

# The use of DNA probes to identify *Ditylenchus dipsaci*

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## SUMMARY

Probes based on genomic DNA from *Ditylenchus dipsaci* were used to discriminate this species from others of the genus *Ditylenchus*. Quantitative differences were revealed by colony hybridisation of clones with probes of radiolabelled DNA from *D. dipsaci* (oat race) and from *D. myceliophagus*. The DNA inserts were recovered from selected clones and used as probes against genomic DNA from four races of *D. dipsaci* plus three other species of *Ditylenchus*. Probes hybridised strongly with DNA from all races of *D. dipsaci* but not with that obtained from other species of *Ditylenchus*. Probes found in this work may have potential diagnostic use.

## RÉSUMÉ

### *Utilisation de sondes d'ADN pour identifier Ditylenchus dipsaci*

Des sondes constituées par l'ADN génomique de *Ditylenchus dipsaci* sont utilisées pour séparer cette espèce des autres espèces du genre *Ditylenchus*. Des différences quantitatives ont été mises en évidence par hybridation de colonies de clones avec des sondes d'ADN marqué aux radio-éléments provenant de *D. dipsaci* (race avoine) et de *D. myceliophagus*. Les fragments d'ADN sont récupérés à partir de clones sélectionnés et utilisés comme sondes vis-à-vis de l'ADN génomique de quatre races de *D. dipsaci* et de trois autres espèces de *Ditylenchus*. Les sondes s'hybrident fortement avec l'ADN de toutes les races de *D. dipsaci*, mais non avec celui obtenu à partir des autres espèces de *Ditylenchus*. Les sondes présentées dans ce travail peuvent donc avoir une utilisation potentielle pour le diagnostic spécifique.

Recently, progress has been made in developing methods for discriminating species and sub-specific forms of plant parasitic nematodes that are not readily distinguished by morphology. Variation within the genus *Globodera* has been examined by several approaches including electrophoresis of both total protein and isoenzymes, by immunochemistry and by pyrolysis gas chromatography (see Fox and Atkinson, 1986 for a review). Additionally, the use of antibodies and genomic DNA has revealed differences (Schotts *et al.*, 1987; Burrows, 1990a), establishing the potential of these approaches. Both genomic and mitochondrial DNA of *Meloidogyne* spp. have been studied (Curran, McClure & Webster, 1986; Powers, Platzer & Hyman, 1986) and lectin probes have revealed variation between species in surface glycoconjugates (Davis *et al.*, 1988).

The genus *Ditylenchus* poses many problems for taxonomists relying solely on morphological characters. Careful observation plus precise biometrics are sometimes necessary to discriminate species and there are several morphologically indistinguishable races of

*D. dipsaci* with dissimilar host ranges. In addition, a form of the latter species from field beans is morphologically quite distinct since adults are larger than adults of other races of *D. dipsaci*. The true status of this giant form is uncertain and its erection to species has been suggested (Sturhan, 1983).

The complex nature of the genus *Ditylenchus* offers considerable scope for examining techniques that are not dependent on morphological characters. This work investigates the potential of probes based on genomic DNA from *D. dipsaci* for discriminating this species from others of the genus *Ditylenchus*.

## Materials and methods

### NEMATODE CULTURE

*D. dipsaci* (giant, lucerne, narcissus, and oat races) were obtained from infected plant-hosts (field beans, lucerne, narcissus and dwarf french beans respectively)

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maintained in cultures at Rothamsted Experimental Station. *D. destructor*, *D. myceliophagus* and *D. trifformis* were cultured at room temperature on *Rhizoctonia cerealis* grown on potato dextrose agar.

#### DNA EXTRACTION

Nematodes (0.5-1.0 g wet weight) were crushed in liquid nitrogen into a fine powder and incubated at 65 °C for 1 h in 0.5 ml of a lysis buffer (0.1 M tris-HCl pH 8.0, 0.05 M ethylene diamine tetraacetic acid (EDTA), 0.2 M NaCl, 1 % sodium dodecyl sulphate (SDS), 2 mg ml<sup>-1</sup> proteinase K). Nucleic acids were extracted three times with 1 vol. phenol/chloroform, and precipitated by 2 vol. ethanol. After centrifugation at 11 000 g for 10 min the pellet was redissolved in 10 mM tris-HCl, 0.1 mM EDTA pH 8.0. RNA was removed by incubation with RNase (50 µg ml<sup>-1</sup> final concentration) and the DNA extracted, precipitated and redissolved as before.

