JOURNAL OF NEMATOLOGY

VOLUME 20 JANUARY 1988 NUMBER 1

Journal of Nematology 20(1):1–8. 1988.
© The Society of Nematologists 1988.

DNA Isolation and GC Base Composition of Four Root-knot Nematode (*Meloidogyne* spp.) Genomes[1]

E. C. PABLEO, A. C. TRIANTAPHYLLOU, AND W. E. KLOOS[2]

Abstract: Phenol extraction and cesium trifluoroacetate ultracentrifugation were compared for efficiency in the extraction of DNA from eggs and second-stage juveniles of four species of *Meloidogyne.* The second method proved to be more satisfactory in that it yielded larger amounts of DNA, shortened the extraction period, and reduced sample handling by eliminating phenol and ether extraction and RNAse treatment. It also made possible the extraction of DNA from more than one sample at a time. The mean base compositions (% GC) of the total DNA of *M. incognita, M. javanica, M. arenaria,* and *M. hapla,* as determined by thermal denaturation tests, were quite similar, as they ranged only between 31 and 33%. Similarly, the thermal stability of the DNA of all four species covered a narrow range from 82.97 to 83.63 C.
Key words: *Meloidogyne,* DNA, genome, thermal denaturation, GC content, thermal stability.

The root-knot nematodes, *Meloidogyne* spp., constitute one of the most important groups of plant-parasitic nematodes. They cause substantial damage to most agricultural crops. An effort has been made during the last 10 years to understand better the root-knot nematodes through extensive studies of their biology, taxonomy, cytogenetics, biochemistry, and other aspects. The International *Meloidogyne* Project (IMP) has played a considerable role in this effort (28). A large number of populations originating from many parts of the world have been critically compared with regard to their morphology, host preferences, mode of reproduction, chromosome numbers, and various isoenzyme systems (10–12,14,32,33). Investigations on such a large scale have demonstrated extensive biological diversity among root-knot nematodes, which undoubtedly is an expression of the changes that have occurred in their genome.

In this paper, we report our initial attempt to investigate the root-knot nematode genome at the molecular level. We have compared two methods of DNA isolation: phenol extraction and cesium trifluoroacetate (CsTFA) ultracentrifugation. Further, we have determined the base composition and thermal stability (Tm) of the DNA of single populations of four *Meloidogyne* species.

MATERIALS AND METHODS

One population of each of the four most common root-knot nematode species—i.e., *M. incognita, M. javanica, M. arenaria,* and *M. hapla*—was used in this study. Species identifications were made on the basis of morphology (9), host range (27,31), enzyme phenotypes (11), chromosome number, and mode of reproduction (33). Stock cultures of each population were maintained on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) grown under normal greenhouse conditions. Each population was periodically propagated on about 30 plants for the harvest of large numbers of eggs. Roots with mature egg masses were

Received for publication 1 May 1987.

[1]Paper No. 11005 of the Journal Series of the North Carolina Agricultural Service, Raleigh, NC 27695-7601. Use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned. This study was supported in part by the International *Meloidogyne* Project, Contract No. AID/ta-C-1234, and by the National Science Foundation Grant BSR-8314908.
[2] Graduate student, Department of Plant Pathology, and professors, Department of Genetics, North Carolina State University, Raleigh, NC 27695.
We thank W. G. Dougherty and J. A. Moorefield for sharing their experience in DNA isolation and determination of GC base composition.

harvested 65–70 days after inoculation. The eggs were dislodged from the roots by clorox extraction (15), and the resulting egg suspension was passed through a series of 200-mesh and 500-mesh sieves. Eggs were further separated from the remaining plant and soil debris by centrifugation in a 20 and 40% (w/v) stepwise sucrose (nonreagent grade) gradient at 1,250 *g* for 15 minutes. They were thoroughly washed on a 500-mesh sieve to remove the sucrose, concentrated by gravity sedimentation at 4 C, and stored in 1-ml portions in labeled vials at −20 C.

