CATALASE AND SUPEROXIDE DISMUTASE ISOZYMES OF INDIAN SPECIES OF *STEINERNEMA* (NEMATODA: STEINERNEMATIDAE)

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Summary. Profiles of catalase isozymes from infective juveniles of six indigenous *Steinernema* spp., obtained by mini slab gel polyacrylamide electrophoresis, revealed species-specific enzyme phenotypes for *S. thermophilum*, characterized by four bands. *Steinernema glaseri* showed two species-specific bands of different Rf values. The banding patterns of *S. carpocapsae, S. siamkayai*, an unidentified strain (IARI-EPN-*mg1*) and *S. riobrave* appeared similar, showing only the same isozyme band. Superoxide dismutase profiles also revealed species-specific phenotypes for *S. thermophilum* (three bands), *S. carpocapsae* (three bands) and *S. glaseri* (one marked band). *Steinernema siamkayai, S. riobrave* and the unidentified strain all showed two isozyme bands of the same Rf values. The combination of catalase and superoxide dismutase isozyme profiles from infective juveniles could be useful for the differentiation of *Steinernema* species.

Key words: Enzyme phenotypes, entomopathogenic nematodes, *Steinernema carpocapsae*, *S. glaseri*, *S. riobrave*, *S. siamkayai*, *S. thermophilum*.

Entomopathogenic nematodes belonging to the genus *Steinernema* are obligate and lethal parasites of insects. They are currently being used as biological control agents for the management of several insect pests of crops. Their species and strains are known to exhibit different efficacies against particular pests in different conditions (Bedding *et al.*, 1983). Therefore, new species are always being sought. Whenever new isolates are discovered, their identification is not always straightforward. Therefore, there has been much confusion over the taxonomy and nomenclature of these nematodes, with many isolates being mis-identified by standard morphological criteria (Poinar, 1990). *Steinernema thermophilum* Ganguly *et* Singh, 2000 is the first new species of this genus described from India. Eight other species of *Steinernema* viz., *S. abbasi* Elawad *et al.*, 1997, *S. bicornutum* Tallosi *et al.*, 1995, *S. carpocapsae* (Weiser, 1955) Wouts *et al.*, 1982, *S. feltiae* (Filipjev, 1934) Wouts *et al.*, 1982, *S. glaseri* (Steiner, 1929) Wouts *et al.*, 1982, *S. riobrave* Cabanillas *et al.*, 1994, *S. siamkayai* Stock *et al.*, 1998, *S. tami* Luc *et al.*, 2000, have been recorded from this country (Ganguly, 2003; Ganguly *et al.*, 2005). Species-specific isozyme patterns, revealed through polyacrylamide slab gel electrophoresis, have been found useful for differentiating species of *Meloidogyne* (Esbenshade and Triantaphyllou, 1985; 1990; Molinari *et al.*, 2005). This biochemical technique was first applied by Herman and Jackson (1963) with entomopathogenic nematodes; later, various workers studied isozyme profiles of different enzymes in attempts to resolve taxonomic problems in steinernematids, especially in the identification of *S. carpocapsae, S. glaseri* and *S. feltiae*, and their strains (Sha, 1985; Artyukhovsky *et al.*, 1997). The isozyme techniques were soon replaced by RAPD (random amplified polymorphic DNA), RFLP (restriction fragment length polymorphism) of ribosomal DNA, and base sequencing of the ITS region of rDNA (Reid and Hominick, 1992; Nguyen *et al.*, 2001; Stock *et al.*, 2001), which are frequently used as supplementary tools in species descriptions.

Although DNA based technologies, such as RFLPs and base sequencing of the entire ITS region of rDNA, have been successfully applied for distinguishing species of *Steinernema*, they remain of limited use for large surveys because they are costly and time-consuming procedures. Instead, isozyme patterns may prove to be an excellent tool for the identification of newly isolated strains of *Steinernema* spp. during routine surveys. Therefore, an investigation was undertaken to characterize the isozyme profiles of catalase (CAT, EC 1.11.1.6) and superoxide dismutase (SOD, E.C.1.15.1.1) of some of the Indian species of *Steinernema*, and to explore their utility for differentiating the species/strains that are being isolated from different parts of the world.

MATERIALS AND METHODS

Live cultures of infective juveniles of entomopatho-

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genic nematodes, i.e. *S. thermophilum* (IARI-EPN1), *S. carpocapsae* (IARI-EPN-*am1*), *S. siamkayai* (IARI-EPN-*ut1*), *S. glaseri* (IARI-EPN-*up2*), an unidentified *Steinernema* sp. (IARI-EPN-*mg1*) and *S. riobrave* (IARI-EPN-*gj1*) were maintained on the last instar larvae of the greater wax moth, *Galleria mellonella* (Kaya and Stock, 1997). Live populations of infective juveniles (IJs) in sterile distilled water were stored at 15 ºC. About 5,000 IJs in 1 ml of suspension were put in 1.5 ml Eppendorf tubes and centrifuged at 2,147 g for 5 min. The supernatant was discarded and the pellets were homogenised with 40 µl of extraction buffer containing 20% glycerol and 2% Triton X-100 (Esbenshade and Triantaphyllou, 1985). Additional extraction medium was added to increase the total volume of crude samples to about 100 µl and centrifuged at 3,354 g for 10 min at 4 ºC. The clear supernatant was introduced immediately into the electrophoretic cell or stored at -80 ºC until use.

