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RFLP Analysis of PCR Amplified ITS and 26S Ribosomal RNA Genes of Selected Entomopathogenic Nematodes (Steinernematidae, Heterorhabditidae)[1]

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Abstract: This study examined the polymerase chain reaction (PCR) amplified internal transcribed spacer (ITS) and 26S ribosomal DNA (rDNA) regions of 15 entomopathogenic nematode isolates including *Steinernema feltiae* syn. *bibionis*, *S. glaseri*, seven strains of *S. carpocapsae*, four strains of *Heterorhabditis bacteriophora*, and two field isolates. RDNA length variation was not observed among the isolates examined. Restriction fragment length polymorphisms (RFLP) of PCR amplified ITS and 26S regions provided specific banding patterns for all isolates but *S. feltiae* syn. *bibionis* and *S. glaseri*. These two species were separated by zymograms of esterase and tetrazolium oxidase. A field trapping method retrieved two isolates of naturally occurring nematodes. One field isolate collected (F1) displayed banding patterns identical to those of *S. carpocapsae* DD136 released in the same location 1 year earlier. The second field isolate (F2) had unique PCR-RFLP profiles compared with all other strains. This study provides a rapid molecular taxonomic method to more fully establish species relationships among members of *Steinernema* and *Heterorhabditis*.

Key words: entomopathogenic nematode, *Heterorhabditis bacteriophora*, internal transcribed spacer (ITS), polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), ribosomal DNA (rDNA), *Steinernema carpocapsae*, *S. feltiae* syn. *bibionis*, *S. glaseri*, taxonomy.

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are successful biological control agents for a wide range of insects (10, 15,18,19,21). Management results, however, are known to vary when different species and strains of nematodes are assayed (8). This variation is thought to be derived from inherent genetic diversity and incorrect population identification. With the increasing interest in use of entomopathogenic nematodes for biocontrol of insect pests, an efficient, reliable technique is required to identify nematode isolates with stable characters.

Classical taxonomy among species of *Steinernema* and *Heterorhabditis* is based primarily on the structure of morphological features including spicules; genital papillae; phasmids; and various ratios involving the distances between the head, excretory pore, nerve ring, and tail (20,27,29, 30). Cross-breeding studies involving inoculation of hanging droplets of blood and inoculation of a single female and male into the larvae of *Galleria mellonella* strengthened species classifications (1,28).

The increasing need to diversify methods of taxonomic resolution has been outlined with reference to the incongruities between morphological and molecular characters for two species of plant-parasitic nematodes, namely *Heterodera schachtii* and *H. glycines* (12).

Thirteen isolates of entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) were separated using total protein and selected isoenzymes (36). Isozyme patterns are useful markers, but they are often limited by an insufficient number of detectable loci and low level of polymorphism. Since proteins are products of genes and are therefore subject to environmental constraints, more stable characters are required to establish solid relationships between nematode isolates. DNA markers are almost unlimited in numbers and allow accurate coverage of the genome (17,23).

Ribosomal DNA (rDNA) has become a useful DNA region for classifying eukaryotes at various taxonomic levels (16). The rDNA is a multi-copy, tandemly repeated array occurring in the nucleolar organizer region at one or several chromosomal sites

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(22). Within the rDNA cistron are coding and noncoding sequences that can be used to study various taxonomic levels, from within species populations to taxa at or above genera. The rDNA coding genes vary in evolutionary conservation from most-conserved 18S, 5.8S, to least-conserved 26S. The spacer regions including ETS (external transcribed spacer), ITS (internal transcribed spacer), and IGS (intergenic spacer) are more variable than the gene regions and are generally used for analysis at or below the species level (4).

Concerns with classical identification methods include the level of sensitivity a technique offers as well as the requirement for sufficient amounts of material necessary for successful analysis. With the advent of the polymerase chain reaction (PCR), even minute amounts of specific target DNA can be exponentially amplified for restriction fragment length polymorphism (RFLP) analysis (2,5,25). The presence of polymorphic bands reflects the amount of DNA sequence divergence between the compared populations (38). RFLP analysis of the PCR amplified ITS region was used to differentiate members of the *Xiphinema americanum* group (37). Recently, species of *Ditylenchus* were separated using PCR-RFLP analysis of ITS rDNA (39). The ITS region was also found to be useful in differentiating members of the plant-parasitic nematode *Heterodera* spp. (13,14). Thirteen entomopathogenic nematodes were compared by PCR-RFLP analysis and, although rDNA markers were useful at the species level, the results were not clear (34).

