

## Complete Elimination of Endosymbiotic Algae from *Paramecium bursaria* and its Confirmation by Diagnostic PCR

Miho TANAKA<sup>1</sup>, Maki MURATA-HORI<sup>1\*</sup>, Takashi KADONO<sup>1</sup>, Takashi YAMADA<sup>2</sup>, Tomonori KAWANO<sup>1</sup>, Toshikazu KOSAKA<sup>1</sup> and Hiroshi HOSOYA<sup>1,3</sup>

<sup>1</sup>Department of Biological Science, Graduate School of Science, Hiroshima University, Higashi-Hiroshima; <sup>2</sup>Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima; <sup>3</sup>PRESTO, Japan Science and Technology Corporation (JST), Higashi-Hiroshima, Japan

**Summary.** The green paramecium, *Paramecium bursaria*, has several hundred green algae in the cytoplasm. Symbiotic algae can be removed from their host cells by treatment with a herbicide, paraquat. The presence of symbiotic algae in *P. bursaria* can be microscopically examined by detecting the red fluorescence of the algal chlorophyll. However, etiolated algae could not be detected by fluorescent microscopic analyses. Therefore, the absence of symbiotic algae should be confirmed by examining the presence or absence of algal DNA in *P. bursaria*. In this study, we tried to detect the DNA of symbiotic algae by polymerase chain reaction (PCR) using plant genome-specific primers designed to amplify the DNA sequence in the ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) encoding gene. Using this technique, it was confirmed that the DNA of endosymbiotic algae was absent in paraquat-treated paramecia.

**Key words:** algae-free paramecia, constant darkness, endosymbiosis, paraquat, ribulose-1,5-bisphosphate carboxylase small subunit, small subunit rDNA.

### INTRODUCTION

*Paramecium bursaria* is an interesting model for the study of a coexisting system in intracellular symbiosis. One cell of *P. bursaria* contains several hundred green algae in the cytoplasm as endosymbionts (Loefer 1936). The host and symbionts can be separated and cultured independently, and the algae-free paramecia

can be re-infected with the ex-symbiotic algae (Siegel 1960, Bomford 1965, Weis and Ayala 1979). Several studies have reported that *P. bursaria* can be freed from symbiotic algae by culturing in constant darkness (Siegel 1960, Karakashian 1963, Weis 1978), by irradiation with X-ray (Wichterman 1943, 1948), or by exposure to photosynthesis inhibitors such as 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) (Reisser 1976). However, in these experiments, reproducibilities are lacking, because defined experimental conditions have not been described.

To study the mechanism of endosymbiosis, it is important to establish a simple model using algae-free paramecia and homogeneous (cloned from a single cell) but not heterogeneous algae. It has not been elucidated whether the symbiotic algae in *P. bursaria* are composed of a

---

Address for correspondence: Hiroshi Hosoya, Department of Biological Science, Graduate School of Science, Hiroshima University, Higashi-Hiroshima 739-8526, Japan; Fax: +81-824-24-0734; E-mail: hhosoya@sci.hiroshima-u.ac.jp

\* Present address: Department of Physiology, University of Massachusetts Medical School, MA 01605, USA

single species or not. We have previously cloned endosymbiotic algae from *P. bursaria* and characterization of each clone was carried out (Nishihara *et al.* 1998). On the other hand, we have reported a new technique to produce algae-free paramecia by treating green paramecia with a herbicide, paraquat (Hosoya *et al.* 1995). We have shown that an appropriate exposure of *P. bursaria* to paraquat produces symbiotic algae-free strains without cellular damage or physiological distortion of cell division and conjugation. The newly established algae-free paramecia showed the same growth rate as that of normal green paramecia. These cloned algae and algae-free paramecia will be useful tools to elucidate a mechanism for the establishment of symbiosis between algae and host paramecia.

In our previous report, the absence or presence of symbiotic algae was simply assessed by observing paramecium cells under a fluorescence microscope, since chlorophylls in symbiotic algae emit red fluorescence (Hosoya *et al.* 1995). However, the presence of some chlorophyll-free algae that survived after the herbicide treatment could not be detected by microscopic observation. Therefore, an alternative method is required to clarify that the symbiotic algae are completely removed from their host cells.