Phenol extraction of DNA: Phenol extraction was patterned after the methods employed by Marmur (20) and Kirby (17), as modified by Emmons et al. (8). Frozen eggs were ground to a paste in a mortar with pestle. One to two milliliters of extraction buffer (0.1 M Tris, 0.1 M EDTA, 0.2 M NaCl; pH 8) was added to the paste and the suspension was refrozen in the mortar. The frozen material was scraped lightly with a rapid motion using a metallic spatula, reground, mixed with 1–2 ml extraction buffer, and refrozen. This procedure was repeated three times to ensure sufficient disruption of the eggshells and the cuticles of juveniles of embryonated eggs. Sodium dodecyl sulfate was added to a concentration of 4% (w/v), and the suspension was incubated for 3 hours at 37 C with occasional agitation. To further lyse the cells, proteinase K was added to a concentration of 200 μg/ml, and the suspension was incubated for another 30 minutes at 50 C with occasional agitation.

Phenol extraction was done by mixing an equal volume of the suspension with redistilled phenol saturated with the extraction buffer and centrifuging the mixture at 4,600 *g* in a fixed angle rotor head for 5 minutes at 4 C. The upper aqueous phase was subjected to two more cycles of phenol extraction, and the remaining phenol was removed by three cycles of diethyl ether extraction. This was done by mixing the aqueous solution with an equal amount of ether and then centrifuging for 5 seconds at 324 *g*. Excess ether was removed by gently blowing the surface of the solution with nitrogen. The nucleic acids (DNA and RNA) in the aqueous solution were precipitated by adding one part of 3 M sodium acetate containing 0.001 M ethylenediaminetetraacetic acid (EDTA) to nine parts of the solution and two parts of 95% ethanol at −20 C for 1 hour or overnight. The precipitated nucleic acids were pelleted by centrifugation at 8,100 *g* for 20 minutes and then dried under vacuum. The pellet was resuspended in 1–2 ml extraction buffer, and the RNA was removed by digestion with 200 μg RNAse at 37 C for 1 hour, followed by phenol and ether extraction and ethanol precipitation. Further purification of the DNA was done by hydroxyapatite (HA) column chromatography (4). All DNA preparations were stored frozen in 0.12 M sodium phosphate containing 0.001 M EDTA at pH 6.8.

Cesium ultracentrifugation: CsTFA was used similarly to cesium chloride and cesium sulfate (1,13). Each method of ultracentrifugation uses a salt cushion with the buoyant density of the DNA being extracted.

Eggshells and larval cuticles were disrupted as described. For further lysis of cells and tissues, the homogenates were incubated with 60 μg elastase, 0.5 ml CsTFA (from 134 g CsTFA in 100 ml stock solution)/ml eggs, and sufficient sarkosyl to obtain a final concentration of 8% (w/v), at 50 C for 20 minutes. The CsTFA was added 10 minutes after the start of incubation. Ethidium bromide was added to a concentration of 40–50 μg/ml and 3.5 ml of the preparation was added onto 1.5 ml of a cushion of CsTFA in extraction buffer (density of 1.60 g/ml) in a polyallomer centrifuge tube. The suspension was centrifuged at 150,000 *g* in a swinging bucket rotor (Sorvall TH-641) for 16 hours at 20–25 C. After centrifugation, the UV-fluorescent DNA band at the interface between the cushion and the suspension was removed with a 16-gauge syringe needle. The DNA was precipitated with two volumes of 95% ethanol, washed with 70% ethanol, resuspended in 0.12 M sodium

phosphate containing 0.001 M EDTA, pH 6.8, and stored at −20 C.

Determination of DNA purity: Presence of protein contamination in DNA preparations was determined by the ratios of absorbance at A_{260}/A_{280} and A_{260}/A_{230}, and the equation "protein(mg/ml) = $1.45E_{280} - 0.74E_{260}$" where E_{260} and E_{280} are absorbances at 260 and 280 nm wavelengths (22,35). The quality of the DNA preparations was further determined by the shape of the hyperchromic shift and the percentage of hyperchromicity during thermal denaturation of the DNA (4). Hyperchromicity is the increase in absorbance at 260 nm during thermal denaturation.

Determination of percent GC content and thermal stability: Tests were performed in a Gilford Model 2527 quartz-cuvette thermoprogrammer system. Each cuvette was loaded with a DNA sample in 0.14 M sodium phosphate buffer (pH 6.4) at an approximate concentration of 65–75 μg/ml. Several DNA preparations and a reference blank containing buffer were analyzed at the same time to check experimental variation.

