measured accurately and are not included.

present in *S. carpocapsae* All strain and two of which (Bands 4,5) were present in *S. feltiae* Umeå strain (Table 3).

Aspartate amino transferase (AAT): The isozyme banding patterns of *S. feltiae* Umeå strain and *S. riobravis* TX strain were identical, as were those for the L1C and NF strains of *S. feltiae. Steinernema carpocapsae* All strain had a distinct banding pattern (Table 3).

Glycerol-3-phosphate dehydrogenase (GPDH): All isolates possessed a single band of GPDH activity that migrated toward the cathode. Only *S. carpocapsae* All strain had a GPDH banding pattern that was distinguishable from the other isolates (Table 3).

Mannose-6-phosphate isomerase (MPI): Isozymes of this enzyme were not strain or species specific. The NF strains of *S. feltiae* possessed the same two cathodal bands (Bands 3,4) as the L1C strain (Table 3). The Umeå strain of *S. feltiae* and the All strain of *S. carpocapsae* possessed two common isozyme bands (Bands 1,2). *Steinernema riobravis* TX strain had a distinctive isozyme pattern.

Phosphoglucoisomerase (PGI): Of the five isolates, only *S. riobravis* TX strain possessed one cathodal band and the remaining four isolates possessed two bands. Only the L1C and NF strains of *S. feltiae* had identical isozyme profiles (Table 3).

Phosphoglucomutase (PGM): With the exception of the L1C and NF strains of *S. feltiae,* isolates possessed distinct zymograms. Two cathodal bands (Bands 2,4) were common to L1C and NF strains of *S. feltiae. Steinernema carpocapsae* All strain possessed three and *S. feltiae* Umeå strain

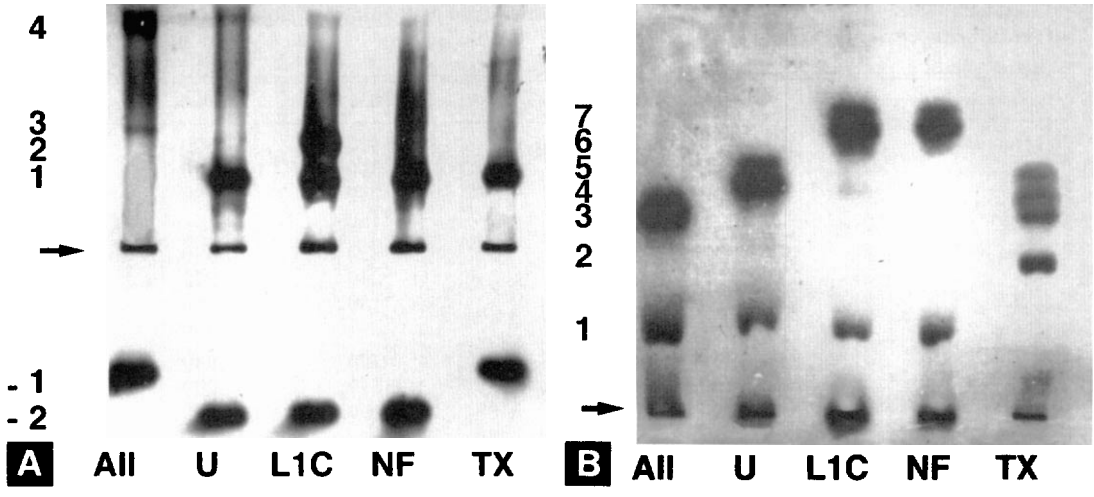

FIG. 1. Photographs of comparative isoenzyme patterns for two representative enzymes exhibited by five nematode isolates. All = *Steinernema carpocapsae* All strain, U = *S. feltiae* Umeå strain, L1C = *S. feltiae* L1C strain, NF = *S. feltiae* NF strain, TX = *S. riobravis* TX strain. A) Arginine kinase (ARK) and B) Fumarate hydratase (FUM). Arrows indicate points of sample application. Bands were numbered cathodal or anodal (−) in increasing numerical order relative to the distance that they migrated away from the origin.

possessed four cathodal bands; two of these bands (Bands 3,5) were common to both species (Table 3).

