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USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) FOR GENERATING SPECIFIC DNA PROBES FOR OXYUROID SPECIES (NEMATODA)

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Summary :

Random amplified DNA markers (RAPD; Williams *et al.*, 1990) were used to obtained specific RAPD fragments characterising different species of oxyuroids. We tested six species of worms parasitizing vertebrates or invertebrates: *Passalurus ambiguus* Rudolphi, 1819, parasite of Leporids; *Syphacia obvelata* (Rudolphi, 1802) Seurat, 1916, a parasite of rodents; *Blatticola blattae* (Graeffe, 1860) Chitwood, 1932 parasite of the cockroach *Blattella germanica*; *Hammerschmidtiella diesingi* (Hammerschmidt, 1838) Chitwood, 1932 and *Thelastoma bulhoesi* (Magalhaes, 1990) Travassos, 1929, parasites of the cockroach *Periplaneta americana*, and an undescribed parasite species of a passalid insect from New Caledonia. Among 15 oligonucleotides tested, nine produced several specific bands allowing the interspecific discrimination.

KEY WORDS : RAPD-PCR, Oxyuroidea, Nematoda, taxonomic identification.

Résumé : UTILISATION DES RAPD (RANDOM AMPLIFIED POLYMORPHIC DNA) POUR GÉNÉRER DES MARQUEURS SPÉCIFIQUES D'ESPECES D'OXYURES (NEMATODA)

La technique du RAPD (Random Amplified Polymorphic DNA; William et al., *1990) a été utilisée pour caractériser différentes espèces d'oxyures. Nous avons testé six espèces d'oxyures de vertébrés ou d'invertébrés :* Passalurus ambiguus *Rudolphi, 1819, un parasite de léporidés;* Syphacia obvelata *(Rudolphi, 1802) Seurat, 1916, un parasite de rongeurs;* Blatticola blattae *(Graeffe, 1860) Chitwood, 1932, parasite de la blatte* Blattella germanica *(L.);* Hammerschmidtiella diesingi *(Hammerschmidt, 1838) Chitwood, 1932, et* Thelastoma bulhoesi *(Magalhaes, 1990) Travassos, 1929, parasites de la blatte* Periplaneta americana, *et une espèce non décrite d'un passalide de Nouvelle-Calédonie. Sur les 15 amorces testées, neuf ont produit plusieurs bandes spécifiques permettant la différenciation interspécifique.*

MOTS CLÉS : *RAPD-PCR, Oxyuroidea, Nematoda, identification taxonomique.*

INTRODUCTION

Oxyurid nematodes are monoxenous parasites of vertebrates and invertebrates (Adamson, 1989; Morand *et al.*, 1996). Their taxonomy and phylogenetic relationships still remain confused, particularly for invertebrate parasites (Adamson, 1989; Adamson & Van Waerebeke, 1992), and we suspect numerous cases of synonymy. These parasites have a haplo-diploid mode of reproduction and show a female-biased sex-ratio (Adamson, 1989). Males are often very small and rare. Moreover, in the case of congeneric species infecting the same host species, such as Dermoptera oxyuroids (Hugot, 1986), tortoises oxyuroids (Petter, 1966) or some invertebrate oxyuroids (Adamson & Noble, 1992), it is difficult to assign a female to its species. More difficulties arise when taxonomic identification is based on individuals of one sex only. The use of molecular technics could hence be helpful to resolve such taxonomic problems (Bandi *et al.*, 1993; Chacon *et al.*, 1994; Andrews *et al.*, 1995; Humbert & Cabaret, 1995). Our aim was to test the use of RAPD markers method for resolving taxonomix problems by applying this method to six oxyuroid species: two species from vertebrate hosts and four from invertebrates.

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MATERIAL AND METHODS

PARASITE RECOVERY

Passalurus ambiguus (11 females and 10 males) was obtained from *Oryctolagus cuniculus* (Laboratory MNHN), *Syphacia obvelata* (50 females and one male) from *Mus domesticus* (Laboratory MNHN), *Hammerschmidtiella diesingi* (25 females and nine males) and *Thelastoma bulhoesi* (43 females and two males) from the cockroach *Periplaneta americana* (originated from one population in Paris), *Blatticola blattae* (93 females and 13 males) from the cockroach *Blattella germanica* (originated

from two populations in Rennes and one population in Paris) and an undescribed species (five females) from a New-Caledonian Passalid.

Parasites were collected from the caecum of each host species, carefully washed in physiological saline and in tampon TE [10 mM TrisHCL pH 8, 1 mM EDTA] before being stored and conserved at – 80 °C.

For each parasite sample, ceacum fluid were recovered and stored as mentioned above.

Males and females nematode were identified with confidence to their morphological characters, and according to Basir (1956) and Adamson & van Waerebeke (1992).