In the present study, we attempted to detect the presence of algal genomic DNA by diagnostic polymerase chain reaction (PCR) using a set of plant-specific primers designed to amplify the DNA sequence in the ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) encoding gene. Using this technique, it was confirmed that paraquat is useful for completely removing the algae from green paramecia.

## MATERIALS AND METHODS

### Strains and culture

One strain of *Paramecium bursaria* syngen 1 (NF-1, mating type I) was used in this work. The NF-1 strain was collected from the Koori-ike pond in Toyota-Gun, Hiroshima, Japan in 1999. The cells were cultured in a lettuce infusion supplemented with *Klebsiella pneumoniae* at 23°C under a light-dark cycle (LD=12:12h) at *ca* 1,000 lx of natural-white fluorescent light or in constant darkness (DD).

### Treatment with paraquat

The NF-1 cells in the stationary phase were treated with paraquat to remove the symbiotic algae. The cells were harvested using a nylon mesh (10 µm mesh size) and washed three times with a fresh lettuce

infusion. The cells were suspended in 50 ml of a fresh lettuce infusion containing 10 µg/ml of paraquat (Wako Pure Chem. Ind. Ltd., Osaka, Japan) at an initial cell density of 1,000 cells/ml and were incubated at 23°C under the LD condition. After treatment for 5 days, the cells were harvested and washed three times with a fresh lettuce infusion. Then the cells were transferred to a bacterized lettuce infusion and grown at 23°C under the LD condition. For the observation of paramecia, a Nikon Nomarski differential interference contrast (DIC) microscope (Nikon, Tokyo, Japan) and a fluorescence microscope (Optiphot BFD2, Nikon) were used.

### Preparation of DNA from *Paramecium*

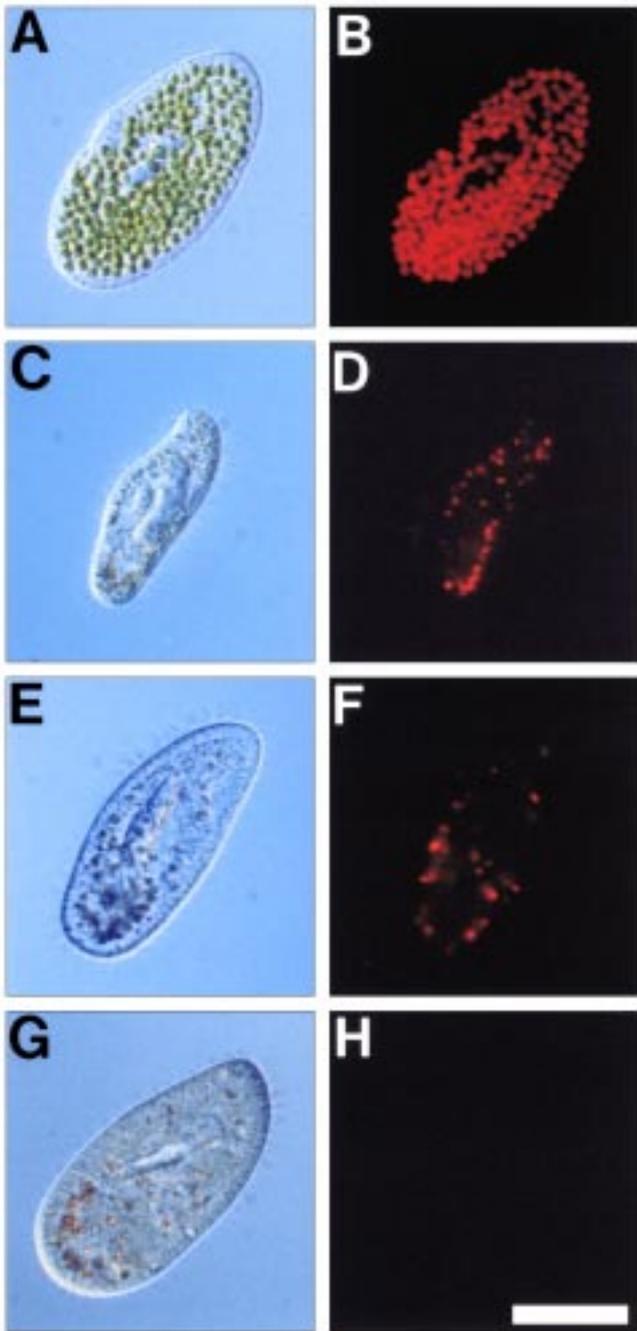
For the preparation of DNA, paramecia cells were harvested using a nylon mesh and washed three times with a CA medium (Nishihara *et al.* 1998). The cells (*ca* 2 x 10<sup>5</sup> cells) were frozen with liquid nitrogen and were crushed with glass beads (GMB-60, Nippon Rikagaku Kikai Co. Ltd., Tokyo, Japan) in DNA extraction buffer (100 mM Tris-HCl, pH 8.0, 10 mM EDTA and 1% sodium N-lauroyl sarcosinate) with phenol. Total DNA was subjected to a standard phenol/chloroform extraction and precipitated with ethanol.