The objective of this study was to determine useful characters from PCR-RFLP analysis of the ITS and 26S rDNA regions in order to describe biological diversity and resolve genus, species, and subspecific groupings for 15 populations of entomopathogenic nematodes.

Materials and Methods

Fifteen entomopathogenic nematode isolates were compared in this study. Thirteen nematode isolates were obtained from various laboratories (Table 1). The two isolates remaining were field isolates collected from nursery soil at Somerville Nurseries, near Alliston, Ontario, Canada. These two field isolates were designated field isolate 1 (F1) and field isolate 2 (F2) and are undescribed beyond this study.

TABLE 1. Origin of the entomopathogenic nematodes used in this study.

Species	Strain	Study code	Source
Steinernema feltiae (Filipjev)	bibionis	F	G. Thurston, McGill University Ste Anne de Bellvue, Quebec, Canada
S. glaseri (Steiner)	glaseri	G	J. M. Webster, Simon Fraser University, Burnaby, B.C., Canada
S. carpocapsae (Weiser)	biosafe	B	Plant Products, Mississauga, Ontario, Canada
	All	A	O. N. Morris, Agriculture Canada, Winnipeg, Manitoba, Canada
	breton	R	O. N. Morris
	42	42	J. M. Webster
	DD136	D	J. M. Webster
	mexican	M	O. N. Morris
	kapow	K	G. Thurston
Heterorhabditis bacteriophora (Poinar)	PheroTech	P	O. N. Morris
	ND	N	G. Thurston
	HP88	Hp	O. N. Morris
	bacteriophora	Hb	O. N. Morris
Field Isolate 1	Undescribed	F1	C. G. Nasmith, Galleria Field trapped; Somerville Nurseries, Conifer plantation: Alliston, Ontario, Canada
Field Isolate 2	Undescribed	F2	C. G. Nasmith (as above)

Nematode field trapping: Ongoing studies concerned with the biocontrol of coniferous tree pests, such as root weevils (*Otiorhynchus* spp.) in forest nurseries, provided the opportunity to determine whether naturally occurring entomopathogenic nematodes could be trapped in the same soil habitat as the root weevil pests (26).

Two windows were cut from a 15-ml Falcon polypropylene tissue culture tube. A removable sock made from aluminum screen was placed inside the tissue culture tube. Four greater wax moth (*Galleria mellonella* L.) larvae were placed in the sock and then sealed. The tube was capped and the trap was placed around six randomly selected Colorado Blue Spruce (*Picea pungens* Englem.) trees. The traps were located 40 cm from the center of the tree, at a depth of 20 cm in each of 4 quadrats (one trap per quadrat). Traps were recovered from the soil 7 days later and placed on White's water traps for entomopathogenic nematode recovery (40). Two isolates were recovered and designated as field sample 1 (F1) and field sample 2 (F2).

Nematode rearing: All isolates used in this study were reared in vivo on final instar larvae of *Galleria mellonella* (Lepidoptera: Pyralidae) (9). Freshly emerged nematodes were collected on White's water traps and stored in 75-mm^2 tissue culture flasks, in the dark, at 10 C (40). Nematodes were allowed to settle to the bottom of the flasks. The volume of water in the flasks was reduced, and the remaining nematodes were dried under vacuum through millipore filters. The nematodes were removed from filters to 1.5-ml Eppendorf centrifuge tubes (ca. 20% volume), freeze-dried overnight, and then stored at −70 C until use.

DNA extraction: DNA was extracted by homogenizing approximately 25 mg of freeze-dried nematodes in 1.5-ml tubes with 500–600 μl of 10% CTAB (cetyltrimethylammonium bromide) lysis buffer containing 0.5M EDTA (ethylenediaminetetraacetic acid) (pH 8.0), 1M Tris-HCl (pH 8.0), 5M NaCl (pH 8.0), 1% β-mercaptoethanol (31). Following centrifugation at 10,000*g* for 15 minutes, the pelleted DNA was finally resuspended in 50 μl of Tris-EDTA. The amount and integrity of total genomic DNA were determined by electrophoresis on 0.8% agarose gels. DNA was stored at 4 C for immediate use and at −20 C for long-term storage.