DNA extraction

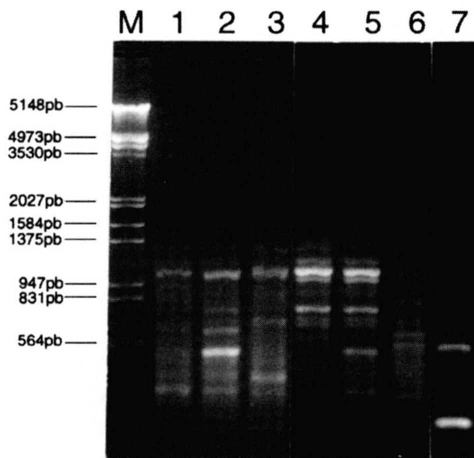
We used a modified protocol from Barral *et al.* (1996). DNA from caecum sample fluid was extracted following the same procedure.

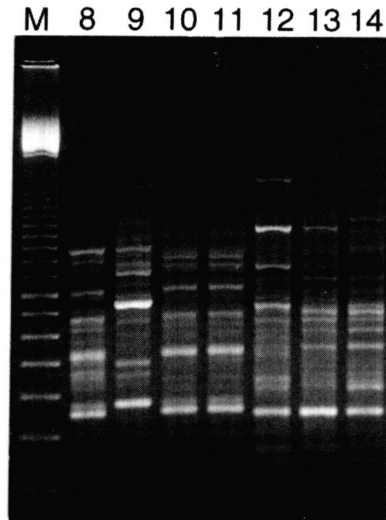
PCR amplifications

Fifteen oligonucleotides were used for the amplification of random DNA markers. Primer sequences were as follows:
RP2 5'-AAGGATCAGA-3'; VG1 5'-ACGTATCTGC-3'; MNH1 5'-ACGTCTATGC-3'; R108 5'-GTATTGCCCT-3'; RP4-2 5'-CACATGCTTC-3'; R28 5'-ATGGATCCGG-3';

Primers	RP2	MNH1	R28	OPAXS	OPB04	OPGB	SB2	OPB11	OPA9
Concentration in MgCl2	2,5	3,5	3,5	1,5	3,5	2,5	1,5	2,5	2,5
Species									
P. ambiguus	1 050 620	875 800 600 575 390	1 050	1 400 500	400 320 150	1 250 700 450			
S. obvelata	1 100	1 050 900 550 450 440	1 150 1 090 1 000 850 800 750 700	950 650 400 350	1 550 1 400 1 320 1 260 925 625 570 350	850 800 550 500		790 500 450	900 820 800 680 600 560 520
H. diesingi	1 700 850 780 680 450	1 200 950 850 675 625 480 300	1 090 950 900 750 500 450 375	1 250 1 100 800 575 500 150	1 250 600 500 270	1 700 1 550 1 120 1 050 600 575 525	1 900 800 675 450 440		
T. bulhoesi	1 750 1 600 1 350 920 770 490 475	1 950 1 250 875 775 650 450 375	1 250 1 050 800 660 525 375 350		1 600 1 300 1 200 900	825 750 700 480 425 325	1 700 1 500 1 100 1 000 850 710 450 300		
B. blattae	1 100 790 580	1 400 925 725 450 275	700 550 360		1 250 700 450 350	1450 700 225	1 150 910 890 750 290	700 590 510 475 400	950 650 560 500
Undescribed species of a passalid insect	1 300 600 350	1 600 1 150 700	850 590 520 250	1 000 600	1 250 1 150	900 775 700 575 500 475			

Table I. — Specific bands generated by nine primers for each species of oxyuroids (optimal concentration in MgCl2, from 1.5 to 3.5 mM, was determined for each primer).





Figs 1-2. — Interspecific differences among 6 oxyurid species revealed with the R28 primer.

Fig. 1. — Column M: DNA-*Eco* RIplus*Hin* DIII marker with a λ molecular weight; column 1: *P. ambiguus* (5 gravid females); column 2: *P. ambiguus* (10 males); column 3: DNA gut fluid of *P. ambiguus* host; column 4: *S. obvelata* (4 gravid females); column 5: *S. obvelata* (4 non-gravid females); column 6: DNA gut fluid of *S. obvelata* host; column 7: Passalidae oxyurids (5 gravid females).

Fig. 2. — Culumn M: marker with a 100PairBaseLadder molecular weight; column 8: *T. bulboesi* (7 gravid females); column 9: *H. diesingi* (10 gravid females); columns 10 and 11: *T. bulboesi* (10 and 5 gravid females respectively); columns 12, 13 and 14: *B. blattae* (19, 21 and 2 gravid females respectively).