### PCR amplification and sequence analysis

Two sets of oligonucleotide primers were used in this experiment. One set of primers designed to amplify the small subunit (SSU) rRNA-encoding gene (rDNA) was used for universal detection. The forward (SSU-5') and reverse (SSU-3') primers were 5'-TTGGAGGGCAAGTCTGGTGC-3' and 5'-TCCTTGGCAAATGCTTTCGC-3', respectively. It was confirmed that these sequences were found in SSU rDNA of green algae *Chlorella* sp. and *P. bursaria* 18S rDNA (DDBJ/EMBL/GenBank databases accession nos. AY004348 and AF100314). For the detection of an algae-specific DNA sequence, a second set of primers was designed using the sequence of *rbcS* encoding gene (cDNA) of *Chlorella vulgaris* (DDBJ/EMBL/GenBank databases accession no. AB058647). The forward (*rbcS*-1-5') and reverse (*rbcS*-1-3') primers were 5'-TTCTCCTACCTGCCCTCTG-3' and 5'-GCGTCAGTGCAGCCGAACAT-3', respectively. Partial sequences of SSU rDNA and *rbcS* DNA were amplified by PCR using the above primers and *Taq* DNA polymerase (TOYOBO Co. Ltd., Osaka, Japan). After denaturation for 3 min at 98°C, amplification was performed for 30 cycles of 45 s at 95°C, 45 s at 57°C and 1 min at 72°C and an additional extension period for 5 min at 72°C. The amplified PCR fragments sub-cloned into the pGEM-T vector (Promega, Madison, USA) were used for sequence analysis. Sequencing was carried out using a DNA sequencer ALF Express II (Amersham Pharmacia Biotech Inc., Uppsala, Sweden). The sequences were analyzed with the software DNASIS (Hitachi Software Engineering, Kanagawa, Japan).

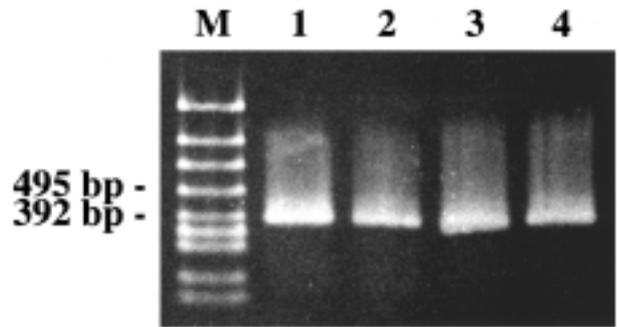
## Results and Discussion

Siegel (1960) reported that culturing green paramecia in DD for 24 days produced algae-free paramecia. However, in our previous study, because no algae-free paramecia were obtained by culturing green paramecia