Thermal denaturation was carried out at increments of 1 C from 50 to 100 C, with a 1-minute dwell time for every temperature increase. The increase in absorbance at 260 nm was recorded at a dwell time of 3 seconds in a Gilford spectrophotometer equipped with Gilford analog multiplexer (Model 6046) and a recorder set for scale absorbance of 0.6. The Tm was graphically determined for each sample from the mid-point of the thermal denaturation profile, and the respective base composition (% GC) was calculated using the formula $GC = 2.44(Tm - 81.5 - 16.6 \log M)$, where M is the molarity of the cation in the buffer (19).

Results

DNA isolation: A critical step in extracting DNA from nematodes is disruption of eggshells, as well as cuticles of juveniles and adults. Satisfactory results were obtained by the physical method of freezing, scraping, and grinding in a mortar with a pestle. The incorporation of elastase, CsTFA, sarkosyl, and proteinase K was effective in lysing the cells once eggshells and cuticles were disrupted. The use of elastase without proteinase K also gave high yields of DNA.

Good thermal denaturation profile could not be obtained for DNA isolated by phenol extraction. When the preparation was purified in a hydroxyapatite column, two fractions were separated, one containing double-stranded and the other single-stranded DNA (Fig. 1). DNA prepared by the CsTFA ultracentrifugation method did not need further purification, and the thermal denaturation test revealed a sharp transition from double-stranded to denatured DNA over a narrow range of temperatures (Fig. 1). A white, milky, opaque band was observed near the DNA band in CsTFA preparations. This band, which could be removed by a 21-gauge syringe needle, dissolved faster than the DNA band in Tris-EDTA buffer, whereas the DNA band appeared glassy while being dissolved.

All DNA preparations isolated by phenol extraction had a ratio of absorbance of 2.02 at 260/280 nm, while those isolated by CsTFA ultracentrifugation had absorbance ratios of 2.00 at 260/280 nm and 2.32 at 260/230 nm.

DNA yield from the four species studied was similar and varied only with the method of isolation. CsTFA ultracentrifugation yielded consistently larger amounts of DNA (350–600 μg DNA/ml of packed eggs) than phenol extraction (200–250 μg/ml of packed eggs).

Percentage of GC and thermal stability: The Tm of the DNA preparations of *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* ranged from 82.97 C to 83.63 C (Fig. 2). The base compositions calculated from these Tm values revealed a homogeneity of GC content percentage ranging from 31.0% to 32.6% (Table 1). The reference bacterial DNA of *Staphylococcus cohnii* subsp. 2 gave a consistent thermal stability of 82.5 C in all runs and had a regular S-shape thermal denaturation profile.

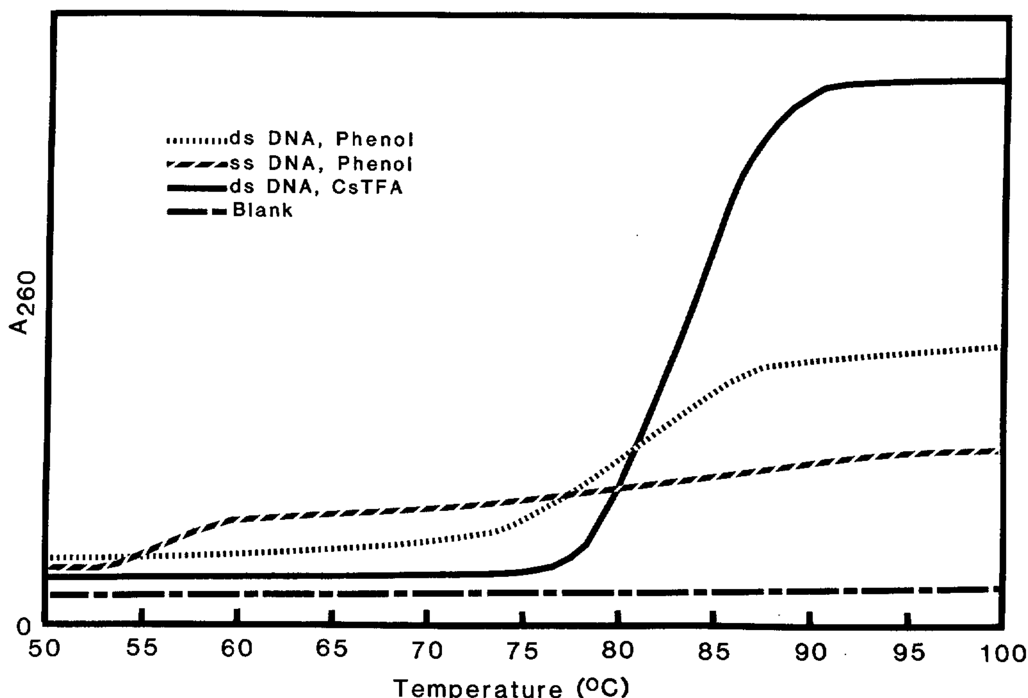

FIG. 1. Thermal denaturation profiles of *Meloidogyne arenaria* DNA preparations in 0.12 M sodium phosphate (NaH_2PO_4-Na_2HPO_4), pH 6.8. DNA was isolated by phenol extraction and CsTFA ultracentrifugation. ds, double-stranded; ss, single-stranded.

