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Esterase and Malate Dehydrogenase Phenotypes in Portuguese Populations of *Meloidogyne* Species[1]

CÉLIA S. PAIS[2] AND ISABEL M. DE O. ABRANTES[3]

Abstract: Nonspecific esterases and malate dehydrogenases of 1–5 females from 40 root-knot nematode populations from Portugal were analyzed by electrophoresis in 0.4-mm-thick polyacrylamide gels. Fourteen major bands of esterase activity were detected, corresponding to 10 distinct phenotypes. *Meloidogyne javanica* and *M. hapla* had distinct species-specific phenotypes. Two phenotypes occurred in *M. arenaria*. The most variability was found among *M. incognita* populations. Of the remaining two phenotypes, one was associated with *M. hispanica* and the other belonged to a new species. Three malate dehydrogenase phenotypes were discerned on the basis of particular combinations of the eight main bands of activity found. As previously found, esterases were more useful than malate dehydrogenases in identification of the major *Meloidogyne* species. The host plant had no effect on the nematode esterase or malate dehydrogenase phenotypes.

Key words: electrophoresis, esterase, isozyme, malate dehydrogenase, *Meloidogyne*, root-knot nematode, taxonomy.

Taxonomy of *Meloidogyne* is difficult because many populations vary greatly in taxonomic characters, especially perineal patterns, karyotypes, and host ranges (13,23,24). Biochemical techniques, particularly polyacrylamide gel electrophoresis, are useful for investigating systematic relationships among nematodes (16). Initially, extracts of many nematodes were examined by disc electrophoresis (5,6,15), but subsequent refined and miniaturized techniques permitted the study of single adult females (1,3) and comparison of enzyme phenotypes (7,11,17). Direct electrophoretic analyses of single specimens from populations of several species (4,8) has established a range of genetic variability within them.

In the present work, adult females from Portuguese populations of root-knot nematodes (*Meloidogyne* spp.) from various geographic regions and with wide host ranges were characterized by their esterase and malate dehydrogenase (Mdh) patterns obtained with a modified thin-slab method (19).

Materials and Methods

Forty populations of *Meloidogyne* from Portugal, the Islands of Madeira, and the Azores were identified on the basis of perineal pattern morphology and differential host tests (20–22). Nematodes were reared on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse at 25–30 C. One of the populations, which did not reproduce on tomato, was propagated on greenhouse-grown olive. After approximately 50 days, young egg-laying females were collected from the roots and prepared for electrophoresis.

Single females were transferred to bottom-sealed microhematocrit tubes containing 5 μl of 20% sucrose and 1% Triton X-100 and macerated with a small glass pestle (19). The macerates were immediately frozen and stored at −10 C for 3–4 months with no noticeable loss of enzyme activity. For malate dehydrogenases, 3–5 females were extracted similarly with a Tris buffer (25).

Shortly before electrophoresis the samples were thawed and centrifuged at 9,000 *g* for 15 minutes. The supernatant was transferred to the stacking gel. The stacking and separating gels were homogeneous 4% and 7% polyacrylamide, respectively (18). Electrophoresis was performed in vertical slabs (8 × 8 cm, 0.4 mm thick) in a Pharmacia GE-2/4 LS apparatus. The bridge buffer was pH 8.3 Tris/glycine (18). After loading all the samples to the stack-

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[2] Teaching Assistant, Universidade do Minho, Area de Biologia, 4719 Braga Codex, Portugal.

[3] Teaching Assistant, Centro de Sistemática e Ecologia—I.N.I.C., Departamento de Zoologia, Universidade de Coimbra, 3049 Coimbra Codex, Portugal.

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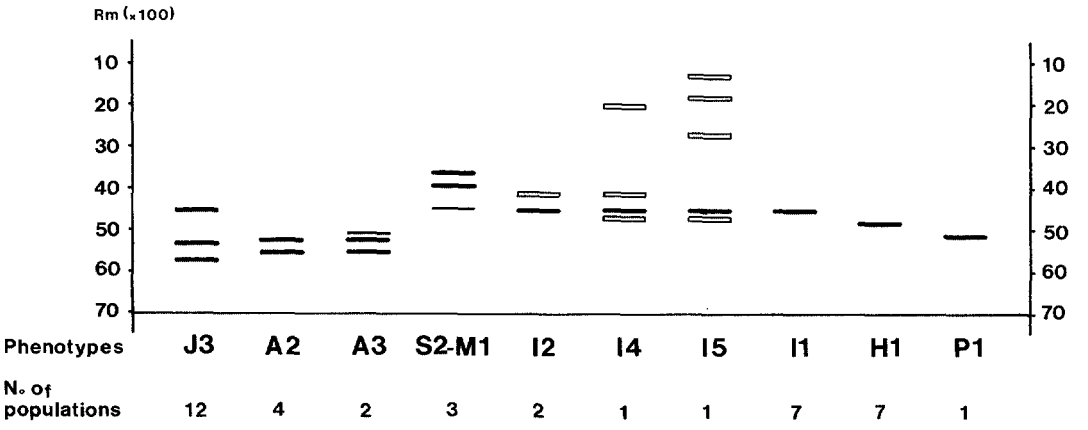