**Fig. 1.** Nomarski differential interference contrast images (A, C, E and G) and their fluorescence images (B, D, F and H) of *Paramecium bursaria*. The images of algae-containing green paramecia (A and B), cells grown in constant darkness for 20 days (C and D) or 50 days (E and F) and symbiotic algae-free paramecia treated with paraquat (G and H) are shown. Symbiotic algae-free paramecia were produced by treatment with 10  $\mu\text{g/ml}$  of paraquat for 5 days. The algal chlorophyll fluoresces red (B, D and F) but no fluorescence was observed in the cells treated with paraquat (H). Scale bar - 40  $\mu\text{m}$

in DD for 22 days, we developed a new technique to produce algae-free paramecia by treatment with paraquat



**Fig. 2.** Diagnostic PCR analyses using primers SSUb-5' and SSUb-3'. The genomes of *Paramecium bursaria* cells treated with paraquat (lane 1), grown under constant darkness for 50 days (lane 2) and 20 days (lane 3) and algae-containing green paramecia (lane 4). PCR products were separated on 2% agarose gels and photographed after ethidium bromide staining. The molecular size markers (lane M) were  $\Phi\text{x}174/\text{Hinc II}$  digest. About 390-bp bands were observed in lanes 1 to 4

(Hosoya *et al.* 1995). In the present study, we compared the algae-eliminating efficiency of the conventional method (culturing in DD) and the newly proposed chemical method (using of paraquat) in green paramecia (NF-1). According to classical studies, we examined the effect of culturing green paramecia in DD for a period of 20 days, and in addition, the impact of 50 days of culturing in DD (the long-term DD condition) was also examined. We prepared the paraquat-treated cells (pqNF-1) and dark-grown cells cultured for 20 days (cd20NF-1) or 50 days (cd50NF-1) in the DD condition. The Nomarski DIC images and their fluorescence images of NF-1, cd20NF-1, cd50NF-1 and pqNF-1 cells are shown in Fig. 1. When NF-1 was observed under a fluorescence microscope, endosymbiotic algae could be visualized as red objects, due to the algal chlorophyll's red fluorescence (Fig. 1B). Notably, no fluorescence was detected in pqNF-1 (Fig. 1H). In contrast, several fluorescent particles were detected in both cd20NF-1 (Fig. 1D) and cd50NF-1 (Fig. 1F). To determine the average number of algae that remained in the dark-grown cells (cd20NF-1 and cd50NF-1), 25 cells were sampled from each culture and the remaining algae were counted under a fluorescence microscope (Table 1). The average number of symbiotic algae in each cd20NF-1 and cd50NF-1 cells was  $41.3 \pm 17.6$  cells and  $37.5 \pm 14.5$  cells, respectively. There were no algae-free paramecia in the dark-grown culture. The number of symbiotic algae was markedly reduced during the long-term culture in DD, but this method failed to complete the

**A**

NF-1  
*P. bursaria* →  
TTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGCTCCAATAGCGTATACTTA  
TTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGCTCCAATAGCGTATACTTA

NF-1  
*P. bursaria* AGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAATTCGGTCAGCCAGCTCTCAGGACATC  
AGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAATTCGGTCAGCCAGCTCTCAGGACATC

NF-1  
*P. bursaria* -ATGGCGGATCTCCGCTTACAATCTACTCGAGCTTTAAACCTTGAGTTGTAGAGTAGGCA  
-ATGGCGGATCTCCGCTTACAATCTACTCGAGCTTTAAACCTTGAGTTGTAGAGTAGGCA

NF-1  
*P. bursaria* GTTTACCTTGAAAAAATTAGAGTGTTCAGGCAGGTTTTCGCCGGAATACATTAGCATG  
GTTTACCTTGAAAAAATTAGAGTGTTCAGGCAGGTTTTCGCCGGAATACATTAGCATG

NF-1  
*P. bursaria* GAATAATGGAATAGGACTTTAGCCTGATGTTGGTTTTGGCTATTGTAATGATTAATAGG  
GAATAATGGAATAGGACTTTAGCCTGATGTTGGTTTTGGCTATTGTAATGATTAATAGG

