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Short Communication

First successful amplification of 18S ribosomal DNA of *Cordyceps* spp. by the PCR method

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The 18S ribosomal DNAs of *Cordyceps* spp. were amplified for the first time by the PCR method. New primers were designed based on the sequence of the 18S ribosomal DNA of *Sclerotinia sclerotiorum.*

Key Words——*Cordyceps* spp.; 18S ribosomal DNA; new PCR primer.

Cordyceps spp., which are used in Chinese traditional medicines, are parasitic fungi belonging to the Pyrenomycetes in Ascomycetes. They infect the larva or imago of insects, kill them, and then form a fruit body on the insect. Because the host selectivity is strict, a *Cordyceps* sp. that infects a cicada does not infect other insects. *Cordyceps* spp. comprise about 400 species (Shimizu, 1994). They are classified morphologically by color, shape, and host insect, but their phylogeny has not been established. Among bacteria and eucaryotes, the comparison of rRNA (rDNA) sequences is the most useful method for deducing phylogenetic relationships (Woese, 1987). In analyzing a DNA sequence, it is necessary to amplify the sequence, and for this the polymerase chain reaction (PCR) is the most useful method because it can amplify a particular DNA sequence region by use of a pair of primers (Medlin et al., 1988; Boettger, 1989; Edwards et al., 1989). Consequently, a pair of primers is essential for amplification. In this study, based on the 18S ribosomal DNA sequence of *Sclerotinia sclerotiorum* (Lib.) de Bary (which belongs to Ascomycetes, the division containing *Cordyceps* spp.), we designed new primers for *Cordyceps* spp. By using these primers we achieved the amplification of 18S rDNA of *Cordyceps* spp. for the first time.

Isaria japonica Yasuda, *Hymenostilbe odonatae* Y. Kobayasi, and *Cordyceps tuberculata* (Leb.) Maire f. *moelleri* (Henn.) Y. Kobayasi were picked up at Ibaraki Prefecture in Japan. *Hymenostilbe odonatae* is an anamorph of *Cordyceps odonatae* Y. Kobayasi. *Isaria japonica*, an anamorph of *Cordyceps takaomontana* Yakushiji et Kumazawa, was grown (25°C, 14 d) on Sabouraud-dextrose agar medium (Difco).

DNAs were isolated from 100 mg of each fruit body of these fungi by using an ISOPLANT DNA extraction kit (Nippon Gene Co., Tokyo, Japan) (Jhingan, 1992). In the case of the strain cultured from the spores of natural *I. japonica*, the mycelium was lyophilized and then used for DNA isolation in the same way. The isolated DNA was suspended in 20 μl of TE buffer (10 mM Tris hydrochloride, 1 mM EDTA, pH 8.0) and used.

The sequences of *Saccharomyces cerevisiae* Meyen ex Hansen are generally utilized to design primers for amplification (Gutell, 1993). But the 18S rDNA of *Cordyceps* spp. could not be amplified or could only be amplified with low efficiency by the use of such primers, suggesting that, the nucleotide sequences of *Cordyceps* spp. are different from that of *S. cerevisiae.* Therefore, we designed a new set of primers by using the sequence of 18S rDNA of *S. sclerotiorum,* because we presumed that it would be similar to the sequences of *Cordyceps* spp., both of them belonging to the same Euascomycetes division. The primers were as follows: 5′-CGACTTCGGAAGGGGTGTATTTAAT (base positions 183-207) and 5′-TAATGATCCTTCCGCAGGTT (bases 2088-2107). The nucleotide numbers are equivalent to those in the primary structure of *S. sclerotiorum* MUCL 11553 (Wilmotte et al., 1993).

Isaria japonica, cultured *I. japonica*, *H. odonatae*, and *C. tuberculata* f. *moelleri* were used for amplification of 18S rDNA (Fig. 1). All strains could be amplified by use of the primers. Approximately <0.2 μg of DNA was amplified in 25 μml of reaction mixture. The conditions for PCR amplification consisted of an initial denaturation step of 94°C for 5 min followed by 30 cycles of 94°C for 0.5 min, 55°C for 0.5 min, and 72°C for 1.5 min, and a final extension step of 72°C for 10 min. These amplifications were performed in a Perkin-Elmer temperature controller. To estimate molecular sizes of the amplified DNA products, they were separated by 1% agarose gel electrophoresis in TAE (Tris-acetate/EDTA buffer) and detected with ethidium bromide stainning. The PCR products of both *I. japonica* strains were about 1.9 kb in molecular size (lanes 1 and 2), whereas those of