DISCUSSION

The difficulty in lysing eggshells of *Meloidogyne* species was observed previously by Bird and McClure (3). Treatment of eggs with chitinase, trypsin, and collagenase did not cause pronounced changes in eggshell morphology as seen under the light and electron microscopes. Similarly, cuticles of *Tylenchorhynchus dubius* treated with chitinase, papain, and lipase were less rigid, more laterally folded and had deeper striations, but remained intact (24). Cox et al. (6) found that elastase could digest the soluble collagen fraction and the resistant noncollagen protein of the cuticle layers of *Caenorhabditis elegans.* Other structural components, however, still held the cuticle relatively intact. In our experiment, we observed that the same enzyme did not digest the eggshells and the cuticles of juveniles of root-knot nematodes. The eggshell of root-knot nematodes contains a chitinous layer, lipoproteins, and glycoproteins that together form a structure very resistant to enzymes (3). The basal layer of the cuticle of *Meloidogyne* juveniles contains a nonsoluble collagen which is also resistant to enzymes (26). Mechanical disruption of eggshells and juvenile cuticles was required for efficient extraction of DNA in our experiments.

Behme and Pasternak (2) observed a satellite band in DNA preparations from the free-living, *Turbatrix aceti.* Distinctive light scattering observed in the Schlieren optical system that was eliminated by alpha amylase treatment indicated that the band contained polysaccharides. Its buoyant density of 1.688 g/cm^3 was very close to that of the DNA of the other free-living nematodes they investigated, which ranged from 1.695 to 1.703. Because of polysaccharide contamination, thermal denaturation data could not be obtained in *T. aceti.* Bands containing polysaccharides in DNA preparations from nematodes possibly contain protein-linked carbohydrates or other complexes, as do similar preparations from bacteria (7). In our experiments, good thermal denaturation profiles could not be obtained for DNA isolated by phenol extraction.