The superoxide dismutase (SOD) and catalase (CAT) isozymes of IJs were separated by negative polyacrylamide mini slab gel electrophoresis in a mini slab gel apparatus (Genei Bangalore, India). A 5% acrylamide stacking gel and 7% acrylamide separation gel were used. About 25 µl of the samples were loaded into the wells of the gel. The electric potential was set at 35 volts for the first 40-45 min to allow stratification of proteins in the sample according to their relative charge. Afterwards, voltage was increased to 150 volts until the marker dye moved to the lower end of the gel.

CAT activity was determined by chilling the gel in a dish resting on a bed of ice for 10 min followed by treatment with 0.01% hydrogen peroxide solution. The gel was rinsed with two changes of distilled water and covered with the stain solution (0.5% potassium ferricyanide + 0.5% ferric chloride) for 1-2 min. The gel was again rinsed with distilled water and stored in 7% acetic acid. The regions of CAT activity appeared as clear areas on a blue-green background (Harris and Hopkinson, 1976).

SOD isozymes were determined by incubating the gel at 37 ºC for 20 min in a stain solution containing 7.5 mg Sodium EDTA, 4 mg Riboflavin and 10 mg NBT in 100 ml Tris buffer pH 8.2 (0.61 g Tris dissolved in 100 ml distilled water). The gel was removed from the incubator and placed under fluorescent light. The bands of SOD activity were resolved as clear areas in the gel (Ravindranath and Fridovich, 1975).

The stained gels were dried, scanned by means of a scan jet (Hewlett Packard), analyzed as digital images and then printed on photo quality paper. The species specific enzyme phenotypes were named with a capital letter of the respective species followed by the number of isozymes, while non-specific enzyme phenotypes were labeled as 'N' followed by the number of isozymes.

RESULTS AND DISCUSSION

Isozyme patterns of CAT and SOD showed polymorphism, which could differentiate some of the species/strains of *Steinernema* (Table I; Figs 1 and 2).

CAT patterns (Fig. 1) showed some polymorphism among the *Steinernema* species. *Steinernema thermophilum* exhibited four distinct bands of Rf values of 0.04, 0.10, 0.21 and 0.27, which represented a species-specific pattern (T4), while *S. glaseri* showed two different species-specific bands (G2) at Rf 0.17 and 0.37. Except for *S. glaseri*, all the strains possessed the isozyme of Rf 0.21, and its presence or absence might be correlated with *Steinernema* species of small size (less than 700 µm long) and large size (about 1000 µm long) infective juveniles, respectively (IJs of *S. glaseri* measured about 1000 µm while the length of IJs of the other five species was less than 700 µm). *Steinernema carpocapsae* (IARI-EPN-*am1*), *S. riobrave*, unidentified *Steinernema* sp. (strain IARI-EPN-*mg1*) and S. *siamkayai* (IARI-EPN-*ut1*) all possessed similar CAT banding patterns, with only one band at Rf 0.21 common to all strains except *S. glaseri*.

Isozyme profiles of SOD (Fig. 2) also revealed three species specific bands (T3) in *S. thermophilum,* with the lower two bands of maximum mobility (Rf 0.45 and 0.61) being specific for this species. *Steinernema carpocapsae* had a species-specific phenotype with three

Table I. Enzyme phenotypes of catalase (CAT) and superoxide dismutase (SOD), for different *Steinernema* species from India.

Species	Strains	Origin (locality, state)	Enzyme phenotypes*	
			CAT	SOD
S. thermophilum	IARI-EPN1	IARI farm, Delhi	T4	T3
S. carpocapsae	IARI-EPN-*am1*	Assam	N1	C3
S. siamkayai	IARI-EPN-*ut1*	Champawat, Uttaranchal	N1	N2
S. glaseri	IARI-EPN-*up2*	Uttar Pradesh	G2	G1
Steinernema sp.	IARI-EPN-*mg1*	Shillong, Meghalaya	N1	N2
S. riobrave	IARI-EPN-*gj1*	Anand, Gujarat	N1	N2

* Species-specific enzyme phenotypes denoted by T (*S. thermophilum*), C (*S. carpocapsae*), G (*S. glaseri*) and R (*S. riobrave*), and non-specific by N; number following these letters indicates the number of bands.