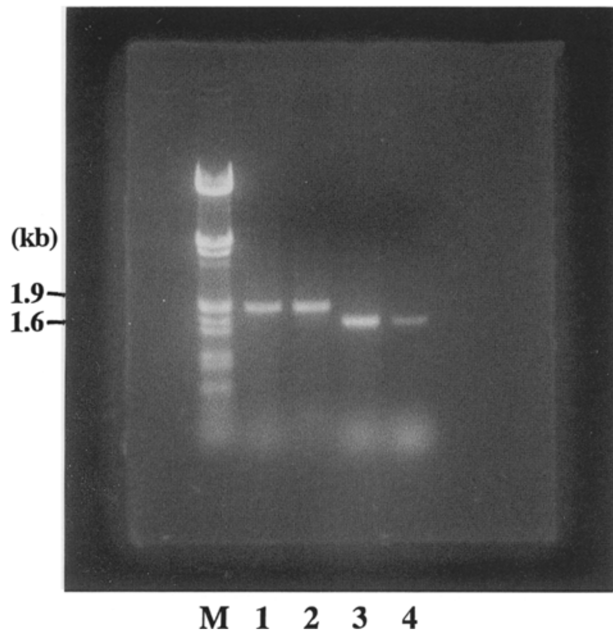

Fig. 1. Ethidium bromide-stained 1% agarose gel displaying amplification products. Lane 1, cultivated strain from the spores of *Isaria japonica*; 2, *Isaria japonica*; 3, *Hymenostilbe odonatae*; 4, *Cordyceps tuberculata* f. *moelleri*; M, *Hind*III and *Eco*RI digests of lambda DNA as molecular size markers.

H. odonatae and *C. tuberculata* f. *moelleri* were about 1.6 kb (lanes 3 and 4).

Many ascomycetes, including *Ascosphaera apis* (Maassen ex Claussen) Olive et Spiltoir, *Neurospora crassa* Shear et Dodge, and *Taphrina deformans* (Berk.) Tul. have a large deletion at positions 1150–1450 of their 18S rDNA, which correspond to the sequence numbers of *S. sclerotiorum* (Wilmotte et al., 1993). We found amplified DNA is of the same size (about 0.5 kb) when we attempted to amplify the region downstream of the large deletion region in 18S rDNAs of the four *Cordyceps* species (data not shown). Therefore, we presume that the *I. japonica* strains have an insertion like *S. sclerotiorum* and that *H. odonatae* and *C. tuberculata* f. *moelleri* have a large deletion like other Ascomycetes.

Having proved applicable to the amplification of 18S rDNA of *Cordyceps* spp., these newly designed primers may offer a useful tool for determining the genetic classification of *Cordyceps* spp.

Literature cited

Boettger, E. C. 1989. Rapid determination of bacterial ribosomal RNA sequences by direct sequencing of enzymatically amplified DNA. FEMS Microbiol. Lett. **65**: 171–176.

Edwards, U., Rogall,T., Bloecker, H., Emde, M. and Boettger, E. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. **17**: 7843–7853.

Gutell, R. R. 1993. Collection of small subunit (16S- and 16S-like) ribosomal RNA structures. Nucleic Acids Res. **21**: 3051–3054.

Jhingan, A. K. 1992. A novel technology for DNA isolation. Methods Mol. Cell. Biol. **3**: 15–22.

Medlin, L., Elwood, H. J., Stickel, S., and Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like r RNA coding regions. Gene **71**: 491–499.

Shimizu, D. 1994. Color iconography of vegetable wasps and plant worms, pp. 137–163. Seibundo Shinkosha, Tokyo. (In Japanese.)

Wilmotte, A., Van de Peer, Y., Goris, A., Chapelle, S., De Baere, R., Nelissen, B., Neefs, J. -M., Hennebert, G. L. and De Wachter, R. 1993. Evolutionary relationships among higher fungi inferred from small ribosomal subunit RNA sequence analysis. System. Appl. Microbiol. **16**: 436–444.

Woese, C. R. 1987. Bacterial evolution. Microbiol. Rev. **51**: 221–271.