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Isozymes and Protein Profiles of Four Species of *Trichogramma* Westwood (Trichogrammatidae : Hymenoptera)

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ABSTRACT

Electrophoresis of proteins and isoenzymes, b esterase and Malate dehydrogenase (MDH) was carried out at the Division of Entomology, Indian Agricultural Research Institute, New Delhi with four species of *Trichogramma viz., T. japonicum* Ashmead, *T. chilonis Ishii, T. brasiliensis* Ashmead and *T. minutum* Riley with a view to differentiate these species based upon their electrophoretic patterns. Species specific qualitative and quantitative differences were observed in the protein fractions of the four species. The species specific protein fraction observed were 5 in *T. japonicum*, 2 in *T. chilonis* and one in *T. brasiliensis*. There were more protein bands common to *T. chilonis* and *T. minutum*. In all the species, three different b esterases with recognizable Rm values were observed but for MDH only one band was observed in all the species, and their Rm values also did not differ significantly. For differentiating these four species, electrophoresis of proteins and b esterase could be employed as a dependable tool.

KEY WORDS : *Trichogramma* spp., isozymes, protein profiles

Many countries are producing the egg parasitoids of the genus *Trichogramma* en-mass more than any other known parasitoids for use in biological control of insect pests (De Bach, 1974). The minute size of trichogrammatids and their relatively uniform morphology make precise identification of the species and strains difficult. This problem has been tackled to some extent by the use of male genitalia as a diagnostic morphological character and cross breeding as a genetic approach in biosystematics (Nagarkatti and Nagaraja, 1977). However, these methods are highly complicated and time consuming. Recently, the biochemical approaches such as gel electrophoresis of isoenzymes proved to be a reliable tool to differentiate different species of *Trichogramma* (Jardak *et al.*, 1979; Pintureau and Babault, 1981; Hung, 1982; Lu Wen Qing *et al.*, 1988 and Pintureau and Keita, 1990). However, in the present investigation, attempts were made to differentiate four common species of *Trichogramma viz., T. japonicum* Ashmead, *T. chilonis* Ishii, *T. brasiliensis* Ashmead and *T. minutum* Riley on the basis of protein profiles and zymograms of b esterase and Malate dehydrogenase (MDH).

MATERIALS AND METHODS

The parasitoids were reared in the laboratory on the eggs of *Corcyra cephalonica* St. at 27 ± 2°C and 60 ± 5% RH. Newly emerged adults were chilled in an ice bath and homogenised in distilled water (1:10 W/V). The extract was centrifuged at 10,000 rpm for 20 minutes. The water soluble proteins from this extract were estimated according to Lowry *et al.* (1951). A standard curve for protein was prepared using Bovine Serum Albumin (BSA Fraction IV; Sigma).

The protein profiles of the tissue homogenate were studied by SDS- PAGE discontinuous electrophoresis on 10% slab gel

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according to the method of Laemmli (1970). Tris glycine was used as electrophoresis buffer and the run was carried out at 15mA initially and then at 40mA till the end. Standard protein molecular weight markers (SDS - 70L; Sigma) were also run along with samples. The gels were stained with 0.1% Coomassie brilliant blue R 250. For determining the molecular weight of proteins, a calibration curve was made using the relative mobility (Rm) values.

For isozyme studies, five male progenies from a single female were used per sample. The extraction of the sample was done in 17% sucrose and Tris.C1 for b esterase and MDH, respectively. Electrophoresis was done in 7% acrylamide minigel pH 8.4 for b esterase, pH 8.0 for MDH. Run was made initially at 10 volts and then at 130 volts till the end of the run. For b esterase, the gel was stained with fast blue RR salt and α napthyl acetate and for MDH, the same was incubated in Tris-Cl buffer pH 7.1 containing L-maleic acid, nitroblue tetrazolium, nicotinamide adenine dihydrogen and phenazine methosulphate according to the method of Dalmasso and Berge (1978).

RESULTS AND DISCUSSION

The protein content expressed as μg per mg of freshly emerged adults (0-2h old) is presented in Table 1. The protein content was maximum in *T. chilonis* followed by *T. minutum* whereas, *T. brasiliensis* and *T. japonicum* had about 100 μg. The molecular weights calculated based on the Rm values of the standard and sample proteins are presented in Table 2. These quantitative and qualitative data on proteins may possibly be used for the segregation of the four species.