FIG. 1. Esterase phenotypes in 40 Portuguese populations of *Meloidogyne* spp. Phenotypes are designated with a letter or letters and a number indicating the number of bands. Solid lines correspond to strongly stained bands. Dotted lines refer to fainter bands. A = *M. arenaria*; H = *M. hapla*; I = *M. incognita*; J = *M. javanica*; P = *Meloidogyne* n. sp.; S2-M1 = *M. hispanica.*

ing gel, the top chamber of the electrophoresis apparatus was filled with buffer containing two or three drops of bromophenol blue (1 mg/ml). Electrophoresis was carried out at 40 volts during the first 15 minutes and then at 130 volts for about 90 minutes. Following electrophoresis the gels were removed from the glass plates, washed, and covered with the staining solution. Gels were stained for esterase and Mdh as described (2). The relative migration of the bands in any particular gel was adjusted using an *M. javanica* extract included in each gel as a reference. Esterase phenotypes were named as described (7).

For each population except the one growing on olive, protein extracts from females grown under the same greenhouse conditions on different host plants ('California Wonder' pepper, 'NC 95' tobacco, 'Charleston Gray' watermelon, 'Deltapine' cotton, and *Impatiens balsamina*) were examined in order to clarify whether the host plant has an effect on the enzyme phenotypes.

RESULTS

Esterases: Two esterase systems, b and β, were detected. The esterases of the b system were strongly stained and hydrolyze both α- and β-naphthylacetate. The β-esterases were fainter and not visible with the β-naphthylacetate. Since the β-esterases were inconsistent and often undetectable in extracts from single females, only the major bands of b-esterases were considered in this study.

In the 40 root-knot nematode populations studied, 14 major bands of esterase activity were detected; these comprised 10 phenotypes on the basis of single bands or combinations. *M. javanica* and *M. hapla* contained distinct, species-specific phenotypes (Figs. 1, 2—J3, H1). Two phenotypes, A2 and A3, occurred in the six populations of *M. arenaria* (Fig. 1). Of the 11 *M. incognita* populations, seven had the same phenotype (Figs. 1, 2—I1), but the other four showed additional constant and reproducible bands, belonging to three different phenotypes (Fig. 1—I2, I4, I5). All 11 populations shared one common band at Rm 0.46. Phenotype S2-M1 (Figs. 1, 2) occurred in three unidentified populations and was the same reported for *M. hispanica* (7). Phenotype P1 (Figs. 1, 2) had a single esterase band at Rm 0.52 and occurred only in the single population from olive trees.

Malate dehydrogenases: Eight main bands of Mdh activity were detected among the 40 *Meloidogyne* populations. Additional, weaker bands occurred in most populations but were not evaluated further because they varied with the number of nematodes analyzed and were not detected with a single female.

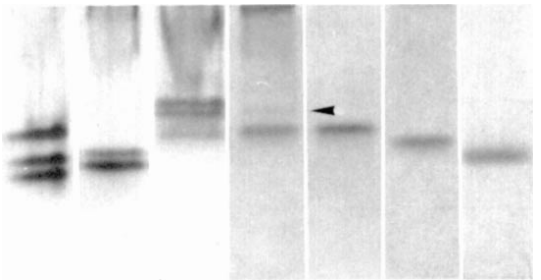

FIG. 2. Seven of the ten esterase phenotypes found in 40 *Meloidogyne* populations. For explanation of phenotype designations see legend of Figure 1. Arrow points to fainter band.

Three different phenotypes were discerned and designated N3, P3, and H2 (Figs. 3, 4). The most common phenotype, N3, had three heavily stained bands of activity and occurred in all populations of *M. incognita, M. arenaria, M. javanica,* and *M. hispanica.* Phenotype P3 occurred only in the undescribed species from olive. It had two very fast migrating bands—one very faint at Rm 0.70 and one heavily stained at Rm 0.55—and a faint, slower band (Fig. 3). Only H2, with one major fast migrating band (Rm 0.62), was species specific. It was associated with all seven populations of *M. hapla.*

