Original

PCR primers for the amplification of the nuclear small subunit ribosomal DNA sequences from polycystine radiolarians

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SUMMARY

Polycystinea (Radiolaria) is a class of planktonic protists widely distributed in tropical, subtropical, and even polar marine environments. Various types of algae occur as intracellular symbionts in the cells of the polycystines (e.g., Anderson, 1976) which causes significant cross-contamination in polycystine DNA extractions, complicating DNA-based molecular analyses. We designed potential primers; Spu 191F and Spu 1731R, specifically for spumellarian polycystines, which are solitary and have spongy or latticed siliceous shells. These primers amplified 18S rDNA sequences directly from the specimens containing symbiotic algae. They will be used to accelerate phylogenetic analyses of polycystine radiolarians, which have not been established in culture and possess symbiotic algae.

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INTRODUCTION

Polycystinea (Radiolaria) is a class of planktonic protists widely distributed in tropical, subtropical, and even polar marine environments. (Currently, many researchers use “Radiolaria” as a conventional name, which comprehensively indicates the Acantharea, the Polycystinea, and the Phaeodarea (Table 1)). The first determination of small-subunit ribosomal DNA (18S rDNA) sequences in the Polycystinea was reported by Amaral Zettler et al. (1997). As templates for polymerase chain reaction (PCR) amplification, they used the genomes of the swarmer cells of the colonial and skeletonless spumellarians that endogenously formed in individual vegetative cells. On the other hand, a molecular technique for the solitary spumellarians with siliceous skeletons and shells has not yet been established due to the difficulty of culturing them.

Various types of algae occur as intracellular symbionts in the cells of the polycystines; dinoflagellates, prasinophytes, and prymnesiophytes (e.g., Anderson, 1976). In all polycystines, algal symbionts are generally found in the rhizopodial network of the ectocytoplasm (Fig. 1)

Table 1 The present system of "Radiolaria" reviewed by Levine et al. (1980)

Kingdom PROTISTA Haeckel, 1866	
Subkingdom PROTOZOA Goldfuss, 1818: emend. Owen, 1858	
Phylum SARCOMASTIGOPHORA Honigberg and Balamuth, 1963	
Subphylum SARCODINA Schmarda, 1871	
Superclass ACTINOPODA Calkins, 1909	
Class HELIOZOA Haeckel, 1866	
Class ACANTHAREA Haeckel, 1881	"Radiolaria"
Class PHAEODAREA Haeckel, 1879	
Class POLYCYSTINEA Ehrenberg, 1838	
Order Spumellarida Ehrenberg, 1875	
Order Nassellarida Ehrenberg, 1875	

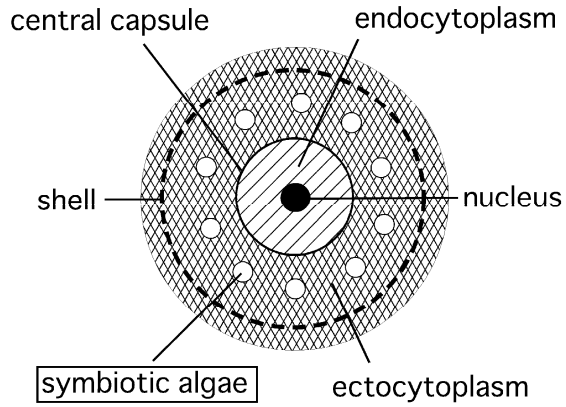

Fig. 1. Schematic representation of the host-symbiont configuration. Polycystine cell is largely divided into two cytoplasmic bodies: endocytoplasm and ectocytoplasm, by porous central capsular wall. The central capsule contains major cytoplasmic organelles: nucleus, mitochondria, and other membranous organelles, exclusive of digestive vacuoles (Anderson, 1983). Symbiotic algae are generally found in the ectocytoplasm.

(Anderson, 1976; Takahashi et al., 2003) and rarely have been observed within the central capsule (Anderson and Matsuoka, 1992). These cause significant cross-contamination in polycystine DNA extractions, complicating DNA-based molecular analyses. PCR amplification using polycystine-specific primers thus may overcome these limitations. Amaral Zettler et al. (1997) used a combination of spumellarian-specific primers in order to obtain the 18S rDNA sequences; however, their primers are specific to the colonial spumellarians. In the present study, we designed primers specifically for the spumellarians, which are solitary and have spongy or latticed siliceous shells.

MATERIALS AND METHODS

Polycystine samples were collected from northwest of Okinawa Island, Japan. The polycystines included in the present study were *Dictyocoryne truncatum* (Ehrenberg), *Dictyocoryne profunda* Ehrenberg, and *Spongaster tetras* Ehrenberg (Family Spongodiscidae), whose sequences were already reported by Takahashi et al. (2004), and *Didymocyrtis tetrathalamus* (Haeckel) (Family Coccodiscidae). They all are classified into Order Spumellarida and they are solitary and have spongy or latticed siliceous shells, about 200 μm in diameter.

