A DNA extraction method with SDS from single nematodes for direct application to PCR amplification

Article *in* Japanese Journal of Nematology · January 2010

DOI: 10.3725/jjn.40.13

1 author:

Hiromichi Sakai
Institute for Plant Protection, NARO, Japan
19 PUBLICATIONS **52** CITATIONS

[SHORT COMMUNICATION]

A DNA extraction method with SDS from single nematodes for direct application to PCR amplification

Hiromichi Sakai [1,2,*]

Various methods for extracting DNA from single nematodes for PCR amplification have been developed by many authors. Such techniques generally do not include a purification step, and direct application of crude lysate to PCR amplification is usual. SDS is not used in DNA extraction methods for PCR amplification from single nematodes, although it is a common detergent used in DNA extraction. This is because SDS has an inhibitory effect on PCR (Gelfand, 1989). The author developed an SDS-based DNA extraction method from single nematodes, where the crude lysate can be applied directly to PCR amplification, and reports it here.

MATERIALS AND METHODS

DNA extraction from single nematodes:

Second-stage juveniles of *Meloidogyne incognita* (Kofoid and White) were used here. A single nematode was placed into a drop of sterile distilled water on a glass slide. After the water dried, the nematode was crushed with a small sterile filter paper chip under the stereo microscope using forceps (Iwahori *et al*., 2000). The paper chip was dropped into a 0.5 ml plastic tube containing 4 µl of 0.1% SDS lysis buffer (10 mM Tris-HCl (pH8.0), 5 mM EDTA (pH8.0), 500 µg/ml Proteinase K, and 0.1% SDS [the contents except for SDS were prepared as a 2 × stock premixed solution and frozen at −20°C until used; SDS was added just before use at room temperature]), and then the tube was incubated under the following conditions: 50°C for 2 hr, followed by 95°C for 10 min. At the end of the incubation time, 196 µl of sterile distilled water (stored at room temperature) was added to the tube, to yield 200 µl of 50-fold diluted lysate. DNA lysates were individually prepared from 10 single juveniles and used in the following PCR amplifications. Two female nematodes each of *Xiphinema* sp. and *Pratylenchus vulnus* Allen and Jensen were also subjected to the above DNA extraction procedure.

PCR amplification:

Demonstrative PCR amplifications of mitochondrial DNA were conducted for *M. incognita* with the set of primers "C2F3" and "1108" described by Powers and Harris (1993). High fidelity PCR enzyme was used after Jeyaprakash *et al.* (2006). The PCR reaction mixture consisted of 0.2 mM dNTPs, 0.3 µM of each primer, 0.5 U PrimeSTAR HS DNA Polymerase with PrimeSTAR Buffer (Mg^{2+} plus) (Takara Bio Inc., Seta, Shiga, Japan), and 5 µl of DNA lysate as PCR template, in a total volume of 20 µl. PCR amplifications were conducted with Program Temp Control System PC-818 A (Astec Co., Ltd. Kasuya, Fukuoka, Japan), under reaction conditions modified from Powers *et al.* (2005) as follows: a single step of pre-denaturation at 94°C for 2 min, followed by 25 cycles of denaturation at 94°C for 30 sec, annealing at 48°C for 30 sec, 0.5°C/sec ramp up to extension temperature, and extension at 72°C for 90 sec.

PCR amplifications of the rDNA ITS region were also conducted for *Xiphinema* sp. and *P. vulnus*. The sets of primers described by Vrain *et al.* (1992) and Ferris *et al.* (1993) were used for *Xiphinema* sp. and *P. vulnus*, respectively. The PCR reaction mixture consisted of 0.2 mM dNTPs, 0.4 µM of each primer, 1.5 U *TaKaRa Ex Taq* Hot Start Version with *Ex Taq* Buffer (Mg^{2+} plus) (Takara Bio), and 20 µl of DNA lysate as PCR template, in a total volume of 50 µl. PCR amplifications were conducted with Program Temp Control System PC-818 A (Astec), under reaction conditions modified from Uehara *et al.* (2005) as follows: a single step of pre-denaturation at 94°C for 2 min , followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min. Electrophoresis was carried out on 1.5% agarose gels (Type II-A, Sigma) and 0.5 × TBE at 100 V with Mupid-exU (ADVANCE Co., Ltd., Tokyo, Japan), by applying 5 µl of each PCR product. The gels were stained with ethidium bromide and visualised with UV illumination.