PCR amplification: All PCR relations were performed in 50- or 100-μl reaction mixtures including: 5–15 ng of DNA; 0.5 μM of each primer; 10 mM Tris-HCl; 50 mM KCl; 1.5 mM $MgCl_2$; 0.02% gelatin; 200 μM each dATP, dCTP, dTTP, and dGTP; and 1.0–2.0 units of *Taq* polymerase (Boehringer Mannheim).

Sequences corresponding to the ITS and 26S subunits of the rDNA cistron were amplified and analyzed for length polymorphisms among the nematode isolates. The sequence of the ITS1 (5′-dTCCGTAGGTGAACCTGCGG) and ITS4 (5′-dTCCTCCGCTTATTGATATGC-3′) primers used in this study is based on the alignment of fungal sequences (41). The sequence of 26S1 (5′-dCTAAAAAGGATTCCCTTAGTAACGGCGAGT-3′) and 26S2 (5′-dGAGCCAATCCTTTTCCCGAAGTTA-3′) primers was derived from the rDNA sequence data of *Caenorhabditis elegans* (11).

All reactions were topped with two drops of mineral oil and run in a thermal cycler (Perkin-Elmer Cetus). PCR reaction conditions are as follows: 3 minutes at 94 C, 60 seconds at 45 C, 10 seconds at 60 C, ramping 5 seconds per degree to 72 C, 60 seconds at 72 C; 30 cycles of 60 seconds at 94 C, 60 seconds at 45 C, 10 seconds at 60 C, ramping 5 seconds per degree to 72 C, 60 seconds at 72 C; and a final extension cycle of 8 minutes at 72 C (26).

Once PCR amplification was complete, 10 μl of the amplified product from each sample and DNA size ladders (1kb [Gibco BRL] as well as 100 bp [Pharmacia]) were analyzed by running the amplified product on a 1.5% agarose gel prepared with Tris-borate-EDTA (TBE) (89 mM Tris-HCl, 89 mM boric acid, and 20 mM EDTA). The gel was stained with ethidium bromide, visualized, and photographed under UV light (32). PCR amplified prod-

ucts were confirmed as ITS and 26S rDNA by transfer hybridization analysis with digoxigenin labelled rDNA isolated from *Fusarium oxysporum* (A. Dugal pers. comm.). Blotting and hybridization procedures followed manufacturers' (Boehringer Mannheim) specifications.

All restriction enzyme procedures followed manufacturers' (Boehringer Mannheim, Pharmacia, and Promega) specifications. Amplified ITS and 26S products were digested with 14 different restriction enzymes (*Aat* II, *Alu* I, *Apa* I, *Bam* HI, *Bgl* II, *Eco* RI, *Hae* III, *Hind* III, *Hinf* I, *Pst* I, *Rsa* I, *Sac* I, *Xba* I, and *Xho* I) electrophoresed in 1.5% agarose gels, stained with ethidium bromide, and visualized under UV light for the presence of DNA.

Results

Procedures for obtaining PCR amplified products were repeated four times for consistency of results. Amplified products were reamplified to ensure they were reasonably free of foreign products. Restriction digests were repeated at least three times for all nematode isolates.

PCR amplification: The entire procedure, from nematode collection to the start of the PCR, could be completed in less than 24 hours, and the extraction steps usually took 3–4 hours to complete. Each 1.5-ml microcentrifuge tube was filled to approximately 25–30% volume with freeze-dried nematodes, yielding approximately 50–75 μg DNA, sufficiently pure for PCR and restriction enzyme analysis.

PCR amplification of the entire ITS region (Fig. 1a) generated one fragment of approximately 0.88 kb for all isolates. This product represents the approximately 1-kb fragment expected from the known sequence of the rDNA of *Caenorhabditis elegans* (11). A fragment of approximately 1.9 kb was generated, representing the 26S region for all isolates (Fig. 1b). This fragment is approximately half of that expected (ca. 3.5 kb) from the known rDNA sequence for *C. elegans,* from which the primers were derived (11). No length variation was observed for both ITS and 26S regions among the isolates; therefore, RFLP analysis was used to determine the sequence variation within the amplified products of each isolate.

