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Identification of some Indian populations of *Steinernema* species (Nematoda) by RFLP analysis of the ITS region of rDNA

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Abstract. Three steinernematid species, *Steinernema tami*, *S. abbasi* and an undescribed *Steinernema* sp. SSL2 from agricultural soil samples in India were recovered from soils using the *Galleria* baiting technique. These nematodes were identified by RFLP analyses of the PCR amplified ITS-rDNA region using seventeen restriction enzymes. These steinernematid species had been previously reported from Asia and are found in India for the first time.

Keywords. India, molecular diagnostics, *Steinernema* sp., *Steinernema abbasi*, *S. tami*.

INTRODUCTION

Entomopathogenic nematodes of the family Steinernematidae are widely used in biological control of different insect pests (Gaugler, 1988; Georgis & Gaugler, 1991). In recent years several new *Steinernema* species have been described in tropical and subtropical regions of the world, *e.g. Steinernema scapterisci* from Uruguay (Nguyen & Smart, 1990), *S. neocurtillae* from Florida, USA (Nguyen & Smart, 1992), *S. puertoricense* from Puerto Rico (Roman & Fugueroa, 1994), *S. abbasi* from Oman (Elawad *et al.*, 1997) *S. karii* from Kenya (Waturu *et al.*, 1997), *S. monticolum* from Korea (Stock *et al.*, 1997), *S. siamkayai* from Thailand (Stock & Kaya, 1998), *S. tami* from Vietnam (Luc *et al.*, 2000) and *S. thermophilum* from India (Ganguly & Singh, 2000).

Indian soils were sampled for the presence of entomopathogenic nematode to understand the species composition and distribution of *Steinernema* occurring in agricultural and natural habitats in India. Accurate identification of these nematodes is not a trivial task and it is time consuming. In recent years molecular methods based on the restriction fragment length polymorphism (RFLP) profiles of ITS region of rDNA became popular tool for characterization of this nematode group (Hominick *et al.*, 1997; Reid *et al.*, 1997). In the present paper we report on the rDNA-RFLPs identification of some Indian *Steinernema* populations recovered using the *Galleria* baiting technique.

MATERIALS AND METHODS

Nematode collection. During October, 1997 and March, 1998, a wide range of soil samples was collected from different regions of India to detect the presence of entomopathogenic nematodes. Each soil sample (approximately 250 ml) was placed in a plastic container and baited with 10 last instar larvae of *Galleria mellonella* (Bedding & Akhurst, 1975). The containers were stored at the room temperature. After 48 h infected *G. mellonella* larvae were removed and placed on to a White trap to collect infective juveniles. The origins of nematode isolates used in this study are given in Table 1. The collected nematode populations are maintained on *G. mellonella* in the laboratory of the Project Directorate of Biological Control, Bangalore, India.

Molecular analysis. For each population, several dozen infective juveniles were transferred into 10 µl of double distilled water in an Eppendorf tube and crushed with a microhomogeniser. The DNA extraction method was used as described by Subbotin *et al.* (2000). After centrifugation 2 µl of the DNA suspension was added to the PCR reaction mixture containing 10 µl of 10X *Taq* incubation buffer, 20µl of 5X Q-solution, 200 µM of each dNTP (*Taq* PCR Core Kit, Qiagen, Germany), 1.5 µM of each primer and double