6-Phosphogluconate dehydrogenase (6PGDH): The Umeå, L1C, and NF strains of *S. feltiae* each possessed two cathodal bands, whereas the All strain of *S. carpocapsae* and the TX strain of *S. riobravis* each possessed one cathodal band. Only the L1C and NF strains of *S. feltiae* had identical zymograms (Table 3).

DISCUSSION

Cellulose acetate electrophoresis proved effective in discriminating between the five isolates of *Steinernema.* Results reported in this study have been replicated on several occasions. One of the eight enzymes studied (ARK) yielded zymograms that were distinctive for each of the isolates, except for the Umeå and NF strains of *S. feltiae,* which had identical banding patterns. Four enzymes (FUM, PGI, PGM, and 6 PGDH) had isozyme profiles that were characteristics for all isolates except for the L1C and NF strains of *S. feltiae,* which were indistinguishable. Three enzymes (AAT, GPDH, and MPI) provided banding patterns that did not permit strain or species separations to be made. In the case of AAT and GPDH, only *S. carpocapsae* All strain had a distinguishing banding pattern, whereas *S. riobravis* TX strain was the only such isolate with respect to MPI zymograms. Thus, while no single enzyme would be sufficient to allow taxonomic identification among the five isolates, a combination of enzymes would permit such a task.

The steinernematid nematode that we have isolated from soil samples in Newfoundland may, on the basis of its morphometry and RFLPs in ribosomal ITS, be regarded as a boreally adapted strain of *S. feltiae.* The fact that the L1C and NF strains of *S. feltiae* had identical isozyme profiles for seven of the eight enzymes is understandable, since these two strains were isolated from soil samples taken approximately 10 km from one another. Since the ribosomal DNA profiles of L1C and NF strains are indistinguishable, we initially had considered them to be the same strain. However, *S. feltiae* L1C strain was isolated in 1983 (7) and has been maintained in laboratory culture since then. It is possible that it may have undergone ge-

TABLE 3. Mean electrophoretic mobility (cm^2/sec./v) of isoenzymes of eight enzymes in five isolates of steinernematid nematodes.

No. of[a] band	*S. c.* All[b]	*S. f.* Umeå	*S. f.* L1C	*S. f.* NF	*S. r.* TX
		Arginine kinase (ARK)			
(−) 1.	2.05×10^{-4}	–	–	–	1.99×10^{-4}
(−) 2.	–	2.69×10^{-4}	2.71×10^{-4}	2.71×10^{-4}	–
1.	–	1.10×10^{-4}	1.10×10^{-4}	1.10×10^{-4}	1.10×10^{-4}
2.	–	–	1.65×10^{-4}	–	–
3.	1.98×10^{-4}	–	–	–	–
4.	3.25×10^{-4}	–	–	–	–
		Aspartate amino transferase (AAT)			
(−) 1.	4.01×10^{-5}	3.78×10^{-5}	–	–	4.01×10^{-5}
1.	–	1.68×10^{-4}	1.54×10^{-4}	1.51×10^{-4}	1.60×10^{-4}
2.	2.07×10^{-4}	–	–	–	–
		Fumarate hydratase (FUM)			
1.	1.08×10^{-4}	1.16×10^{-4}	1.16×10^{-4}	1.08×10^{-4}	–
2.	–	–	–	–	1.55×10^{-4}
3.	2.05×10^{-4}	–	–	–	1.97×10^{-4}
4.	–	2.31×10^{-4}	–	–	2.31×10^{-4}
5.	–	2.54×10^{-4}	–	–	2.57×10^{-4}
6.	–	–	2.90×10^{-4}	2.89×10^{-4}	–
7.	–	–	3.15×10^{-4}	3.17×10^{-4}	–
		Glycerol-3-phosphate dehydrogenase (GPDH)			
1.	–	2.40×10^{-4}	2.40×10^{-4}	2.40×10^{-4}	2.48×10^{-4}
2.	2.98×10^{-4}	–	–	–	–
		Mannose-6-phosphate isomerase (MPI)			
1.	1.50×10^{-4}	1.48×10^{-4}	–	–	–
2.	1.80×10^{-4}	1.72×10^{-4}	–	–	–
3.	–	–	1.99×10^{-4}	1.93×10^{-4}	–
4.	–	–	2.25×10^{-4}	2.23×10^{-4}	2.17×10^{-4}
5.	–	–	–	–	2.36×10^{-4}
		Phosphoglucoisomerase (PGI)			
1.	1.67×10^{-4}	–	–	–	–
2.	2.04×10^{-4}	–	–	–	–
3.	–	2.21×10^{-4}	–	–	–
4.	–	2.48×10^{-4}	2.45×10^{-4}	2.48×10^{-4}	–
5.	–	–	2.72×10^{-4}	2.69×10^{-4}	–
6.	–	–	–	–	3.08×10^{-4}
		Phosphoglucomutase (PGM)			
1.	–	5.65×10^{-5}	–	–	–
2.	–	8.53×10^{-5}	8.29×10^{-5}	8.22×10^{-5}	–
3.	1.41×10^{-4}	1.47×10^{-4}	–	–	1.42×10^{-4}
4.	–	–	2.64×10^{-4}	2.80×10^{-4}	2.84×10^{-4}
5.	3.73×10^{-4}	3.70×10^{-4}	–	–	–
6.	3.92×10^{-4}	–	–	–	–
		6-Phosphogluconate dehydrogenase (6PGDH)			
1.	–	–	2.41×10^{-4}	2.41×10^{-4}	–
2.	–	–	2.65×10^{-4}	2.65×10^{-4}	–
3.	3.11×10^{-4}	2.99×10^{-4}	–	–	–
4.	–	3.54×10^{-4}	–	–	3.47×10^{-4}