NF-1  
*P. bursaria* GACAGATGGGGCATTAGTATTTAATTGTCAGAGGTGAAATTCCTGGATTTATTAAGAC  
GACAGATGGGGCATTAGTATTTAATTGTCAGAGGTGAAATTCCTGGATTTATTAAGAC

NF-1  
*P. bursaria* ←  
TAACTTATGCCAAAGCATTGCCAAGGA  
TAACTTATGCCAAAGCATTGCCAAGGA

**B**

NF-1  
*Chlorella* →  
TTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGCTCCAATAGCGTATACTTA  
TTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGCTCCAATAGCGTATACTTA

NF-1  
*Chlorella* AGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAATTCGGT-----C-T-G-CCAGTC-T  
AGTTGTTGCAGTTAAAAAGCTCGTAGTTGAAATTCGGTAGGGTTCCTGCGGTCC-SCGGT

NF-1  
*Chlorella* T-CGGA--CA-TCAATGGCG--ATCTC-C-GCCT--ACAATCTACTCGAGCTTTAAAC  
TTCCGTTGTGCACTGACGGCGCTATCTTGCTGCCGGGGACGGGCTCTGGGC--TTAA-

NF-1  
*Chlorella* CT-T-GAGT-T-GTAGAGTAGGC-AGTTTACCTTGAAAAAATTAGAGTGTTTTCAG-GC  
CTGTCCGGGACTCGG--AGYCGGCAGGTTACTTTGAGTAATTAATAGTG-TTCAAAGC

NF-1  
*Chlorella* AGGTTTTTCGC-CGGAATACATTAGCATGGAATAATG-CATAGGACTTTAGCCTGAT--  
AGGCTTACGCTCTGAATACATTAGCATGGAATAACAGCATAGGACTCTGGCCT-ATCT

NF-1  
*Chlorella* -GTTGGTTTTT--GG-CT--ATTGTAATGATTAATAGGGACAGATGGGGCATTAGTATT  
TGTGGTCTGTAGGACC-GGA-GTAATGATTAATAGGGACAGTGGGGCATTAGTATT

NF-1  
*Chlorella* TAATTGTCAGAGGTGAAATTCCTGGATTTATTAAGACTTAACTTATGCGAAAGCATTGTC  
TCATTTGTCAGAGGTGAAATTCCTGGATTTATTAAGACTTAACTTATGCGAAAGCATTGTC

NF-1  
*Chlorella* CAAGGA  
CAAGGA

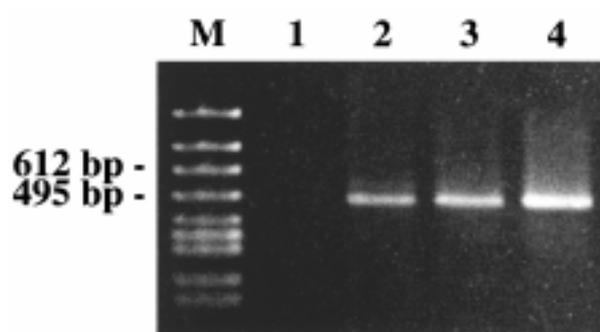
**Fig. 3.** Partial sequences of SSU rDNA amplified from NF-1 genome, 18S rDNA of *Paramecium bursaria* and *Chlorella* sp. SSU rDNA. A - comparison of nucleotide sequences between NF-1 SSU rDNA and 18S rDNA of *P. bursaria*. B - comparison of nucleotide sequences between NF-1 SSU rDNA and *Chlorella* sp. SSU rDNA. The SSU rDNA sequence of *Chlorella* sp. and the reported sequence of 18S rDNA of an unnamed *P. bursaria* strain collected from S. Nation River (Ontario, Canada) (Strüder-Kypke *et al.* 2000) were available from DDBJ/EMBL/GenBank databases (accession nos. AY004348 and AF100314, respectively). The differences in sequence length were compensated for by introducing alignment gaps (-) in the sequences and matched sites are framed. Arrows indicate the primer positions designed from 18S rDNA of *P. bursaria* and *Chlorella* sp. SSU rDNA. The sequences of NF-1 SSU rDNA shared 97.9% homology with *P. bursaria* 18S rDNA and relatively much weaker homology (74.6%) with *Chlorella* sp. SSU rDNA.