#### CONSTRUCTION OF RECOMBINANT PLASMIDS

Cloning of *D. dipsaci* (oat race) was carried out using standard methods (Maniatis, Fritsch & Sambrook, 1982). DNA was partially digested with Sau3A to give 2-10 kb fragments and pBR322 was linearised using BamHI. Plasmid and nematode DNA were ligated together; *Escherichia coli* cells (strain DH5) were transformed with recombined plasmid and tetracycline sensitive, ampicillin resistant colonies were selected.

#### HYBRIDISATION

Recombinant *E. coli* colonies, containing fragments of *D. dipsaci* (oat race) nematode DNA, were prepared for colony hybridisation as described previously (Mason & Williams, 1985). Briefly, colonies were first grown for two days on duplicate nitrocellulose filters overlaid on top of agar containing ampicillin and nutrients. Filters were then removed from the agar, the colonies lysed and the DNA (including the recombined plasmid DNA) was denatured and bound permanently to the filter.

Two probes from total DNA extracted from *D. dipsaci* (oat race) and *D. myceliophagus* were prepared for use in colony hybridisation as a means of differential screening. The DNA (40 ng) from each of the above mentioned nematode species was digested separately with Sau3A to give 0.5 Kb fragments, and labelled with [<sup>32</sup>P]dCTP by oligolabelling (Feinberg & Volgelstein, 1983).

Duplicate filters prepared (above) for colony hybridisation were prehybridised at 65 °C for 6 h in 100 ml of prehybridisation fluid which contained the following: 6X SSC (SSC = 0.15M sodium chloride, 0.15M sodium citrate), 5X Denhardt's solution (Denhardt's solution = 0.2 % polyvinyl pyrrolidone, 0.2 % ficoll, 0.2 % bovine serum albumen), 0.1 % sodium dodecyl sulphate (SDS),

200 µg denatured salmon sperm DNA, 1 mM ethylene diamine tetra-acetic acid. The filters were then hybridised at 65 °C for 12-24 h using 10 ml hybridisation fluid (prehybridisation fluid containing radiolabelled probe). One of each duplicate pair of filters was hybridised using radiolabelled *D. dipsaci* (oat race) DNA, whilst the other was hybridised using radiolabelled *D. myceliophagus* DNA. After hybridisation, filters were washed with a solution of 2X SSC, 0.1 % SDS for 30 min at room temperature, and for 30 min at 65 °C with fresh solution before autoradiography.

Some colonies were chosen for use as probes against total DNA extracted from a selection of nematode species. DNA from these colonies was recovered from the recombinant plasmids by double digestion with EcoRI and Sall and radiolabelled as before. These new probes were used separately to probe dot blots of 2 µg total DNA from each of the following nematode species: *D. dipsaci* (giant, lucerne, narcissus and oat races), *D. destructor*, *D. myceliophagus* and *D. trifformis*. After hybridisation at 65 °C for 12-24 h the dot blots were rinsed four times in a solution of 2X SSC, 0.1 % SDS, washed in fresh solution for 60 min at 65 °C and finally washed in 0.5X SSC, 0.1 % SDS at 65 °C for 40 min before autoradiography for 2-3 days at -70 °C.

#### Results

All colonies screened with *D. myceliophagus* and *D. dipsaci* (oat race) probes hybridised with both of these probes (Fig. 1). No absolute differences were revealed at the stringency used, but some colonies showed quantitative differences, binding more to *D. dipsaci* (oat race) probe than to *D. myceliophagus* probe (□, Fig. 1) or vice versa (○, Fig. 1). Colonies demonstrating the greatest quantitative differences were selected for use as probes in dot blot hybridisation. Two such colonies yielded three fragments of nematode DNA after double digestion. Electrophoresis established that these fragments I, II and III chosen to probe dot blots were approximately 5 kb, 1.8 kb and 1.2 kb respectively. All three probes hybridised strongly with DNA from all four races of *D. dipsaci* tested but did not hybridise significantly under the same conditions with DNA from the other three *Ditylenchus* species (Fig. 2).