PCR-RFLP analysis: Four of the 14 restriction enzymes used to digest the ITS and 26S amplified products produced polymorphic fragments. RFLPs were found when the ITS product was digested with *Aat* II, *Hinf* I, *Hind* III, and *Rsa* I (Fig. 2, Table 2). With all four enzyme digests, *Steinernema feltiae* syn. *bibionis* (F) and *S. glaseri* glaseri (G) showed identical RFLPs. *Steinernema carpocapsae* strains (B, A, R, 42, D, M, K), *Heterorhabditis bacteriophora* strains (P, N, Hp, Hb), and field isolate 1 (F1) had identical RFLPs with all enzyme digests of ITS. *Steinernema glaseri* glaseri (G), *S. feltiae* syn. *bibionis* (F), and field isolate 2 (F2) revealed no cutting site when

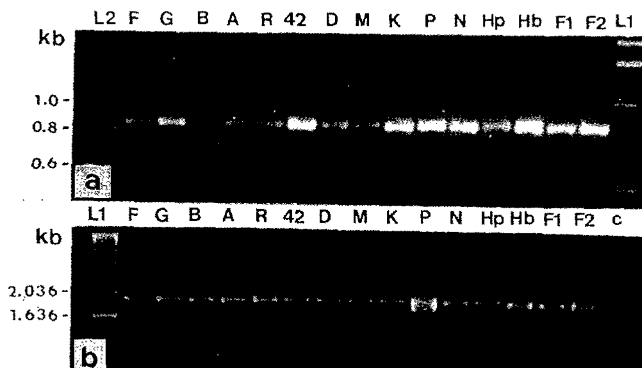

Fig. 1. Agarose gel of the polymerase chain reaction (PCR) amplified internal transcribed spacer (ITS) (a); and 26S rDNA (b); regions for Steinernematid, Heterorhabditid, and field isolated nematodes (as abbreviated in Table 1). L1 = 1 kb ladder. L2 = 100 bp ladder. c = control with no DNA.

