Bulletin OEPP/EPPO Bulletin **24**, 447-451 (1994)

Identification of plant-parasitic nematodes by PCR amplification of DNA fragments[1]

by C. DE GIORGI*, M. FINETTI SIALER*, M. DI VITO† and F. LAMBERTI†

*Dipartimento di Biochimica e Biologia Molecolare, Università di Bari, Via Amendola 165/A, 70126 Bari (Italy)

†Istituto di Nematologia Agraria, CNR, Via Amendola 165/A, 70126 Bari (Italy)

A study concerning the detection and characterization of a DNA fragment from plant-parasitic nematodes to be used as a molecular marker for the identification of different nematodes is described. A fragment of DNA, which is known to consist of a variable region flanked by two conserved regions, has been studied by using PCR amplification. A portion of about 600 nucleotides at the 5′ end of the larger rRNA gene has been amplified in different nematodes, using heterologous primers which hybridize with the conserved regions. The results obtained clearly indicate that the same primers can be used for the amplification of this segment in nematodes of different species and of different genera: *Meloidogyne artiellia*, *M. incognita*, *Xiphinema index*, *X. diversicaudatum* and *Globodera pallida*. The amplified regions have been partially sequenced. The nucleotide sequences have been analysed by comparison with the published sequence of *Caenorhabditis elegans*.

Introduction

Molecular approaches for the identification of nematodes have recently been increasing considerably. Several studies have been carried out in different laboratories to detect DNA restriction fragments that can be used as molecular markers in order to discriminate different nematodes at the genomic level. Digestion of DNA with restriction endonucleases generates a unique set of restriction fragments dependent upon the base sequence of the genome. The size distribution of the restriction fragments, analysed by agarose gel electrophoresis, is characteristic of a given genotype. Thus, genetic differences between various organisms can be visualized by observing differences in the restriction fragment length.

This approach has been used on both nuclear DNA and mitochondrial DNA (mtDNA). The size distribution of restriction fragments in ethidium bromide-stained agarose gel allowed a direct visualization of the fragment-length differences (Curran *et al.*, 1986; Powers & Sandall, 1988; Radice *et al.*, 1988). Furthermore, the sensitivity of this technique can be increased when a labelled DNA fragment is used to prove restriction fragments. In this case, the different hybrids are shown by autoradiogram (Powers *et al.*, 1986; Curran & Webster, 1989; Castagnone-Sereno *et al.*, 1991; De Giorgi *et al.*, 1991; Piotte *et al.*, 1992; Stratford *et al.*, 1992; Xue *et al.*, 1992).

Although very interesting results have been obtained, this technique suffers from the major limitation of requiring a relatively large amount of material.

The most powerful molecular approach available today seems to be the method of polymerase chain reaction (PCR) (Harris *et al.*, 1990; Vrain *et al.*, 1992; Powers & Harris 1993). This technique allows the characterization of genetic variation at the DNA level by using

[1]Paper presented at the EPPO/CIHEAM Conference on Plant Nematology in the Mediterranean Region, Valenzano (IT), 1993-03-30/04-01.

a very small amount of material, virtually a single DNA molecule. Organisms that cannot be grown in pure culture have been detected, identified and characterized phylogenetically by using PCR amplification and sequencing of specific DNA fragments.

PCR is an *in vitro* synthesis of a specific target DNA segment, bordered by two regions whose DNA sequences are known. It is made possible by the specific annealing of oligonucleotides complementary to the regions flanking the segment: each oligonucleotide binds to one DNA strand and acts as a primer in the synthesis of a new DNA strand complementary to the old one, thus producing a new double-stranded DNA fragment. The 'amplification' is achieved because after each round of replication the newly synthesized DNA molecule acts as a template for further replication.

This paper presents some preliminary results from the application of PCR to a number of genera and species of plant-parasitic nematodes.