In the thermal denaturation tests, a 23–

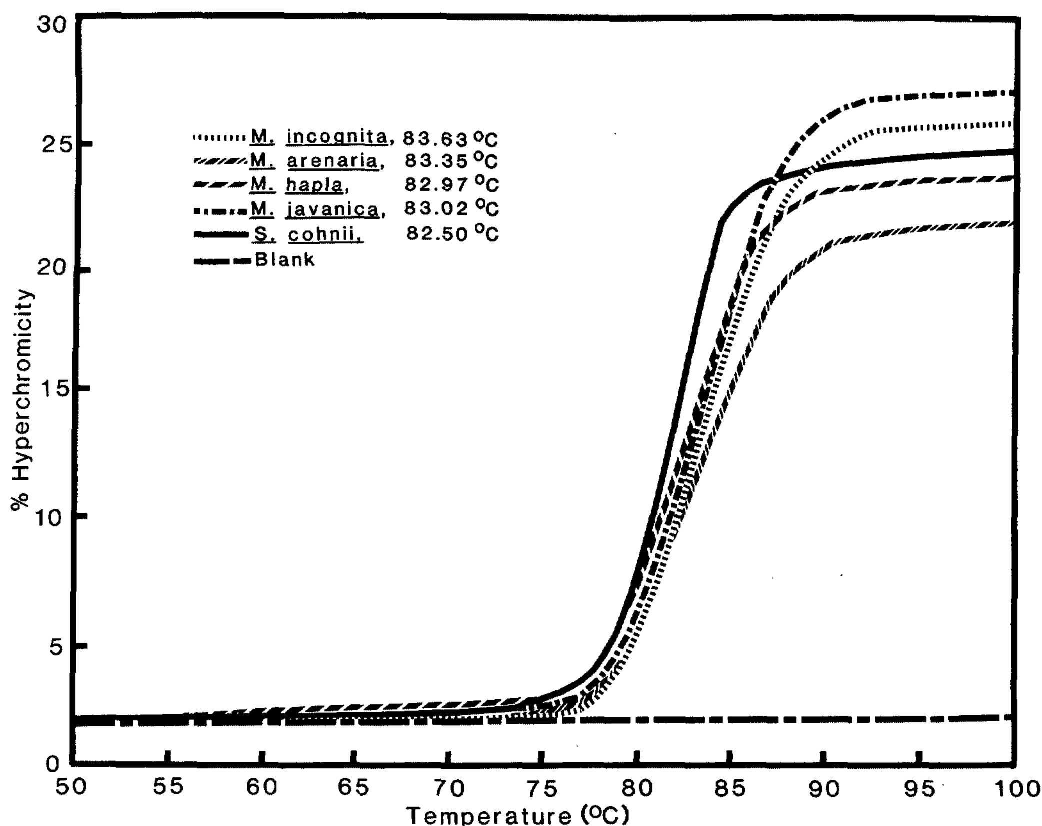

FIG. 2. Thermal denaturation profiles of DNA in 0.14 M NaH_2PO_4-Na_2HPO_4, pH 6.4, from four species of *Meloidogyne* and a *Staphylococcus cohnii* reference. DNA samples were isolated by CsTFA ultracentrifugation. The temperatures indicated in the figures are the thermal stabilities of the different DNA and correspond to the mid-point of the denaturation profiles.

27% increase in hyperchromicity was observed in DNA preparations obtained by CsTFA centrifugation (Fig. 2). The observed increase in hyperchromicity is acceptable, according to Britten et al. (4). In CsTFA ultracentrifugation, the RNA is pelleted at the bottom of the centrifuge tubes and is effectively separated from the DNA which bands at the interface of the two CsTFA solutions. No gradual or early rise of the thermal denaturation profile was observed in DNA preparations isolated by this method. This implies that there was no RNA and denatured DNA which would have caused an early rise of the profile (4). Therefore, RNA and denatured DNA are not major contaminants of DNA preparations isolated by CsTFA ultracentrifugation. A band in CsCl gradients similar to our opaque band in CsTFA DNA preparations has been attributed to the presence of polysaccharides (5). The suspected polysaccharide band can be easily removed from centrifuge tubes with a 21-gauge syringe needle, whereas polysaccharides in DNA preparations isolated by phenol extraction can be eliminated effectively only through

TABLE 1. Base composition (% GC) of nematode DNA.

Species	% GC	Reference
Caenorhabditis elegans	36.0	(30)
Caenorhabditis briggsae	36.0	(2)
Panagrellus silusiae	44.0	(2)
Panagrellus redivivus	44.0	(2)
Rhabditis anomala	42.0	(2)
Turbatrix aceti	40.0	(2)
Ascaris suum	38–41	(16)
Meloidogyne incognita	32.6	Present study
Meloidogyne javanica	31.2	Present study
Meloidogyne arenaria	32.0	Present study
Meloidogyne hapla	31.0	Present study

a HA column. We did not determine the identity or amount of polysaccharide contamination in our DNA preparations, but the sharp transition of the thermal denaturation profiles suggests that there was no significant contamination.

DNA prepared by the CsTFA ultracentrifugation method did not need further purification, and the thermal denaturation test revealed a sharp transition from ds DNA to ss DNA over a narrow range of temperatures (Fig. 1). This pattern of thermal denaturation indicated the presence of clean, intact ds DNA. During ultracentrifugation, the trifluoroacetate ions promote the hydration and solubilization of both nucleic acids and proteins (34). The nucleic acids band at lower densities than proteins, however, and this property leads to their effective disassociation from proteins during CsTFA centrifugation. Absorbance ratios indicate that protein contamination in the DNA preparations isolated by both methods was neligible (35). The values resulting from the equation of Mayr-Harting et al. (22) also revealed that the DNA preparations contained insignificant protein contamination.

In addition to higher DNA yield and purity, CsTFA ultracentrifugation was preferable to phenol extraction because it shortened the extraction period and reduced handling of the samples by eliminating phenol and ether extractions and RNAse treatment. Also, it made possible the extraction of DNA from more than one sample at a time.