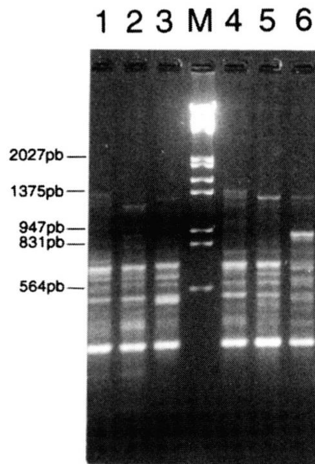


Fig. 3. — Intraspecific differences among 6 *B. blattae* (6 females) with R28 primer.
Column M: DNA-*Eco* RIplus*Hin* DIII marker with a λ molecular weight; columns 1 to 6: 6 different females of *B. blattae.*

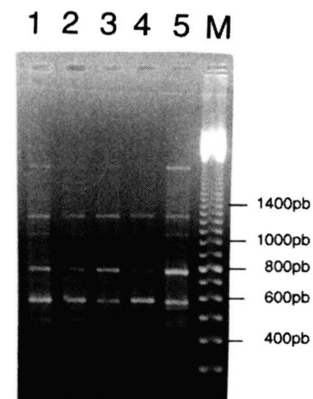


Fig. 4. — Intraspecific differences among 5 *B. blattae* (5 males) with RP2 primer.
Column M: marker with a 100PairBaseLadder molecular weight; columns 1 to 5: 5 different males of *B. blattae.*

OPAXS 5'-AGTGCACACC-3'; OPB04 5'-GGACTGGAGT-3'; OPB11 5'-GTAGACCCGT-3'; SB1 5'-AGGTCCCTGC-3'; SB2 5'-TGCACCCTGC-3'; OPGB 5'-GAGCCCTCCA-3'; OPA9 5'-GGGTAACGCC-3'; A2 5'-TGGTCGCGGC-3'; NS33 5'-GCCAGCAGCC-3'.

The PCR reaction was carried out in a volume of 25 µl containing 10 mM Tris HCl pH 8.3, 50 mm KCl 1U of *Taq* DNA polymerase (Boehringer Mannheim GmbH, Germany), 200 µM of each dNTP (dATP, dCTP, dGTP, dTTP), 50 pmole primer and a final concentration of MgCl2 depending of the primer used. From 25 to 50 ng total nematode DNA was used as a template for the PCR.

The PCR cycle was carried for 30 seconds at 94 °C, for 30 seconds at 36 °C and for 75 seconds at 72 °C for a total of 44 cycles followed by an extension polymerisation reaction of five minutes at 72 °C. Amplification was performed in a Perkin Elmer (9600) thermo cycler.

The amplified DNA fragments resulting from PCR were analysed directly on 1.5 % agarose gels by ethidium bromide staining (0.5 µg/ml). The gels were run in TBE

1X (BioProbe) at a constant voltage (120 v) and photographed. The molecular sizes of the fragments were determined using the 100 bp DNA Ladder (Pharmacia Biotech, USA) or the λDNA-*Eco*RIplus*Hin*dIII (Boehringer, Mannheim) as references.

Amplifications were performed twice to asses reproducibility and blanks were done without DNA template. To detect potential contamination of nematode DNA we performed RAPD experiments DNA sample extracted from host digestive fluid.

RESULTS

The choice of primers were assessed in regard to the number of generated bands, the quality of the profiles and their reproducibility. The optimal concentration in MgCl2 was determined for each primer (Table I).

DNA patterns of host gut fluid control differed completely from nematode DNA patterns except in the case of *P. ambiguus* for which a number of fragments were similar in both control and DNA template.

Interspecific differentiation was easily assessed. Nine of 15 oligonucleotides assayed revealed unambiguous profiles (Table I). Each of these primers generated patterns that were specific of each nematode species. An example is given for primer R28 (Figs 1, 2). Similar results were obtained either by using individual or pooled nematodes.

Intraspecific variability was low and concerned non specific bands as showed in Figures 3 and 4. Patterns obtained by RAPD method for *B. blattae* from different origins were similar.

DISCUSSION

The RAPD method allows to distinguish the six studied species of oxyurids. For example, its possible to clearly distinguish the two oxyuroid species, *H. diesingi* and *T. bulhoesi*, which occur in the same host, the cockroach *P. americana*.

Patterns obtained for males and females were identical. Therefore, this method is appropriate for resolving problems of taxonomic identification either in the case of synonymy or when only members of one sex (generaly females) were collected from their hosts (Adamson & Noble, 1992).

We recorded a similarity in patterns generated by RAPD between *P. ambiguus* and its host environment (control). We hypothesize that these results arose because of the particular behaviour of the females of this species, which release their eggs into the internal environment. Hence, there is some chance that we may have also amplified also parasite egg DNA with host gut fluid.

Finally, the RAPD method revealed genetic variability among the six species of worms studied. This preliminary methodological study should enable further investigations of inter and intraspecific genetic variability.

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