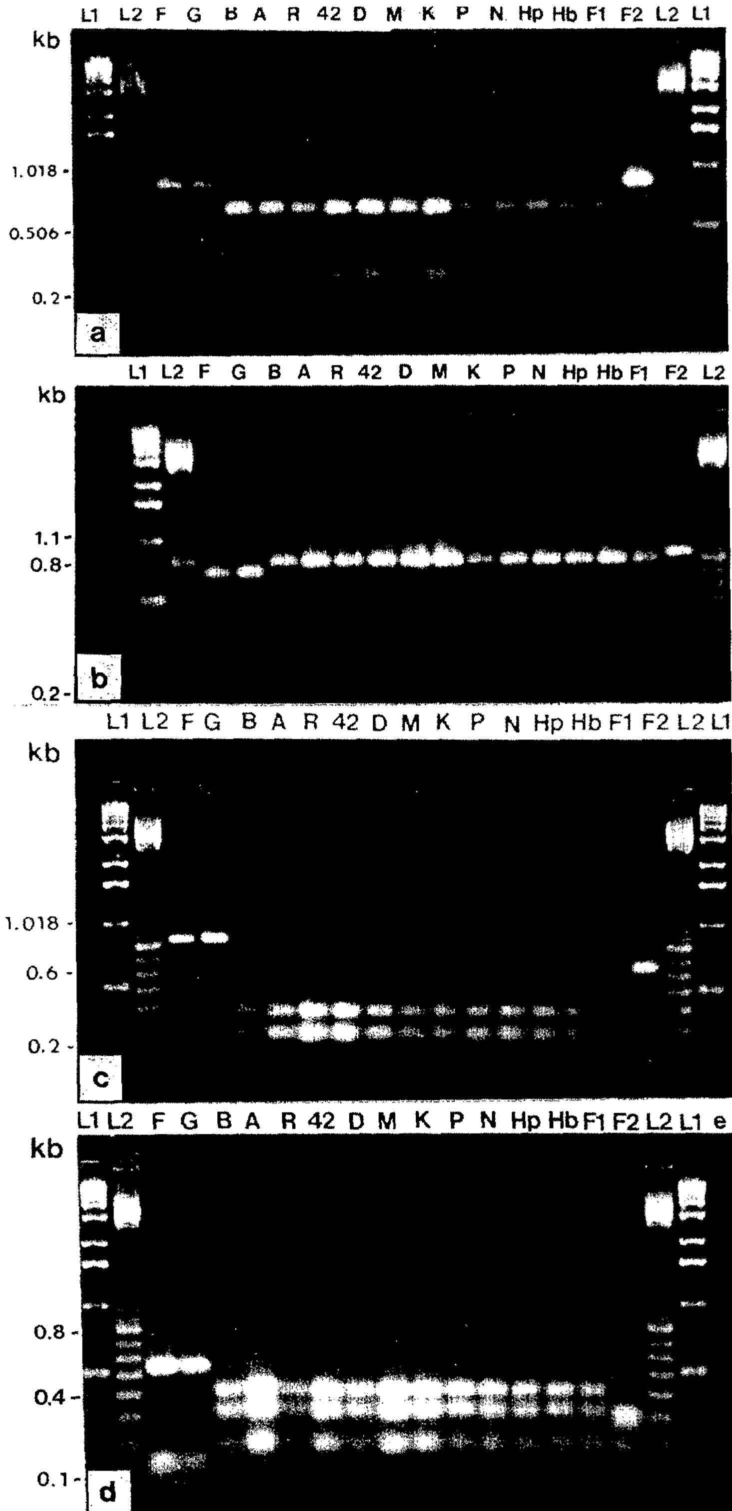

FIG. 2. Agarose gel of PCR amplified ITS, digested with *Aat* II (a); *Hind* III (b); *Hinf* I (c); and *Rsa* I (d) for Steinernematid, Heterorhabditid, and field isolated nematodes (as abbreviated in Table 1). L1 = 1 kb ladder. L2 = 100 bp ladder. e = empty lane, no DNA loaded.

TABLE 2. Estimated restriction fragment sizes (bp) of PCR amplified ITS and 26S rDNA.

		RFLPs of selected entomopathogenic nematode isolates														
DNA	Enzyme	F	G	B	A	R	42	D	M	K	P	N	Hp	Hb	F1	F2
ITS	AatII	880	880	—[a]	—	—	—	—	—	—	—	—	—	—	—	880
		—	—	650	650	650	650	650	650	650	650	650	650	650	650	—
		—	—	230	230	230	230	230	230	230	230	230	230	230	230	—
	HindIII	—	—	—	—	—	—	—	—	—	—	—	—	—	—	880
		—	—	780	780	780	780	780	780	780	780	780	780	780	780	—
		730	730	—	—	—	—	—	—	—	—	—	—	—	—	—
		150	150	—	—	—	—	—	—	—	—	—	—	—	—	—
		—	—	100	100	100	100	100	100	100	100	100	100	100	100	—
	HinfI	880	880	—	—	—	—	—	—	—	—	—	—	—	—	—
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	680
		—	—	420	420	420	420	420	420	420	420	420	420	420	420	—
		—	—	290	290	290	290	290	290	290	290	290	290	290	290	—
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	200
	RsaI	550	550	—	—	—	—	—	—	—	—	—	—	—	—	—
		—	—	410	410	410	410	410	410	410	410	410	410	410	410	—
		—	—	310	310	310	310	310	310	310	310	310	310	310	310	310
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	270
		—	—	170	170	170	170	170	170	170	170	170	170	170	170	170
		140	140	—	—	—	—	—	—	—	—	—	—	—	—	110
26S	BamHI	1,900	1,900	—	—	—	—	—	—	—	—	—	—	—	—	1,900
		—	—	1,860	1,860	1,860	1,860	1,860	1,860	1,860	—	—	—	—	1,860	—
		—	—	—	—	—	—	—	—	—	1,200	1,200	1,200	1,200	—	—
		—	—	—	—	—	—	—	—	—	700	700	700	700	—	—
	HaeIII	—	—	960	960	960	960	960	960	960	—	—	—	—	960	—
		790	790	790	790	790	790	790	790	790	—	—	—	—	790	790
		540	540	—	—	—	—	—	—	—	540	540	540	540	—	540
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	420
		—	—	—	—	—	—	—	—	—	400	400	400	400	—	—
		—	—	—	—	—	—	—	—	—	360	360	360	360	—	—
		340	340	—	—	—	—	—	—	—	—	—	—	—	—	—
	HindIII	1,900	1,900	—	—	—	—	—	—	—	1,900	1,900	1,900	1,900	—	1,900
		—	—	1,340	1,340	1,340	1,340	1,340	1,340	1,340	—	—	—	—	1,340	—
		—	—	650	650	650	650	650	650	650	—	—	—	—	650	—
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	SacI	—	—	1,050	1,050	1,050	1,050	1,050	1,050	1,050	1,900	1,900	1,900	1,900	1,050	—
		1,020	1,020	—	—	—	—	—	—	—	—	—	—	—	—	1,020
		850	850	850	850	850	850	850	850	850	—	—	—	—	850	850

[a] (—) Fragment absence.