Single cells of each specimen were rinsed

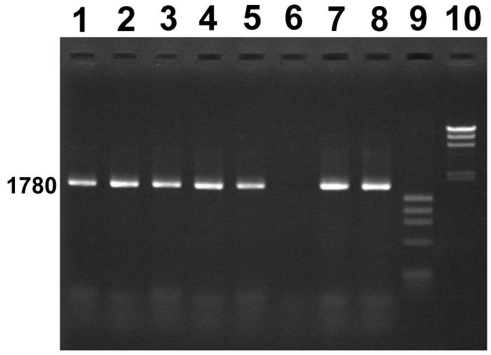

Fig. 2. Agarose gel electrophoresis of PCR products from both a central capsule and ectocytoplasm of each spumellarian species, amplified with an eukaryote-specific primers 90F/B. Templates of lanes 1, 2, 3, and 4 are from the central capsules of *Dictyocoryne truncatum*, *D. profunda, Spongaster tetras,* and *Didymocyrtis tetrathalamus*, respectively. Templates of lanes 5, 6, 7, and 8 are from the ectocytoplasm of *D. truncatum*, *D. profunda, S. tetras,* and *D. tetrathalamus*, respectively. The all amplified fragments are 18S rDNAs. Molecular weight standards: ØX174RF *Hae* III digest in Lane 9. λ-*Hin*d III digest in Lane 10.

twice in filtered seawater and the central capsule was physically separated by a sterilized razor blade from the ectocytoplasm, which contained endosymbiotic algae, as mentioned above. Each central capsule and the ectocytoplasm was rinsed twice again in distilled water, and then incubated for 30 minutes at 37°C in 0.2 μg/μl Proteinase K solution. This sample was used as a template for the amplification of 18S rRNA coding regions. Polymerase chain reaction (PCR) was accomplished using an eukaryotic-specific forward primer 90F (Hendriks et al., 1989): 5'-GAAACTGCGAATGGCTCATT-3' and a reverse primer B (Medlin et al., 1988): 5'-CCTTCTGCAGGTTCACCTAC-3'. The PCR amplification was performed using an initial denaturation step of 95 °C for 3 minutes, followed by 35 amplification cycles each consisting of 95 °C for 1 minute, 55 °C for 2 minutes, and 72 °C for 3 minutes. Each PCR product was purified by the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) and then cloned in the pGEM-T Easy Vector System (Promega) using *E. coli* JM109 Competent Cells (Promega). Five clones for each of the amplified fragments from the inserts were sequenced using flanking vector primers. The DDBJ accession numbers for the 18S rDNA sequence data of *D. truncatum, D. profunda*, *S. tetras*, and *D. tetrathalamus* are AB101540- AB101542, and AB193605, respectively.

RESULTS AND DISCUSSION

The sequences of PCR products from ectocytoplasm of *Dictyocoryne truncatum* (Fig. 2, lane 5) were branched in a clade of Class Haptophyta, and those from *Spongaster tetras* and *Didymocyrtis tetrathalamus* (Fig. 2, lanes 7, 8) were branched in a clade of Class Chlorophyta (data not shown). These sequences of the amplified fragments probably originate from the symbionts in each spumellarian ectocytoplasm. On the other hand, *Dictyocoryne profunda* does not possess symbiotic algae (Takahashi et al., 2003); therefore, the PCR products from ectocytoplasm of *D. profunda* were not obtained (Fig. 2, lane 6). Finally, the sequences of the amplified fragments were aligned with the corresponding region from the host spumellarians by Clustal W ver. 1.81 (Thompson et al., 1994) (Fig. 3). Then, potential primer sequences were designated Spu 191F: 5'-GCGACTYACGAAGCCCTGTA-3' and Spu 1731R: 5'-ACTTCGRGCCTCCCGTT-3'. The primers Spu 190F and Spu 1731R, when paired with each other, using an initial denaturation step of 95 °C for 3 minutes, followed by 35 cycles of 95 °C for 1 minute, 57°C for 2 minutes, 72 °C for 3 minutes, amplified single fragments from the four spumellarian species (Fig. 4, lanes 1-4). 18S rDNAs from each symbiotic algae in the ectocytoplasm of *D. truncatum*, *S. tetras*,