**Table 1.** The number of symbiotic algae that remained in a single *Paramecium bursaria* sampled from three groups, cd20NF-1, cd50NF-1 and pqNF-1. The number of algae was counted under a fluorescence microscope. n - number of individuals examined, SD - standard deviation

Character	No. of symbiotic algae/paramecium (cells)				Rate of algae-free paramecium (%)	n
	Minimum	Maximum	Mean	SD		
cd20NF-1	13	81	41.3	17.6	0	25
cd50NF-1	9	64	37.5	14.5	0	25
pqNF-1	0	0	0	-	100	25

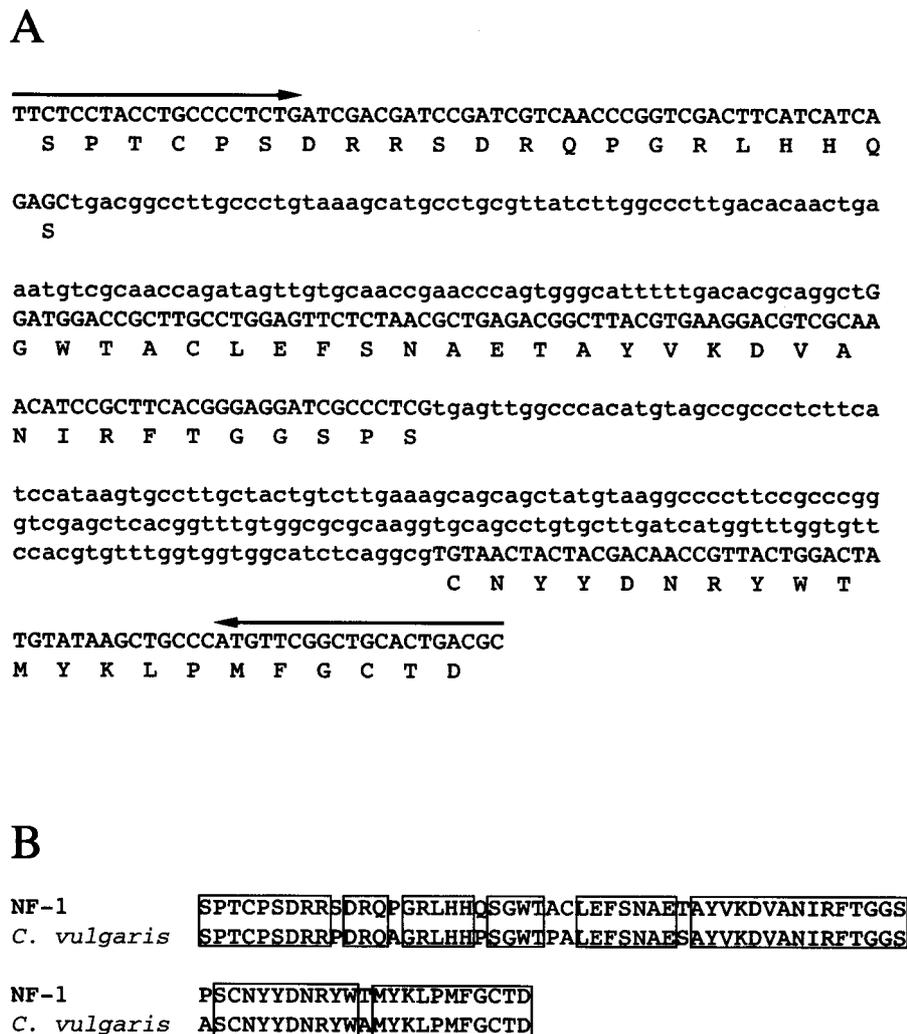
elimination of algae from the host cells. In contrast, no symbiotic algae were detected in the pqNF-1 cells (Table 1).