Table 1. Protein content of different species of *Trichogramma*

Species	Protein content μg/mg of insect (± S.D. (n))
T. brasiliensis	103.94 ± 5.67
T. chilonis	145.67 ± 9.47
T. minutum	125.17 ± 4.72
T. japonicum	101.31 ± 4.60

Note : Values are based on three estimations of Protein for the same extract

Very clear differences and similarities were observed in the protein pattern of the four species. *T. brasiliensis* had low protein content and fewer fractions (8) but the

Table 2. Molecular weights of different protein fractions of *Trichogramma* spp. (in daltons)

T. brasiliensis	*T. chilonis*	*T. minutum*	*T. japonicum*
127000	–	127000	124000
–	–	–	–
116000			113500
–	111000	111000	108500
–	106000	–	–
–	101200	103500	–
–	–	–	95500
–	–	–	89000
–	–	–	83000
–	–	–	76000
–	72500	–	–
69000	69000	69000	69000
68500	–	–	–
–	–	–	64000
–	53000	52000	–
44000	44000	43000	42000
41700	40700	39800	39800
–	36700	–	36700
–	35000	35900	35900
–	–	34300	–
–	33500	33500	–
32000	31300	31300	31300
–	–	–	30500
–	26300	27500	27500
–	24500	–	25700
–	–	22900	22900
–	–	20600	20600
19700	19300	18400	18400
–	17600	–	–

68500d protein fraction was rather specific to this species. In contrast, though *T. japonicum* had low protein content, 20 fractions could be located, of which five (95500, 89000, 83000, 76000 and 30500d) were specific. *T. chilonis* and *T. minutum* had very close resemblances with regard to their protein fractions. Probably the best clue to differentiate them was the presence of two proteins (106000 and 72500d) exclusively in *T. chilonis*. In addition, one high molecular weight protein (124000-127000d) recognized in the other three species was absent in *T. chilonis*.

The electrophoretic pattern and Rm values of b esterases is shown in Fig. 1(A). Though each species had only three bands, species specific differences in their Rm values could easily be recognised. The two high molecular weight esterases with Rm 0.12 - 0.15 and 0.25 - 0.28 were present in all the species except *T. chilonis* but instead only a complex esterase fraction of Rm 0.23 was present in *T. chilonis*. Both *T. minutum* and *T. brasiliensis* had one low molecular weight esterase with Rm 0.63 and 0.75 respectively but the same was faint in *T. japonicum* (Rm 0.52). Here again, *T. chilonis* was unique in the sense that it had two closely moving low molecular weight esterases of Rm 0.55 and 0.58. Similar differences were also reported in the case of esterases with four species of *Trichogramma* by Hung (1982).

There was greater intensity of MDH in *T. chilonis* and *T. japonicum* as compared to *T. brasiliensis* and *T. minutum* (Fig. 1(B)). Only one band was observed in all the species and their Rm values also did not differ significantly. Similarly Guanliang *et al.* (1988) reported no clear cut bands for MDH, but differential bands with high resolution with regard to esterases in their study with nine species of *Trichogramma*.

Based on this study, it may be concluded that the banding patterns of general proteins and b esterases may also be used as a reliable

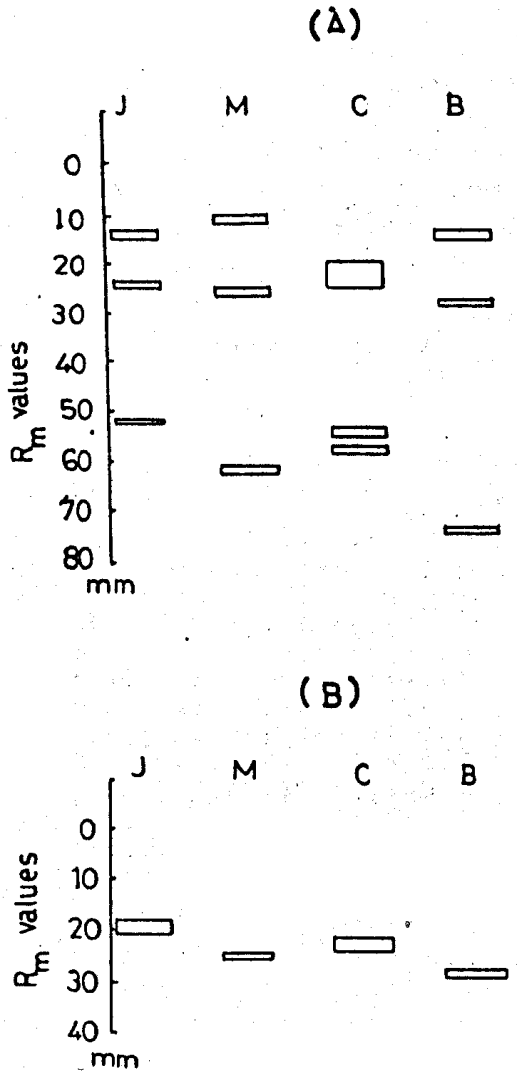

Fig. 1. Electrophoretic pattern and Rm values : (A) b esterases (B) Malate dehydrogenase J = *T. japonicum*; M = *T. minutum*; C = *T.chilonis*; B = *T. brasiliensis*.

tool for identification of different species of *Trichogramma*.

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