Values are the means of three replicate plates, each replicate representing a separate IJ homogenate.

[a] Bands were numbered cathodal or anodal (−) in increasing numerical order relative to the distance that they migrated away from the origin. Bands among isolates were considered the same if their relative electrophoretic mobility values were within 10% of one another. Dashes indicate absence of bands.

[b] *S. c.* = *Steinernema carpocapsae*, *S. f.* = *S. feltiae*, *S. r.* = *S. riobravis*. All, Umeå, L1C, NF, and TX are strain designations.

netic and consequent biochemical changes resulting from repetitive laboratory recycling and that the loss of one of the cathodal bands of ARK activity constitutes one manifestation of such transformation. The possibility that the two closely related strains may have always been distinct from the time of collection cannot be investigated because the original collection site for the L1C strain has been paved over and we have determined that soil from adjacent areas is free of entomopathogenic nematodes.

Based on its morphometry (Jagdale and Gordon, unpubl. obs.) and RFLPs in the ribosomal ITS spacer as reported herein, the nematode first isolated from soil in Sweden (21) and since marketed commercially as *S. carpocapsae* Umeå strain should be reclassified as *S. feltiae*. This designation further accords with studies done on its bacterial symbiont. On the basis of biochemical and physiological characters, Boemare and Akhurst (3) designated the bacterial symbiote of the Umeå isolate as *Xenorhabdus bovienii,* a bacterial species normally associated with *S. feltiae*. The overall isozyme profiles of this nematode contained elements of all three species. For example, it had a zymogram for ARK identical to *S. feltiae* NF strain, lacking all three isozymes present in *S. carpocapsae* All strain. However, its MPI zymogram was identical only to *S. carpocapsae* All strain, and its GPDH zymogram consisted of the same isozyme produced by *S. feltiae* and *S. riobravis,* but not by *S. carpocapsae*. A wider array of isolates and enzymes would need to be tested before species-diagnostic isozyme patterns could be discerned.

Electrophoretic procedures, starch gel (1,12), polyacrylamide gel electrophoresis (13,17), and isoelectric focusing (8,18) have been widely deployed for taxonomic separations of species and subordinate taxa of animal-parasitic and plant-parasitic nematodes. Cellulose acetate electrophoresis is a relatively inexpensive procedure that has the added benefit of speed. Prerun times are reduced to a minimum by using pre-manufactured plates, and running times are as low as 20 to 30 minutes. Based on our experience, it affords the necessary sensitivity to allow good resolution of isozymes from small amounts of nematode material. The current interest in commercializing steinernematids has resulted in a burgeoning inventory of isolates from many parts of the world (19). While based on only three species that embody five strains, the present study nevertheless suggests that cellulose acetate electrophoresis could be of practical use to researchers, enabling species and even strain designations of isolates to be made. More extensive studies are warranted to provide for the cataloging of a broad array of isolates and enzymes.

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