It is possible that some etiolated algae which lost chlorophyll survived in the pqNF-1 cells. Thus, the absence or presence of such algae should be proven by examining the presence of algal genomic DNA in the host paramecia. Electron microscopic observation may be applicable for this purpose, but the evidence is weaker compared to that obtained by a molecular method. Therefore, we attempted to detect the DNA of endosymbiotic algae that survived in host paramecia by diagnostic PCR using plant gene-specific primers. Total genomes were collected from pqNF-1, cd20NF-1, cd50NF-1 and NF-1 cells. The SSU primers were used as a positive control so that the yield of PCR products from four different samples could be normalized. As seen in Fig. 2, about 390-bp fragments were amplified from all of the pqNF-1, cd20NF-1, cd50NF-1 and NF-1 genomes. The size of the amplified DNA was consistent with the expected size of the partial sequence of *P. bursaria* 18S rDNA to be amplified by SSU primers. The PCR products were subjected to DNA sequencing. In Fig. 3, the determined nucleotide sequence of SSU rDNA amplified from the NF-1 genome was compared with the *P. bursaria* 18S rDNA sequence and *Chlorella* sp. SSU rDNA sequence. The sequence of NF-1 SSU rDNA shared 97.9% similarity with *P. bursaria* 18S rDNA, while only 74.6% similarity in the DNA sequence was found between NF-1 SSU rDNA and *Chlorella* sp. SSU rDNA. These results suggested that the sequence of SSU rDNA amplified from the NF-1 genome corresponded to 18S rDNA of the host paramecia. Although it was expected that the PCR products amplified from cd20NF-1, cd50NF-1 and NF-1 would include both the *P. bursaria* 18S rDNA and

**Fig. 4.** Diagnostic PCR analyses using primers *rbcS*-1-5' and *rbcS*-1-3'. The genomes of *Paramecium bursaria* cells treated with paraquat (lane 1), grown under constant darkness for 50 days (lane 2) and 20 days (lane 3) and algae-containing green paramecia (lane 4). The molecular size markers (lane M) were the same as Fig. 2. About 500-bp PCR products were observed in lanes 2 to 4 but not in lane 1

alga SSU rDNA fragments, the sequence analysis showed that only 18S rDNA was amplified.

Secondly, we carried out algal DNA-specific detection using the *rbcS* primers. About 500-bp PCR products were obtained from the cd20NF-1, cd50NF-1 and NF-1 genomes, but no signal was detected in pqNF-1 (Fig. 4). The 500-bp fragments obtained from cd20NF-1, cd50NF-1 and NF-1 were subjected to sequence analysis. The determined nucleotide sequence in the 500-bp fragment amplified from the NF-1 genome is shown in Fig. 5. Since the 500-bp fragment was amplified by genomic PCR, the obtained nucleotide sequence included the introns. The deduced amino acid sequence of the encoded protein was compared with that of *Chlorella vulgaris* C-169 *rbcS*. The deduced amino acid sequence of the NF-1 PCR product shared 88.8% similarity with that of *C. vulgaris* *rbcS*. The deduced



**Fig. 5.** Nucleotide and deduced amino acid sequences of *rbcS* DNA amplified from the NF-1 genome. A - the determined nucleotide sequence of *rbcS* DNA amplified from the NF-1 genome is shown, together with the deduced amino acid sequence of the encoded protein. The sequences estimated to be introns were uncapitalized. Arrows indicate the primer positions designed from *Chlorella vulgaris* C-169 *rbcS* cDNA (DDBJ/EMBL/GenBank databases accession no. AB058647). B - deduced amino acid sequence of NF-1 *rbcS* was compared with that of *C. vulgaris* C-169 *rbcS*

amino acid sequence of 500-bp fragments obtained from cd20NF-1 and cd50NF-1 were identical to that of the NF-1 PCR product. These results suggested that the *rbcS*-targeting primers are adequate for symbiotic algae-specific detection.