ITS was digested with *Aat* II (Fig. 2a). Field isolate 2 (F2) was the only isolate with no cutting site when ITS was digested with *Hind* III (Fig. 2b). *Hinf* I digest of ITS revealed no cutting site for both *S. feltiae* syn. *bibionis* (F) and *S. glaseri* glaseri (G) (Fig. 2c). Field isolate 2 (F2) revealed two fragments (680 bp and 200 bp) separating it from all other isolates when ITS was digested with *Hinf* I. *Rsa* I digests of ITS revealed that the field isolate 2 (F2) profile was distinct from all other isolates; however, it shared a 180-bp band with *S. carpocapsae* strains (B, A, R, 42, D, M, K), field isolate strain 1 (F1), and *H. bacteriophora* strains (N, P, Hp, Hb) (Fig. 2d). ITS digested with *Rsa*I also revealed that field isolate 2 (F2) shared a 100-bp fragment size with *S. feltiae bibionis* (F) and *S. glaseri* glaseri (G) (Fig. 2d).

RFLPs were found when the 26S rDNA product was digested with *Bam* HI, *Hae* III, *Hind* III, and *Sac* I (Figs. 3, 4; Table 2). In all polymorphic enzyme digests of 26S, *S. carpocapsae* strains (B, A, R, 42, D, M, K) and field isolate 1 (F1) had identical RFLPs. Furthermore, with the exception of *Hind* III, all *Heterorhabditis bacteriophora* isolates (N, P, Hp, Hb) displayed a group profile distinct from all *Steinernema* isolates when 26S rDNA was digested with *Bam* HI, *Hae* III, and *Sac* I. *S. feltiae* syn. *bibionis* (F), *S. glaseri* glaseri (G), and field isolate 2 (F2) possessed no cutting site in *Bam* HI

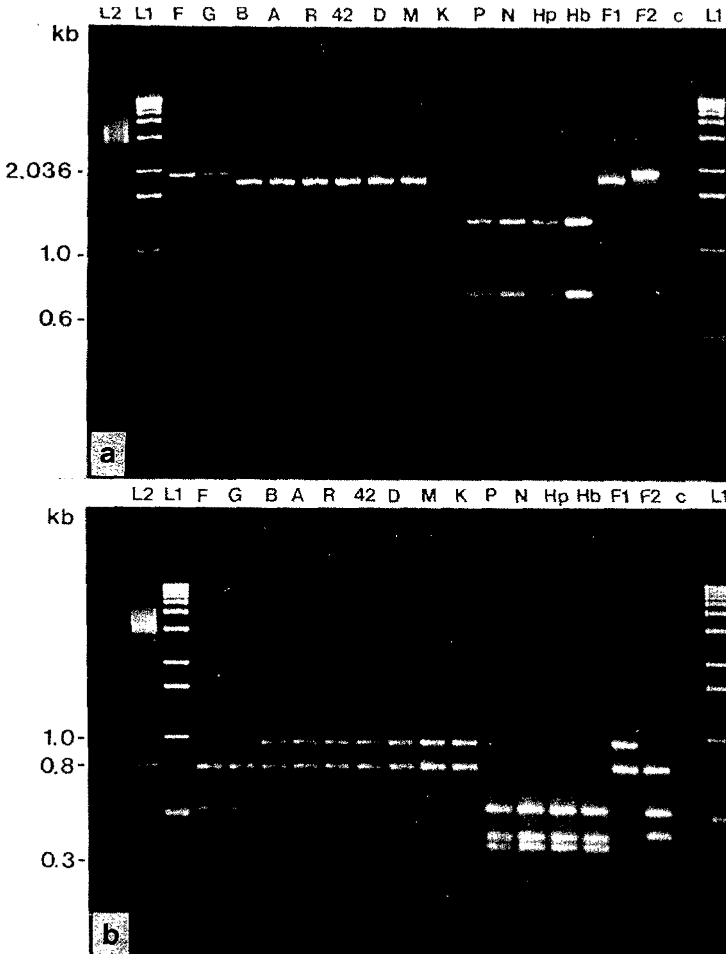

FIG. 3. Agarose gel of PCR amplified 26S rDNA, digested with *Bam* HI (a) and *Hae* III (b) for Steinernematid, Heterorhabditid, and field isolated nematodes (as abbreviated in Table 1). L1 = 1 kb ladder. L2 = 100 bp ladder. c = control with no DNA.