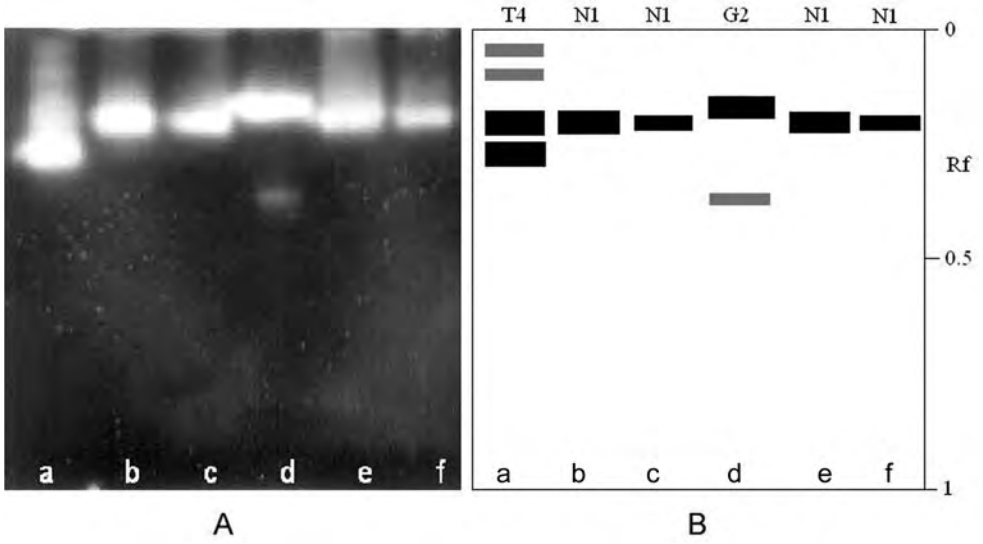

Fig. 1. Catalase isozyme profiles (A) and their enzyme phenotypes (B), of infective juveniles of six Indian species of *Steinernema*. a: *S. thermophilum*; b: *S. carpocapsae*; c: *S. siamkayai*; d: *S. glaseri*; e: *Steinernema* sp. (strain IARI-EPN-*mg1*); f: *S. riobrave* (IARI-EPN-*gj1*).

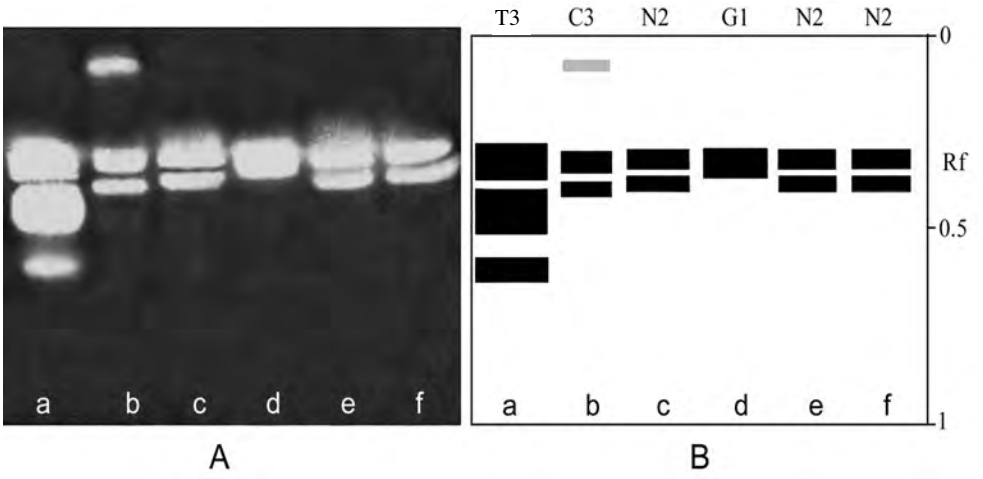

Fig. 2. Superoxide dismutase isozyme profiles (A) and their enzyme phenotypes (B), of infective juveniles of six Indian species of *Steinernema*. a: *S. thermophilum*; b: *S. carpocapsae*; c: *S. siamkayai*; d: *S. glaseri*; e: *Steinernema* sp. (strain IARI-EPN-*mg1*); f: *S. riobrave* (IARI-EPN-*gj1*).

isozymes (C3), of which the first band (Rf 0.08) was unique to this species. *Steinernema glaseri* possessed only one band at Rf 0.35, which represented a species-specific pattern (G1). *Steinernema siamkayai*, an unidentified *Steinernema* sp. (strain IARI-EPN-*mg1*) and *S. riobrave* (IARI-EPN-*gj1*) showed similar banding patterns, each having two non-specific bands (N2) at Rf 0.33 and 0.39.

The present study has demonstrated the utility of CAT and SOD isozymes of IJs for the differentiation of some of the species of *Steinernema*, as evident from the species-specific enzyme phenotypes obtained for *S. thermophilum, S. carpocapsae* and *S. glaseri*, using both enzymes. This is in conformity with earlier findings of Sha (1985) and Artyukhovsky *et al.* (1997), who also differentiated the species based upon enzyme phenotypes. Further studies on isozyme profiles of several populations of *Steinernema*, comprising different species and strains, will yield useful information for the identification of species in this genus.

Compared to DNA based technologies, this technique is simpler, less time-consuming and less costly and therefore can be widely applied for identifying nematode samples obtained from large surveys. Because of the great biocontrol potential of *Steinernema* species, more strains are continuously being isolated from different parts of the world and several of them are still to be unequivocally identified. The combination of CAT and SOD isozymic profiles from IJs, supplemented with morphological details, could be useful for differentiating species of *Steinernema*.

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