In conclusion, it became clear that treatment of *P. bursaria* with paraquat only for 5 days completely eliminated the genomic DNA of the symbiotic algae, indicating that algae-free *P. bursaria* were produced. In contrast, the present study showed that no algae-free paramecia were produced by a conventional method in which *P. bursaria* was cultured in DD. In addition, the rapid growth of the host cells in DD will accelerate the

clonal aging. These results suggest that culturing in DD is not suitable for producing algae-free paramecia. The new technique using paraquat can produce algae-free paramecia without physiological damage or distortion in cell division and conjugation (Hosoya *et al.* 1995, Nishihara *et al.* 1996). As presented here, treatment with paraquat is a powerful technique to produce completely algae-free paramecia.

### Perspectives

Our results also showed that symbiotic algae could survive without photosynthesizing under the long-term DD condition. It is possible that the host cells supply the

least amount of nutrition required for survival of symbiotic algae. To elucidate the endosymbiotic mechanism of *P. bursaria* and symbiotic algae, it is important to investigate the flow of nutrition and metabolites between hosts and symbionts.

## REFERENCES

- Bomford R. (1965) Infection of alga-free *Paramecium bursaria* with strains of *Chlorella*, *Scenedesmus*, and a Yeast. *J. Protozool.* **12**: 221-224
- Hosoya H., Kimura K., Matsuda S., Kitaura M., Takahashi T., Kosaka T. (1995) Symbiotic algae-free strains of green *Paramecium bursaria* produced by herbicide paraquat. *Zool. Sci.* **12**: 807-810
- Karakashian M. W. (1963) Growth of *Paramecium bursaria* as influenced by the presence of algal symbionts. *Physiol. Zool.* **36**: 52-68
- Loefer J. B. (1936) Isolation and growth characteristics of the "zoochlorella" of *Paramecium bursaria*. *Am. Midl. Nat.* **70**: 184-188
- Nishihara N., Takahashi T., Takahashi T., Kosaka T., Hosoya H. (1996) Characterization of symbiotic algae-free strains of *Paramecium bursaria* produced by the herbicide paraquat. *J. Protozool. Res.* **6**: 60-67
- Nishihara N., Horiike S., Takahashi T., Kosaka T., Shigenaka Y., Hosoya H. (1998) Cloning and characterization of endosymbiotic algae isolated from *Paramecium bursaria*. *Protoplasma* **203**: 91-99
- Reisser W. (1976) Die stoffwechselfysiologischen Beziehungen zwischen *Paramecium bursaria* Ehrbg. und *Chlorella* spec., in der *Paramecium bursaria*-Symbiose. I. der Stickstoff- und der Kohlenstoff-Stoffwechsel. *Arch. Mikrobiol.* **107**: 357-360
- Siegel R. W. (1960) Hereditary endosymbiosis in *Paramecium bursaria*. *Exp. Cell Res.* **19**: 239-252
- Strüder-Kypke M. C., Wright A. D. G., Fokin S. I., Lynn D.H. (2000) Phylogenetic relationships of the genus *Paramecium* inferred from small subunit rRNA gene sequences. *Mol. Phylogenet. Evol.* **14**: 122-130
- Wichterman R. (1943) Conjugation and fate of exconjugants of zoochlorella-free *Paramecium bursaria*. *Anat. Rec.* **87**: 50
- Wichterman R. (1948) The biological effects of X-rays on mating types and conjugation of *Paramecium bursaria*. *Biol. Bull.* **94**: 113-127
- Weis D. S. (1978) Correlation of infectivity and Concanavalin A agglutinability of algae exsymbiotic from *Paramecium bursaria*. *J. Protozool.* **25**: 366-370
- Weis D. S., Ayala A. (1979) Effect of exposure period and algal concentration on the frequency of infection of aposymbiotic ciliates by symbiotic algae from *Paramecium bursaria*. *J. Protozool.* **26**: 245-248

Received on 2nd January, 2002; accepted on 25th April, 2002