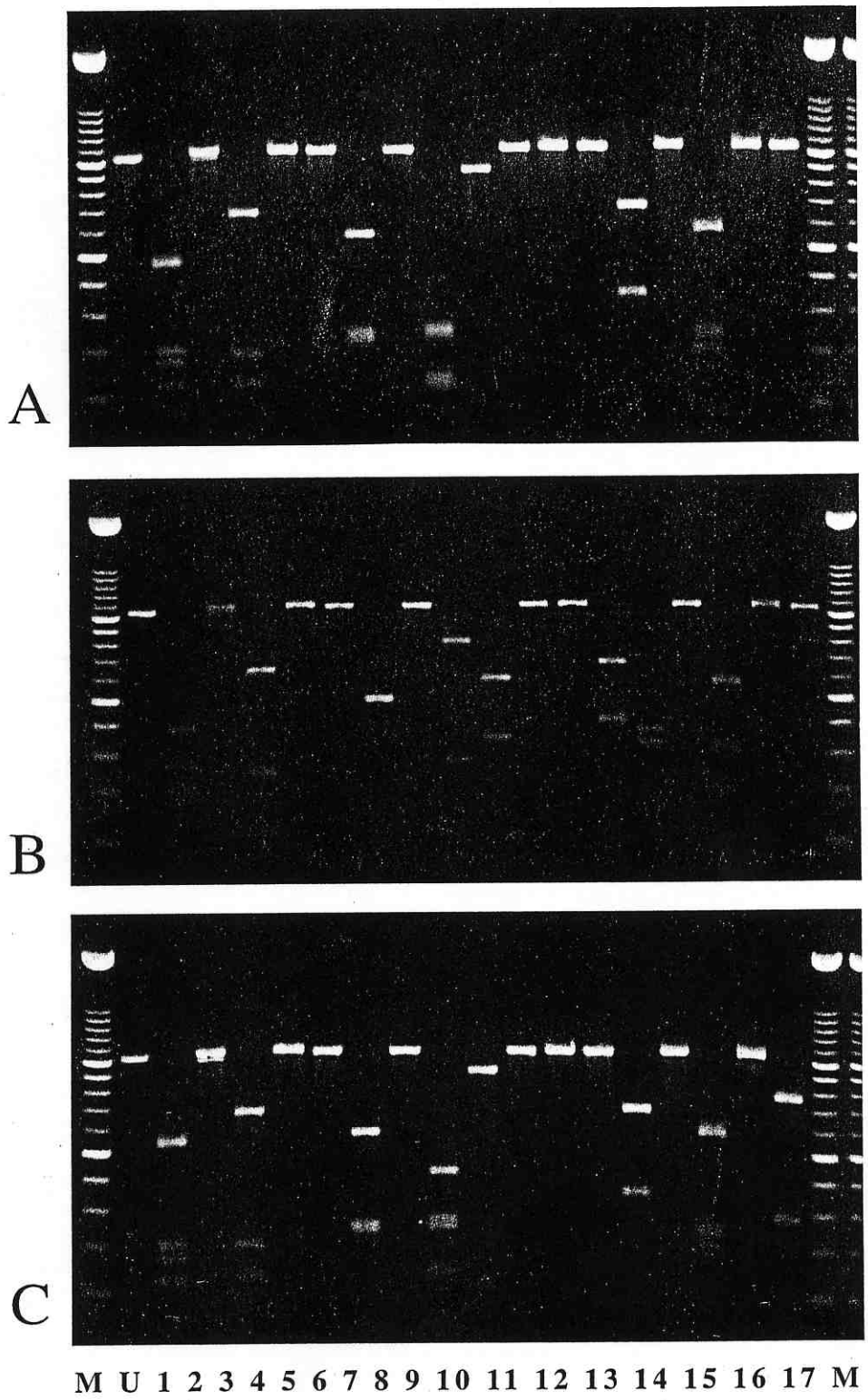

Fig. 1. RFLP patterns of PCR product of the ITS region digested by seventeen restriction enzymes for three *Steinernema* species isolated in India. A: *Steinernema tami*, B: *Steinernema abbasi*, C: Undescribed *Steinernema* sp. SSL2. The first and last lane M - 100-bp DNA marker, U - unrestricted fragment. The other lanes are the PCR product cut by the following restriction enzymes: 1, *Alu*I, 2, *Mva*I, 3, *Dde*I, 4, *Eco*RI, 5, *Hae*III, 6, *Cfo*I, 7, *Hin*dIII, 8, *Hin*fI, 9, *Msp*I, 10, *Kpn*I, 11, *Pst*I, 12, *Pvu*II, 13, *Rsa*I, 14, *Sal*I, 15, *Nde*II, 16. *Bsi*ZI, 17, *Xba*I.

Table 1. Origins of steinernematid isolates from India.

Species identification based on RFLPs	Code number	Origin
Steinernema tami	EN2.1	Mustard field, Jorhat, Assam
Steinernema abbasi	EN3.1	Green gram field, New Delhi
Steinernema sp. SSL2	EN13.21	Sugarcane plantation, Coimbatore, Tamil Nadu
Steinernema sp. SSL2	EN13.22	Sugarcane plantation, Coimbatore, Tamil Nadu
Steinernema sp. SSL2	EN7.2	Coconut field, Minicoy, Lakshadweep

distilled water to a final volume of 100 µl. Primers 18S (5'-TTGATTACGTCCCTGCCCTTT-3') and 26S (5'-TTTCACTCGCCGTTACTAAGG-3') as described by Hominick *et al.* (1997) were used in the PCR. The reactions were placed in GeneE (New Brunswick Scientific, Wezembeek-Oppem, Belgium) and subjected to the following cycling profile: one cycle of 94°C for 4 min, then 35 cycles of 94°C for 1 min, 55°C for 1.5 min, and 72°C for 2 min and followed by a final elongation step at 72°C for 10 min. After DNA amplification five µl of product was run on a 1% agarose gel. Five to 7 µl of each PCR product was digested with one of the following seventeen restriction enzymes: *Alu*I, *Mva*I, *Dde*I, *Eco*RI, *Hae*III, *Cfo*I, *Hin*dI, *Hin*fI, *Msp*I, *Kpn*I, Pst*I*, *Pvu*II, *Rsa*I, *Sal*I, *Nde*II, *Bsi*ZI and *Xba*I in the buffer stipulated by the manufacturer. The digested DNA was loaded on 1.5% TBE buffered agarose gel. DNA molecular XIV marker (Boehringer Mannheim Ltd) was used as a size standard. Products visualised with ethidium bromide, were photographed under UV-light.

RESULTS AND DISCUSSION

Several samples collected from fields showed positive results on the presence of the entomopathogenic nematodes of the genus *Steinernema*. Five nematode samples were taken for further molecular analysis. PCR products were successfully obtained from all these samples.

The RFLP patterns yielded from PCR product of the population EN2.1 with seventeen restriction enzymes are shown in Fig. 1A. The RFLP profiles of this population was identical to those of recently described *S. tami* found in lowland forest in Vietnam (Luc *et al.*, 2000).

The restriction patterns obtained from the population EN3.1 differed from RFLP of previous population by eight restriction enzymes (Fig. 1B). The RFLP profile of this population was similar to those of *S. abbasi* described from the south of the Sultanate of Oman (Elawad *et al.*, 1997).

Digestion of amplified PCR products from three *Steinernema* populations EN13.21, EN13.22, and EN7.2 produced similar RFLP profile. Comparison of this RFLP profile (Fig. 1C) with published data revealed that it is identical to those for undescribed species of *Steinernema* sp. SSL2 from Sri Lanka (Reid *et al.*, 1997; Luc *et al.*, 2000).

Thus, RFLP study revealed three *Steinernema* species from India, all these species had been previously reported from Asia and were recovered from agricultural soils in India for the first time.

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