The regular S-shaped thermal denaturation profiles of DNA from the four *Meloidogyne* species and *S. cohnii* indicated a uniform distribution of nitrogenous bases in the DNA molecule (Fig. 2). This shape was observed in all DNA preparations from the four species of root-knot nematodes. The formula used in determining the GC content applies to buffers containing Na^+ and K^+ cations in the range of 0.01–0.20 M salt concentration in buffered solutions near neutrality. The sodium phosphate solution that was used was buffered to pH 6.4 and is within the linear relationship between the logarithm of salt concentration and Tm allowed by the formula $GC = 2.44(Tm - 81.5 - 16.6 \log M)$ (19,29). The relationship is such that the higher the salt concentration in the buffer, the higher the Tm. In our experiments, we observed that the Tm increased by 5 C when there was a change from 0.18 to 0.42 M Na^+ cationic concentration in the buffer. When sodium citrate buffer was used during thermal denaturation experiments, the Tm decreased by 16.35 C but the formula appropriate for this buffer, $GC = (Tm - 53.9)2.44$ (19), gave similar base compositions as those obtained with 0.14 M sodium phosphate buffer.

The base composition is known for only seven nematode species, belonging to five genera (Table 1) (2,16,25,30). Lee et al. (18) first recognized the possibility of using DNA base analysis as a taxonomic aid and suggested that base composition could be employed as an index of genetic relatedness. Indeed, subsequent analyses of bacterial DNA demonstrated that forms that are genetically closely related have similar DNA base composition (21,23). Platzer (25) suggested that base composition studies may be helpful in separating nematodes into genera and families.

The striking similarity in DNA base composition observed among the four species of *Meloidogyne* examined in this study suggests a high similarity in nucleotide sequences of their DNA. Consequently, it may be concluded that the genomes of the four *Meloidogyne* species have undergone only a conservative type of diversification. Nonetheless, these species exhibit considerable morphological, biological, and cytogenetic diversity. They have different host preferences and show various ecological adaptations. *M. hapla* is primarily a diploid species that reproduces by amphimixis or meiotic parthenogenesis, whereas *M. incognita, M. javanica,* and *M. arenaria* are polyploid and reproduce by obligatory mitotic parthenogenesis (33). Apparently, the conservative diversification of the genome has not precluded an accumulation of gene and chromosomal mutations that have

brought about the extensive biological and cytogenetic evolution in the genus *Meloidogyne.*

Literature Cited

1. Barbour, W. M., J. N. Mathis, and G. H. Elkan. 1985. Evidence of plasmid- and chromosome-borne multiple *nif* genes in *Rhizobium fredii.* Applied and Environmental Microbiology 50:41–44.

2. Behme, R., and J. Pasternak. 1969. DNA base composition of some free-living nematode species. Canadian Journal of Genetics and Cytology 11:993–1000.

3. Bird, A. F., and M. A. McClure. 1976. The tylenchid (Nematoda) egg shell: Structure, composition and permeability. Parasitology 72:19–28.

4. Britten, R. J., D. E. Graham, and B. R. Neufeld. 1974. Analysis of repeating DNA sequences by reassociation. Pp. 363–419 *in* L. Grossman and K. Modalve, eds. Methods of enzymology, vol. 29. New York: Academic Press.

5. Brunk, C. F., and P. C. Hanawalt. 1966. Glycogen satellite bands in isopycnic CsCl gradients. Experimental Cell Research 42:406–408.

6. Cox, G. N., M. Kusch, and R. S. Edgar. 1981. Cuticle of *Caenorhabditis elegans*: Its isolation and partial characterization. Journal of Cell Biology 90:7–17.

7. Darby, G. K., A. S. Jones, J. F. Kennedy, and R. T. Walker. 1970. Isolation and analysis of the nucleic acids and polysaccharides from *Clostridium welchii.* Journal of Bacteriology 103:159–165.

8. Emmons, S. W., M. R. Klass, and D. Hirsch. 1979. Analysis of constancy of DNA sequences during development and evolution of the nematode *Caenorhabditis elegans.* Proceedings of the National Academy of Sciences of the U.S.A. 76:1333–1337.

9. Eisenback, J. D., H. Hirschmann, J. N. Sasser, and A. C. Triantaphyllou. 1981. A guide to the four most common species of root-knot nematodes (*Meloidogyne* spp.), with a pictorial key. Raleigh: North Carolina State University Graphics.

10. Eisenback, J. D. 1985. Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). Pp. 95–112 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne,* vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.

11. Esbenshade, P. R., and A. C. Triantaphyllou. 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. Journal of Nematology 17:6–20.