Results and discussion

In Fig. 1, the analysis of the amplified product from *Caenorhabditis elegans* (Maupus) Dougherty, and from *Meloidogyne artiellia* Franklin and *M. incognita* (Kofoid & White) Chitwood, are shown. Since information on the nucleotide sequences of plant-parasitic nematodes is still limited, the sequence of the free-living nematode *C. elegans* was used as a model sequence (Ellis *et al.*, 1986). The selected region is a portion of the 5′ end of the large subunit of the rRNA gene. The two flanking segments, destined to anneal the primers, were selected in regions of the gene which are known to be highly conserved in many different organisms, so that the same oligonucleotide pair will bind to corresponding regions in different nematodes. On the other hand, the region included within the primers is variable and, therefore, differences in the nucleotide sequence can be used as diagnostic differences.

From data presented in Fig. 1, it can be seen that the amplification product is present in relatively large amounts since it is clearly visible on the gel. Furthermore, in all cases, the size of

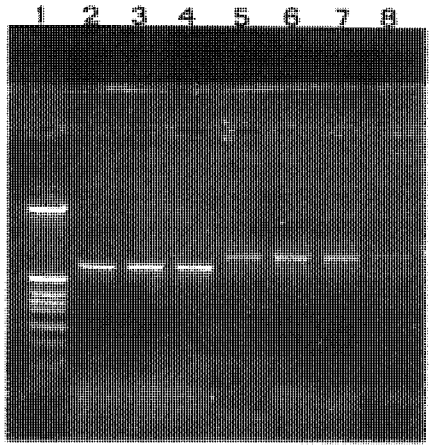

Fig. 1. Ethidium bromide-stained agarose gel of amplified products obtained from *Caenorhabditis elegans* (2, 3, 4), *Meloidogyne artiellia* (5, 6), and *M. incognita* (7, 8) DNAs. In slot 1, molecular weight markers, whose size were 1,631, 517, 396, 344, 298, 220, 154 and 75 nt, were run.
Gel d'agarose coloré au bromure d'éthidium des produits d'amplification pour les ADN obtenus de *C. elegans* (2, 3, 4), *M. artiellia* (5, 6), et *M. incognita* (7, 8). Dans le puits 1 ont été placés les marqueurs de poids moléculaire de taille 1631, 517, 396, 344, 298, 220, 154 et 75 nt.