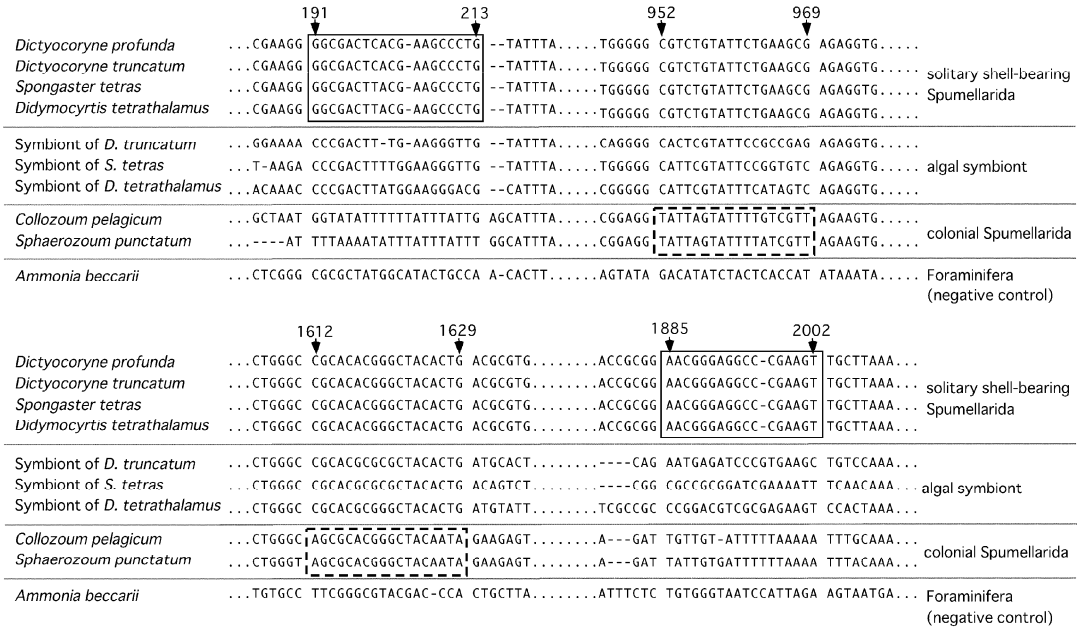

Fig. 3. Comparison of designated primer positions by both the authors and Amaral Zettler et al. (1997). The open squares by solid lines indicate designated primer regions by the authors, which are the forward primer Spu 191F (positions 191-213) and the reverse primer Spu 1731R (positions 1885-2002) (primer sequences see text). The open squares by broken lines indicate designated primer regions by Amaral Zettler et al. (1997), whose sequences are: the forward primer R906 (positions 952-969), 5'-TATTAGTATTTTRTCGTT-3'; the reverse primer R1451bio (positions 1612-1629), 5'-TATTGTAGCCCGTGCGCT-3'. *Ammonia beccarii* (Foraminifera) is added in this alignment as negative control.

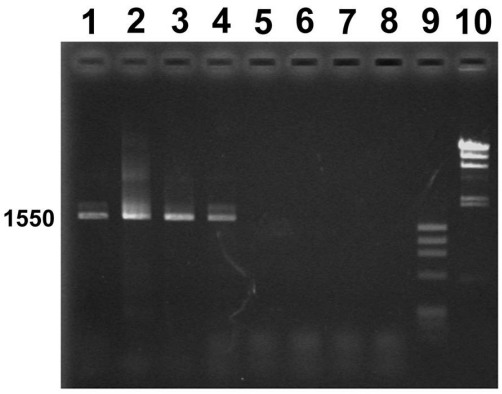

Fig. 4. Agarose gel electrophoresis of PCR products from both a central capsule and ectocytoplasm of each spumellarian species amplified with spumellarian-specific primers 191F/1731R. Templates of lanes 1, 2, 3, and 4 are from the central capsules of *Dictyocoryne truncatum*, *D. profunda, Spongaster tetras,* and *Didymocyrtis tetrathalamus*, respectively. Templates of lanes 5, 6, 7, and 8 are from the ectocytoplasm of *D. truncatum*, *D. profunda, S. tetras,* and *D. tetrathalamus*, respectively. The all amplified fragments are 18S rDNAs. Molecular weight standards: ØX174RF *Hae* III digest in Lane 9. λ-*Hin*d III digest in Lane 10.

and *D. tetrathalamus* could not be amplified by these primers (Fig. 4, lanes 5, 7, and 8), and the ectocytoplasm of *D. profunda* also could not be amplified as expected (Fig. 4, lane 6).

These results given in the Figures 2, 3, and 4 show that the primers Spu 190F and Spu 1731R are specific to the spumellarian polycystine sequences and amplified the 18S rDNA sequences directly from the specimens containing symbiotic algae. At present, there are very small numbers of polycystine species for molecular analyses. The specific primers that were designated in the present study will be used to accelerate phylogenetic analyses and the *in situ* hybridization of polycystines, which have not been established in culture and possess symbiotic algae.

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