12. Esbenshade, P. R., and A. C. Triantaphyllou. 1987. Enzymatic relationships and evolution in the genus *Meloidogyne* (Nematoda: Tylenchida). Journal of Nematology 19:8–18.

13. Gilsin, V., R. Crkvenjakov, and C. Byus. 1974. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry 13:2633–2637.

14. Hirschmann, H. 1985. The genus *Meloidogyne* and morphological characters differentiating its species. Pp. 79–93 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne,* vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.

15. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 59:1025–1028.

16. Kilejian, A., and A. McInnis. 1976. Density distribution of DNA from parasitic helminths with special reference to *Ascaris lumbricoides.* Rice University Studies 62:161–174.

17. Kirby, K. S. 1968. Isolation of nucleic acids with phenolic solvents. Pp. 87–98 *in* L. Grossman and K. Modalve, eds. Methods in enzymology, vol. 12B. New York: Academic Press.

18. Lee, K. Y., R. Wahl, and E. Barbu. 1956. Contenu en bases puriques et pyrimidiques des acides desoxyribonucleiques des bacteries. Annales de l'Institut Pasteur, Paris 91:221–224.

19. Mandel, M., and J. Marmur. 1968. Use of ultra-violet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. Pp. 195–206 *in* L. Grossman and K. Modalve, eds. Methods in enzymology, vol. 12B. New York: Academic Press.

20. Marmur, J. 1961. A procedure for the isolation of DNA from micro-organisms. Journal of Molecular Biology 3:208–218.

21. Marmur, J., S. Falkow, and M. Mandel. 1963. New approaches to bacterial taxonomy. Annual Review of Microbiology 17:329–372.

22. Mayr-Harting, A., A. J. Hedges, and R. C. W. Berkeley. 1972. Methods in studying bacteriocin. Pp. 315–422 *in* J. R. Norris and D. W. Ribbons, eds. Methods in microbiology, vol. 7A. New York: Academic Press.

23. McCarthy, B. J. 1965. The evolution of base sequences in polynucleotides. Pp. 129–160 *in* J. N. Davidson and W. E. Kohn, eds. Progress in nucleic acid research and molecular biology, vol. 4. New York: Academic Press.

24. Miller, P. M., and D. C. Sands. 1977. Effects of hydrolytic enzymes on plant parasitic nematodes. Journal of Nematology 9:192–197.

25. Platzer, E. G. 1981. Potential use of protein patterns and DNA nucleotide sequences in nematode taxonomy. Pp. 3–21 *in* B. M. Zuckerman and R. A. Rohde, eds. Plant parasitic nematodes, vol. 3. Biochemistry, cytochemistry, and genetics. New York: Academic Press.

26. Reddigari, S. R., P. L. Jansma, D. Premachandran, and R. S. Hussey. 1986. Cuticular collagenous proteins of second-stage juveniles and adult females of *Meloidogyne incognita*: Isolation and partial characterization. Journal of Nematology 18:294–302.

27. Sasser, J. N. 1972. Physiological variation in the genus *Meloidogyne* as determined by differential hosts. OEPP/EPPO Bulletin 6:41–48.

28. Sasser, J. N., J. D. Eisenback, C. C. Carter, and A. C. Triantaphyllou. 1983. The International *Meloidogyne* Project—its goal and accomplishments. Annual Review of Phytopathology 21:271–288.

29. Schildkraut, C., and S. Lifson. 1965. Dependence of the melting temperature of DNA on salt concentration. Biopolymers 3:195–208.

30. Sulston, J. E., and S. Brenner. 1974. The DNA of *Caenorhabditis elegans.* Genetics 77:95–104.

31. Taylor, A. L., and J. N. Sasser. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh: North Carolina State University Graphics.

32. Triantaphyllou, A. C. 1984. Polyploidy in meiotic parthenogenetic populations of *Meloidogyne hapla* and a mechanism of conversion to diploidy. Revue de Nematologie 7:65–72.

33. Triantaphyllou, A. C. 1985. Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. Pp. 113–126 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne,* vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.

34. Tunis, M. J., and J. E. Hearst. 1968. On the hydration of DNA. I. Preferential hydration and stability of DNA in concentrated trifluoroacetate solution. Biopolymers 6:1325–1344.

35. Warburg, O., and W. Christian. 1941. Isolierung und kristallisation des garungsferments enolase. Biochemische Zeitschrift 310:400–403.