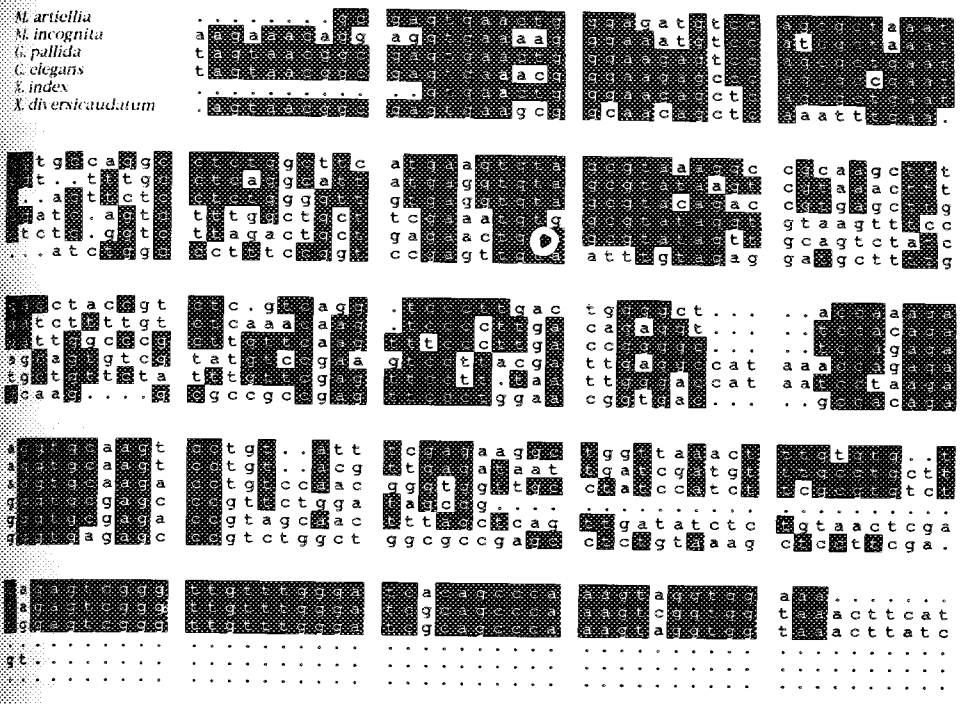

Fig. 2. Computer-aided multi-alignment of sequenced regions of rRNA gene from different nematode species. The program used was the Pretty Program of the GCG Package (release 7.1).
Comparaison obtenue par ordinateur des régions séquencées du gène de l'ADNr pour différentes espèces de nématodes. Le programme utilisé est Pretty Program de GCG Package (version 7.1).

the fragment is about 650 nucleotide (nt) long. The *Meloidogyne* fragment is slightly bigger than that of *C. elegans* which has been reported to be 645 nt long (Ellis *et al.*, 1986), and thus the length difference may reflect specific peculiarity.

Once the DNA fragment is available in relatively large amounts, three different means of DNA typing can be conducted: the determination of length differences (Powers & Harris, 1993), the determination of restriction fragment differences (Vrain *et al.*, 1992; De Giorgi *et al.*, 1992; Powers & Harris, 1993), and the determination of sequence differences. The comparison of sequenced DNA fragments from different organisms provides a molecular basis for the assessment of phylogenetic relationships between the organisms. In order to study the feasibility of PCR to discriminate among different nematode populations, we decided to sequence a DNA fragment of the large rRNA gene in different genera.

In Fig. 2 the sequences of the same regions in different nematode species are displayed. From *M. artiellia*, *M. incognita*, *Xiphinema index* Thorne & Allen, *X. diversicaudatum* (Micoletzky) Thorne and *Globodera pallida* (Stone) Behrens, the same fragment was amplified and sequenced. All the sequences were compared with that of *C. elegans*. In order to calculate the similarity between sequence strings from different organisms, a computer-aided program searched the best alignment between the different related nucleotide sequences. The multi-alignment of the different sequences presented in Fig. 2 has been obtained to highlight conserved and diverging regions. In order to maximize the display of homology of the multi-alignment of the nucleotide sequences, shaded boxes around the conserved regions are shown. It can be seen that some regions are well conserved in all the nematodes investigated, while

some other regions show variability from species to species, thus demonstrating that the technique can be used for nematode identification and taxonomy. Furthermore, it is clear that, within the same block of conserved sequence, there are several differences between different species of the same genus. It is, therefore, conceivable that small nucleotide changes can differentiate different species or races of nematodes within the same taxonomic group. Further studies are required in order to clarify this point. Moreover, a further development of the technique, directly oriented to the identification of the base/bases changes, will allow a more rapid and efficient diagnosis procedure.

Acknowledgements

The skilful collaboration of student Giuseppe Capozza is gratefully acknowledged.

Identification de nématodes phytoparasites à l'aide de l'amplification PCR de fragments d'ADN

L'étude concerne la détection et la caractérisation d'un fragment d'ADN provenant de nématodes phytoparasites, utilisé comme marqueur moléculaire pour l'identification de différents nématodes. Un fragment d'ADN, comportant une région variable entourée de deux régions conservées, a été étudié à l'aide de la PCR. Une portion d'environ 600 nucléotides située à l'extrémité 5' du plus gros gène de l'ARNr a été amplifié chez différents nématodes à l'aide d'amorces hétérologues qui s'apparient avec les régions conservées. Les résultats obtenus démontrent clairement que les mêmes amorces peuvent être utilisées pour l'amplification de ce segment chez des nématodes appartenant à différentes espèces et à différents genres: *Meloidogyne artiellia, M. incognita, Xiphinema index, X. diversicaudatum, Globodera pallida*. Les régions amplifiées ont été partiellement séquencées. Les séquences de nucléotides ont été analysées par comparaison avec la séquence publiée pour *Caenorhabditis elegans*.