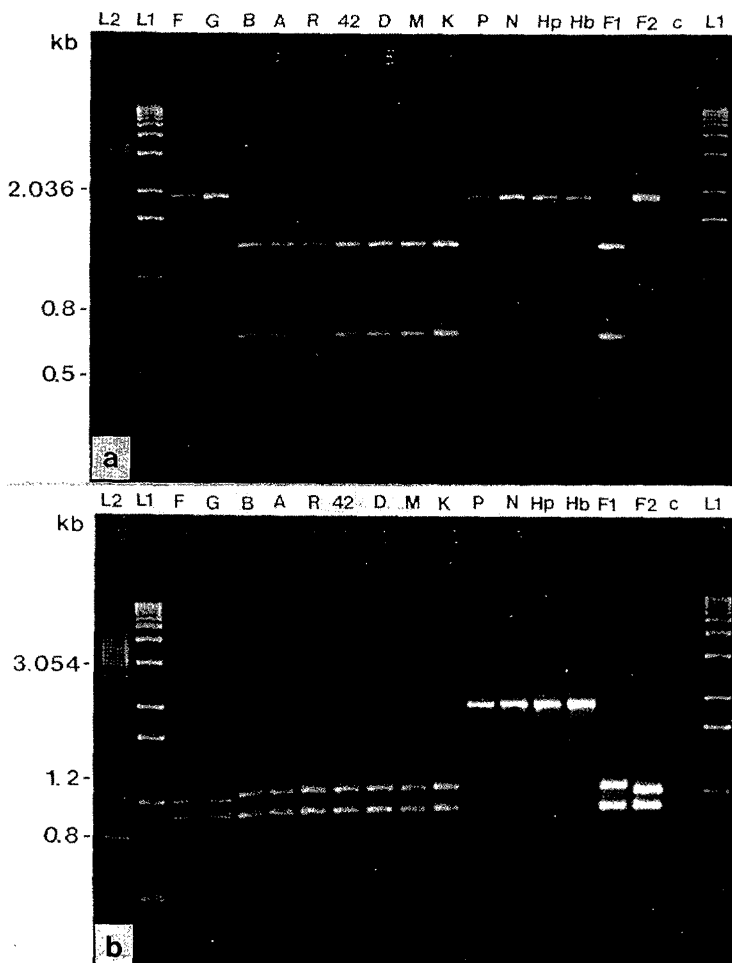

FIG. 4. Agarose gel of PCR amplified 26S rDNA, digested with *Hind* III (a), and *Sac* I (b), for Steinernematid, Heterorhabditid, and field isolated nematodes (as abbreviated in Table 1). L1 = 1 kb ladder. L2 = 100 bp ladder. c = control with no DNA.

digested 26S (Fig. 3a). The 26S amplified product digested with *Hae* III separated the 15 isolates into four groups including: 1) *S. feltiae* syn. *bibionis* (F) and *S. glaseri* glaseri (G); 2) *S. carpocapsae* isolates and field isolate 1 (F1); 3) *Heterorhabditis bacteriophora* isolates; and 4) field isolate 2 (F2). When 26S was digested with *Hae* III, field isolate 2 (F2) shared a 790-bp fragment size with *S. feltiae* syn. *bibionis* (F), *S. glaseri* glaseri (G), *S. carpocapsae* strains, and field strain 1 (F1) (Fig. 3b). In the same digest, field isolate 2 (F2) shared a 540-bp fragment size with *S. feltiae* syn. *bibionis* (F), *S. glaseri* glaseri (G), and *Heterorhabditis bacteriophora* strains. *S. feltiae* syn. *bibionis* (F), *S. glaseri* glaseri (G), field isolate 2 (F2), and *H. bacteriophora* strains possessed no cutting site in *Hind* III (Fig. 4a). *Sac* I digested 26S resulted in identical banding profile for *S. feltiae* syn. *bibionis* (F), *S. glaseri* glaseri (G), and field strain 2 (F2) (Fig. 4b). Banding profiles that are not additive to the total undigested ITS or 26S are due to enzymes that recognize 4 (*Rsa* I and *Hae* III) or 5 (*Hinf* I) base pair sequences. These enzymes commonly digest rDNA regions, resulting in some fragments that are too small to map or measure accurately. Only distinct repeatable polymorphic bands were analyzed.

DISCUSSION

Systematics, the field of biology engaged in the analysis of biological diversity, is a

process of classifying objects into groups such that members of a group bear a closer resemblance to each other than to members of another group (35). Recently, research priorities have been outlined that propose to help secure the future of nematology (3). The results of this study address the need to develop molecular taxonomic characters to augment and refine existing methods. Currently, RFLP data alone cannot assign a Steinernematid or Heterorhabditid population to a given taxonomic level without supporting crossbreeding and morphological data (7).