#### Discussion

Cloning of DNA from *D. dipsaci* (oat race) in bacterial plasmids allowed amplification of separate fragments of the genome for differential hybridisation with probes to detect differences. Colony hybridisation was of value demonstrating some qualitative differences. For some colonies a stronger signal resulted from the heterologous (*D. myceliophagus*) probe than from the homologous probe (*D. dipsaci* oat race). This suggested that the insert fragment was more abundant in *D. mycelio-*

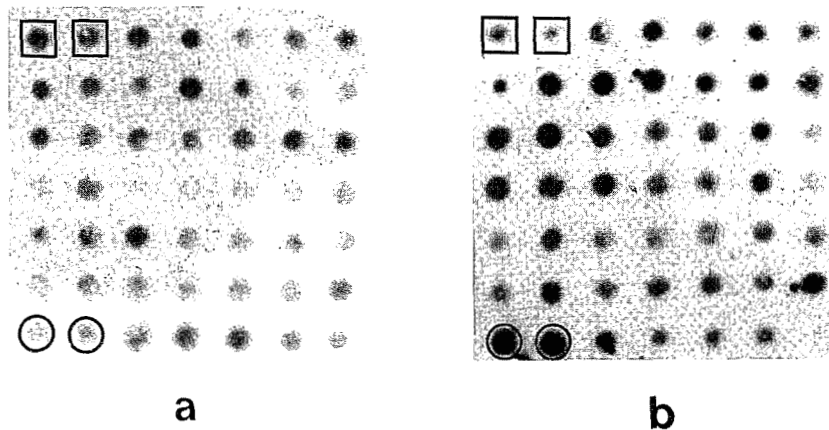


Fig. 1. Colony hybridisation of *Ditylenchus dipsaci* (oat race) clones with radiolabelled DNA from (a) *D. dipsaci* (oat race) and (b) *D. myceliophagus*.

□ indicates examples of colonies showing strong hybridisation with the homologous probe and ○ indicates examples of those showing strong hybridisation with the heterologous probe.

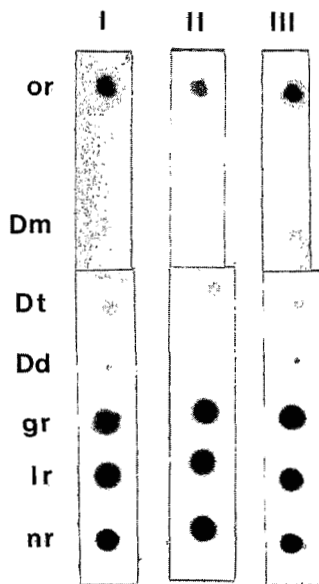


Fig. 2. Dot blots of genomic DNA from four races of *Ditylenchus dipsaci*: oat race (or), giant race (gr), lucerne race (lr) and narcissus race (nr), *D. destructor* (Dd), *D. myceliophagus* (Dm), and *D. trififormis* (Dt) all probed with three cloned fragments (I, II & III) of *D. dipsaci* DNA.

*phagus* than in *D. dipsaci* (oat race) from which it was cloned. Conversely, other colonies gave the strongest signal when probed with homologous *D. dipsaci* (oat race) probe. This method of screening did not reveal colonies that specifically bound only one probe. The low stringency may have produced relatively high background signals because DNA can bind non-specifically

to cell debris and *E. coli* DNA on the filter (Mason & Williams, 1985). Therefore, hybridisation of some unique fragments could be masked by this effect but this limitation did not prevent the approach from providing a simple rapid screen for identifying candidate clones of interest.

Two cloned fragments selected by the initial screen provided three fragments after double digestion that were of value as species-specific probes. All three gave positive hybridisation with each of the four test races of *D. dipsaci* under conditions that resulted in virtually no hybridisation with genomic DNA from the other three species. DNA fragments when isolated from the genome have proved a powerful tool for distinguishing between closely related organisms and gene probes have been developed for a number of parasites: *Plasmodium falciparum* (Franzen *et al.*, 1984), *Echinococcus* (McManus & Simpson, 1985), *Brugia malayi* (McReynolds, Desimone & Williams, 1986), and *Leishmania* (Wirth & Pratt, 1982). Diagnosis using *in situ* hybridisation with biotin labelled probes, which requires only a few or single individuals, has made molecular identification of parasites possible in the field (Barker & Gibson, 1988).

Detection of species-specific DNA fragments for economic plant parasitic nematodes such as *Globodera* (Burrows & Perry, 1988), *Meloidogyne* (Curran, McClure & Webster, 1986; Powers, Platzer & Hyman, 1986) and now *Ditylenchus*, marks a significant step towards the production of diagnostic kits especially if biotinylated probes, rather than those incorporating radioactivity are used. A biotin labelled DNA probe differentiated *G. pallida* from *G. rostochiensis* and was sensitive enough to detect a single second stage juvenile of *G. pallida* (Burrows, 1990b). Development of a biotinylated probe would be useful, both for advisory

work and for plant health regulations, for discrimination of *D. dipsaci* from other members of the genus. More work is required with field populations to establish that such probes are invariably reliable species-specific indicators and, more importantly, the benefit of such an approach would be enhanced considerably if race discrimination within *D. dipsaci* could be achieved.

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