RESULTS AND DISCUSSION

The single target fragments of *ca.* 1.6 kb for *M. incognita* were successfully obtained for all 10 specimens (Fig. 1). It was also possible to amplify the rDNA region for *Xiphinema* sp. and *P. vulnus* from the lysates prepared by this method (Fig. 2). The DNA extraction method developed here is easy to use; the composition of the lysis buffer is simple and no special kit is required for successful PCR amplifications. The procedure is labor-saving and sufficiently effective so that no freezing step is required to facili-

[1] Yokohama Plant Protection Station, Yokohama, Kanagawa 231-0801, Japan.
[2] Present address: National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan.
[*] E-mail: sakaihm@affrc.go.jp

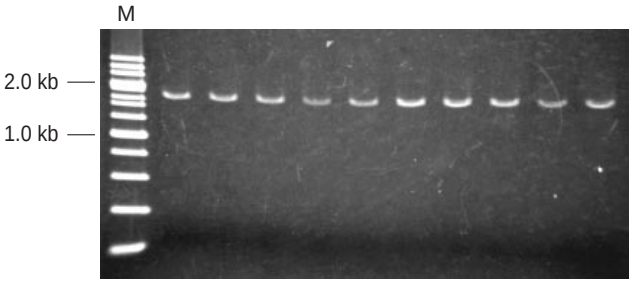

Fig. 1. PCR products of mitochondrial DNA from 10 single *M. incognita* juveniles obtained by the DNA extraction method with 0.1% SDS lysis buffer. M: 200 bp DNA ladder (Takara Bio).

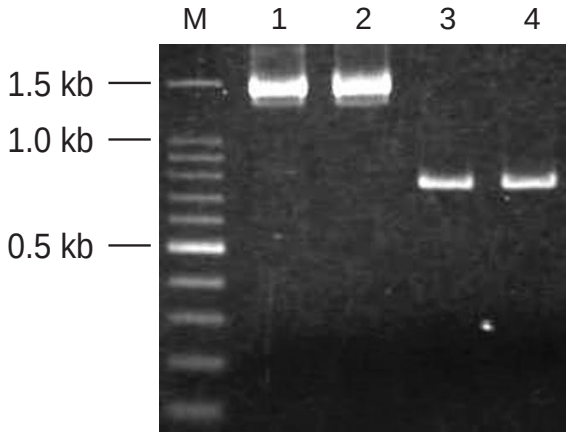

Fig. 2. PCR products of the rDNA ITS region from single female nematodes obtained by the DNA extraction method with 0.1% SDS lysis buffer. 1-2: *Xiphinema* sp.; 3-4: *Pratylenchus vulnus*; M: 100 bp DNA ladder (Takara Bio).

tate cell destruction. Furthermore, the SDS preparation is simplified when a manufactured solution (10-20%) is used. In the demonstration assays, successful amplifications were obtained from crude DNA lysates including SDS, even though SDS has been reported to inhibit the PCR reaction (Gelfand, 1989). In the present method, however, the SDS concentration of the lysate was 0.002% after the dilution step, and that of the PCR reaction mixture was less than 0.001%, at which concentration SDS has no inhibitory effect on PCR (Gelfand, 1989). The DNA extraction method described here can be used sufficiently for diagnostic purposes because mitochondrial DNA and rDNA are genetic regions prevailingly used for discriminating species of nematodes.

ACKNOWLEDGEMENT

The author thanks Laura Cortada for her helpful comments on the manuscript.

LITERATURE CITED

Ferris, V. R., Ferris, J. M. and Faghihi, J. (1993) Variation in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. Fundamental and Applied Nematology 16, 177-184.

Gelfand, D. H. (1989) Taq DNA Polymerase. In: PCR Technology Principles and Applications for DNA Amplification. (Erlich, H. A., ed.), Stockton Press, New York, 17-22.

Iwahori, H., Kanzaki, N. and Futai, K. (2000) A simple, polymerase chain reaction–restriction fragment length polymorphism-aided diagnosis method for pine wilt disease. Forest Pathology 30, 157-164.

Jeyaprakash, A., Tigano, M. S., Brito, J., Carneiro, R. M. D. G. and Dickson, D. W. (2006) Differentiation of *Meloidogyne floridensis* from *M. arenaria* using high-fidelity PCR amplified mitochondrial AT-rich sequences. Nematropica 36, 1-12.

Powers, T. O. and 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., Mullin, P. G., Harris, T. S., Sutton, L. A. and Higgins, R. S. (2005) Incorporating molecular identification of *Meloidogyne* spp. into a large-scale regional nematode survey. Journal of Nematology 37, 226-235.

Uehara, T., Kushida, A., Itou, K., Narabu, T. and Momota, Y. (2005) Discrimination of three cyst-forming nematodes of the genus *Globodera* (Nematode: Heteroderidae) from Japan based on PCR-RFLP of ribosomal DNA. Applied Entomology and Zoology 40, 537–543.

Vrain, T. C., Wakarchuk, D. A., Lévesque, A. C. and Hamilton, R. I. (1992) Intraspecific rDNA Restriction Fragment Length Polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15, 563-573.

Received October 31, 2009