Идентификация фитопаразитных нематод амплификацией фрагментов ДНК при участии полимеразной цепной реакции

Изложено выявление и описание фрагмента ДНК фитопаразитных нематод, используемого в качестве молекулярного маркера для идентификации различных нематод, паразитирующих на растениях. Фрагмент ДНК, который, как известно, содержит вариабельную область с примыкающими консервативными областями, изучался методом амплификации за счет полимеразной цепной реакции. У нескольких нематод был укрупнен участок, содержащий около 600 нуклеотидов, с 5'-конца наибольшего гена рРНК, используя гетерологусные затравки, образующие гибриды с консервативными областями. Полученные результаты четко показывают, что одни и те же праймеры могут быть использованы для амплификации данного сегмента у различных видов и родов нематод: *Meloidogyne artiellia, M. incognita, Xiphinema index, X. diversicaudatum, Globodera pallida*. Укрупненные области были частично секвенсированы. Последовательность нуклеотидов была изучена путем сравнения с опубликованной последовательностью нуклеотидов нематоды *Caenorhabditis elegans*.

References

Castagnone-Sereno, P., Piotte, A., Abad, P., Bongiovanni, M. & Dalmasso, A. (1991) Isolation of a repeated DNA probe showing polymorphism among *Meloidogyne incognita* populations. *Journal of Nematology* **23**, 316–320.

Curran, J. & Webster, J.M. (1989) Genotypic analysis of *Heterorhabditis* isolates from North Carolina. *Journal of Nematology* **21**, 140–145.

Curran, J., McClure, M.A. & Webster, J.M. (1986) Genotypic differentiation of *Meloidogyne* population by detection of restriction fragment length difference in total DNA. *Journal of Nematology* **18**, 83–86.

De Giorgi, C., De Luca, F., Finetti Sialer, M., Di Vito, M. & Lamberti, F. (1991) DNA fragments as molecular probes for the diagnosis of plant parasitic nematodes. *Nematologia Mediterranea* **19**, 131–134.

De Giorgi, C., Finetti Sialer, M., Di Vito, M. & Lamberti, F. (1992) A fragment of the large subunit of the rRNA gene amplified by polymerase chain reaction in individual nematodes. *Nematologia Mediterranea* **20**, 149–152.

Ellis, R.E., Sulston, J.E. & Coulson, A.R. (1986) The rDNA of *C. elegans*: sequence and structure. *Nucleic Acids Research* **14**, 2346–2364.

Harris, T.S., Sandall, L.J. & Powers, T.O. (1990) Identification of single *Meloidogyne* juveniles by polymerase chain amplification of mitochondrial DNA. *Journal of Nematology* **22**, 518–524.

Piotte, C., Castagnone-Sereno, P., Uijthof, J., Abad, P., Bongiovanni, M. & Dalmasso, A. (1992) Molecular characterization of species and populations of *Meloidogyne* from various geographic origins with repeated-DNA homologous probes. *Fundamental and Applied Nematology* **15**, 271–276.

Powers, T.O. & Harris, T.S. (1993) A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology* **25**, 1–6.

Powers, T.O. & Sandall, L.J. (1988) Estimation of genetic divergence in *Meloidogyne* mitochondrial DNA. *Journal of Nematology* **20**, 505–511.

Powers, T.O., Platzer, E.G. & Hyman, B. (1986) Species-specific restriction site polymorphism in root-knot nematode mitochondrial DNA. *Journal of Nematology* **18**, 288–293.

Radice, A.D., Powers, T.O., Sandall, L.J. & Riggs, R.D. (1988) Comparison of mitochondrial DNA from the sibling species *Heterodera glycines* and *H. schachtii*. *Journal of Nematology* **20**, 443–450.

Stratford, R., Shields, R., Goldsbrough, A. & Fleming, C. (1992) Repetitive DNA sequences from potato cyst nematodes and their use as diagnostic probes. *Molecular Plant Pathology* **82**, 881–886.

Vrain, T.C., Wakarchuk, D.A., Levesque, A.C. & Hamilton, R.I. (1992) Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* **15**, 562–573.

Xue, B., Baillie, D.L., Beckenbach, K. & Webster, J.M. (1992) DNA hybridization probes for studying the affinities of three *Meloidogyne* populations. *Fundamental and Applied Nematology* **15**, 35–41.