This study provides some of the first molecular characters useful for separating species of entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae. Results from this study indicate that rDNA analysis allows the separation of 15 strains of entomopathogenic nematodes into one of four groups. The PCR-RFLPs of selected rDNA regions provide stable taxonomic criteria for identifying these increasingly important organisms for biological control.

One of the most interesting aspects of this study was the absence of rDNA polymorphic variation between *S. feltiae* syn. bibionis (F) and *S. glaseri* glaseri (G). These strains are thought to represent two separate species and yet, in this study, results show no differences following all restriction enzyme digests. As species, their similarity should be expected to be lower. The present rDNA profiles, along with future sequence data of these conserved regions, will undoubtedly resolve the question of species identity. *S. glaseri* glaseri (G) is considered to be the type strain for the genus, and its taxonomic position has not been contested. *Steinernema feltiae* syn. bibionis (F) has had many previous synonyms and was once considered to be a species of its own, distinct from other *S. feltiae* strains (29).

Morphologically, *S. glaseri* glaseri (G) is much larger than *S. feltiae* syn. *bibionis* (F), with distinguishing characters. *S. feltiae* syn. *bibionis* (F) has never been considered to be in the same species as *S. glaseri* glaseri, because they will not hybridize when allowed to mate. We decided to search for variation by reviewing a study on biological control of termites that compared selected entomopathogenic nematodes for isoenzyme variation (36). This study found that *S. feltiae* syn. *bibionis* and *S. glaseri* glaseri could be separated clearly by both esterase and tetrazolium oxidase band profiles following recognized staining methods (33). We repeated these conditions, and the zymograms for esterase and tetrazolium oxidase confirmed that *S. feltiae* syn. *bibionis* (F) could be distinctly separated from *S. glaseri* (G) (data not shown). Isozyme analysis of entomopathogenic nematode infective juveniles can be useful since the infective juvenile is neither a feeding nor a developmental stage until it enters a host. During a stage without development, isozymes would not be influenced by physiological changes due to maturation and, therefore, could be used in population analysis. However, long-term stability of these markers is a concern and must be monitored due to environmental influences such as laboratory rearing parameters.

The field trapping method used to investigate naturally occurring entomopathogenic nematodes was successful in obtaining two isolates of nematodes designated field isolate 1 (F1) and field isolate 2 (F2). Some important prospects may be entertained when considering the PCR-RFLPs of both field isolates. Field isolate 1 (F1) in all enzyme digests has the same profile as the *Steinernema carpocapsae* strains. This is interesting because in the same field location where field isolate 1 (F1) was trapped during this study, a sample of *S. carpocapsae* DD136 was released a year earlier to determine its virulence against root weevil pests (6). With other population markers to further resolve the present rDNA results, the field isolate F1 may prove to have successfully established itself in the soil since its introduction previous to the present study. The DNA methods used in this study, therefore, may also provide a means of monitoring field-released isolates. The PCR-RFLP analysis of field isolate 2 (F2) indicates that although it shares

some band fragment sizes with other isolates, its overall rDNA profile is different than all other nematode isolates analyzed in this study. Recently, cold-active strains of *Steinernema* were identified during a survey in Western Canada (24). Since both field isolates (F1 and F2) were isolated from field soil in temperate climate regimes, they both may present characters useful in selective breeding, especially for cold-activity.

The PCR-RFLP analysis of rDNA in this study shows that taxonomic resolution was successful to species-level designation, with the exception of *S. feltiae* syn. *bibionis* (F) and *S. glaseri* glaseri (G). The results indicate some further study is needed to relate conventional species determinations to molecular markers. The methods used in this study provide a quick, reliable tool to identify species and genus designations for the Steinernematid and Heterorhabditis isolates. Using the same battery of enzymes, the 26S region was more variable than the ITS region. This is surprising since the ITS region has proven to be less conserved in other organisms when used for species comparisons. Since both the ITS and 26s rDNA regions provided species- and genus-level resolution, the analysis of the 18S rDNA was not pursued. Other studies have found the 18S region to be too conserved to be informative at the species level (39). From the PCR-RFLP results in this study, variable regions have been determined that will aid in direct sequencing of both the ITS and 26S rDNA regions. Sequencing of these variable regions would provide the opportunity to develop species-specific probes.

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