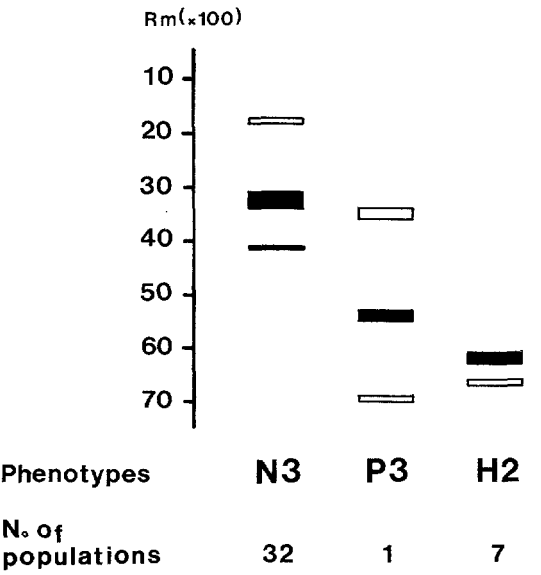

FIG. 3. Phenotypes of malate dehydrogenase (Mdh) in 40 Portuguese populations of *Meloidogyne* spp. Phenotypes are designated with a letter indicating the species and a number indicating the number of bands. Solid lines correspond to strongly stained bands. Dotted lines refer to fainter bands. N = nonspecific; P = *Meloidogyne* n. sp.; H = *M. hapla.*

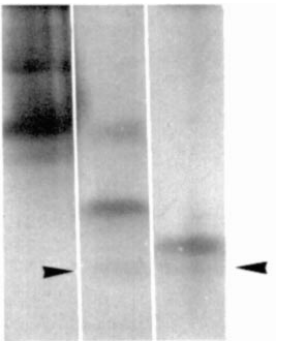

FIG. 4. Malate dehydrogenase phenotypes found in 40 *Meloidogyne* populations. For explanation of phenotype designations see legend of Figure 3. Arrows point to fainter bands.

No effect of the host on esterase or Mdh phenotypes was observed in these experiments. Females of each of the 39 nematode populations propagated on each of the six host plants showed the same esterase and Mdh patterns without noticeable variation in the rate of migration of the major bands.

DISCUSSION

Our studies confirm previous reports that the esterase phenotype is a useful biochemical character for identification of the four most common *Meloidogyne* species and possible characterization of new forms (3,7). Similar studies carried out with several other nematode species have demonstrated the usefulness of enzyme phenotypes for taxonomic purposes (9,12,14).

Although the observed enzyme patterns were stable and repeatable, the mobility of a given band in different gels varied over narrow limits. Also, because of slight variations in techniques and laboratory conditions, the relative mobility of the enzymic bands in our study differed slightly from that in other reports (1,3,4,7,11,17).

All populations of *M. javanica* showed a single esterase phenotype not found in any other *Meloidogyne* population. Esbenshade and Triantaphyllou (7) suggested that the

esterase pattern is a very reliable character for identification of *M. javanica.* Similarly, all populations of *M. hapla* had a characteristic esterase phenotype. *M. incognita,* however, showed some variability among the populations, suggesting that this species can be associated with different phenotypes. Nevertheless, those variations occurred only in minor and fainter bands. The main band, which is usually used to characterize *M. incognita* (7,11,17), was present in all the phenotypes.

Three populations had an esterase phenotype (S2-M1) like the one found in *M. hispanica* and referred to as "Seville population" by Dalmasso and Bergé (3) and Janati et al. (17). Results of the North Carolina differential host test conducted on the above populations showed that tobacco, watermelon, and pepper were slightly infected and no reproduction occurred on peanut and cotton. Only tomato was a good host. This host response was similar to *M. incognita* race 2 or *M. arenaria* race 2. In fact, one of the populations was previously identified as *M. arenaria* race 2 on the basis of the perineal pattern morphology and differential host test (22). Esterase phenotypes revealed that the phenotypes found for these populations were characteristic for *M. hispanica* and further morphological studies confirmed the biochemical data.

Esterase phenotype P1 was associated with only the population from olive trees. Esbenshade and Triantaphyllou (7) reported a similar phenotype (A1) associated with *M. arenaria.* Morphobiometric, host range, and other biochemical studies indicate, however, that the olive population belongs to an undescribed species of *Meloidogyne* (Abrantes, unpubl.). This population also showed a specific and unique Mdh phenotype (P3).

Whereas *M. arenaria, M. incognita,* and *M. javanica* contained similar Mdh phenotypes, *M. hapla* had a characteristic Mdh phenotype useful for differentiating it from the other major species.

In this study we have considered only esterase and Mdh phenotypes and assessed the utility of these systems to characterize *Meloidogyne* species. Multienzyme phenotypic profiles may provide a more reliable method of characterization than phenotypes of single enzymes (7).

Our results confirm the stability of the esterase phenotype of populations propagated on different hosts (7,10). Consequently, esterase phenotypes can be useful for identification of populations of *Meloidogyne* spp. collected from different hosts and for separation of individual species from mixed field populations.

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