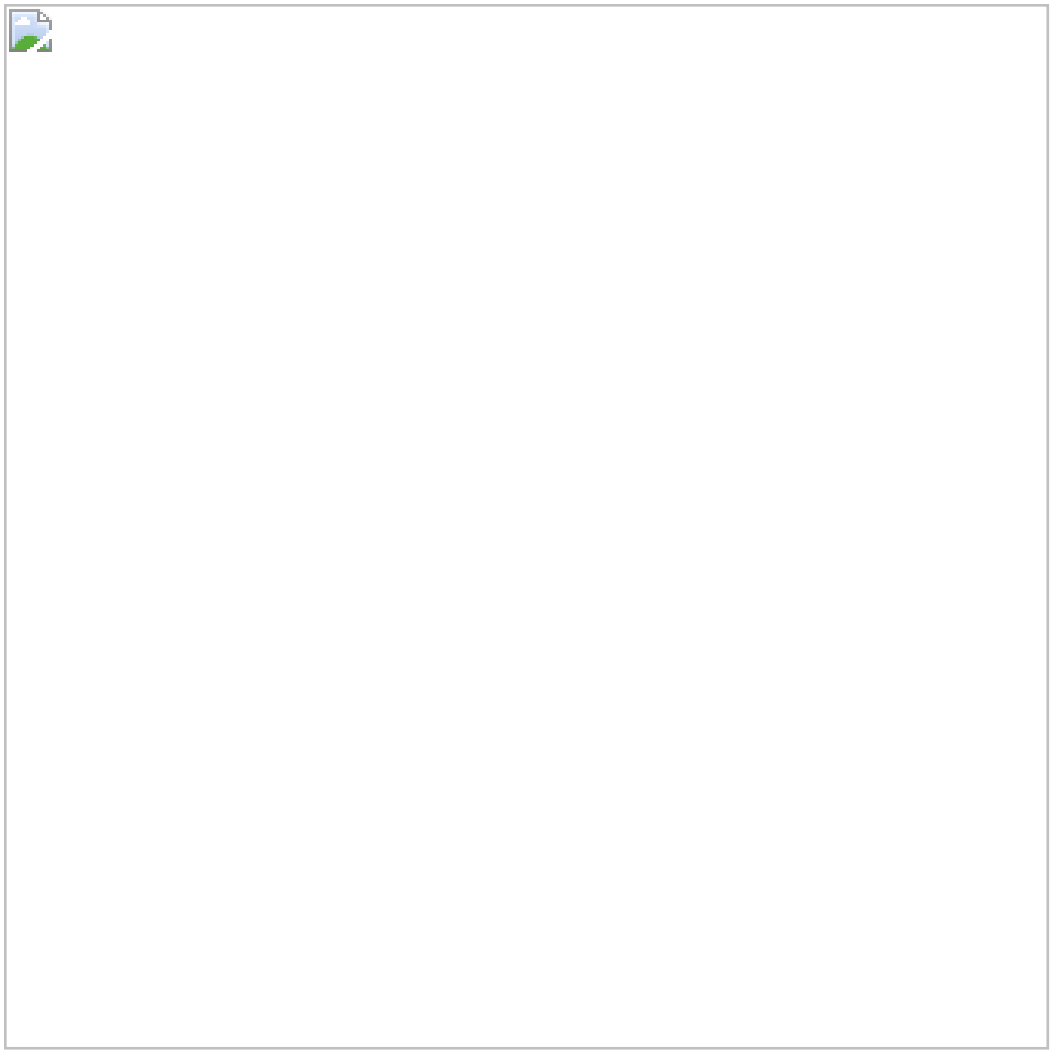

Symbiotic Algae-free Strains of The Green Paramecium Paramecium bursaria Produced by Herbicide Paraquat

Authors: Hosoya, Hiroshi, Kimura, Kouki, Matsuda, Seiji, Kitaura, Miyuki, Takahashi, Tadao, et al.

Source: Zoological Science, 12(6) : 807-810

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.12.807

ZOOLOGICAL SCIENCE 12: 807–810 (1995)

[RAPID COMMUNICATION]

Symbiotic Algae-free Strains of The Green Paramecium *Paramecium bursaria* Produced by Herbicide Paraquat

HIROSHI HOSOYA, KOUKI KIMURA[1], SEIJI MATSUDA[2], MIYUKI KITAURA[3], TADAO TAKAHASHI and TOSHIKAZU KOSAKA

Department of Biological Science, Faculty of Science, Hiroshima University, Higashi-Hiroshima, 739, Japan

ABSTRACT—The green paramecium, *Paramecium bursaria*, has endosymbiotic algae in the cytoplasm. Here, we report that all endosymbiotic algae are destroyed, producing symbiotic algae-free strains of paramecia, when green paramecia are cultivated in the presence of a herbicide paraquat.

INTRODUCTION

A single green paramecium, *Paramecium bursaria*, has several hundred algae which may belong to the genus *Chlorella* ([8], for reviews see refs. [1, 13]). These endosymbiotic algae, which are individually enclosed in special vacuoles of the host, the perialgal vacuoles, are retained during conjugation and transmitted to both daughter cells at cell division.

While it is well known that the symbiotic algae can grow independently of their host, symbiotic algae-free strains of *P. bursaria* have been cultivated as well. The resulting strains can be reinfected by exsymbiotic algae or even by other microorganisms (for review see ref. [1]). Jennings found that the endosymbiotic algae in *P. bursaria* can be removed from the host by a series of rapid fissions of paramecia [2]. Thereafter, several investigators reported that the algae can be removed by various means, for example, by cultivating *P. bursaria* in continuous darkness [3, 6, 11], by X-ray irradiation [12], and by exposure to an inhibitor of photosynthesis, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) [7]. Unfortunately, most experiments were restricted to a phenomenological description of the eventually formed algae-free strains. Furthermore, reproducibilities in these experiments as well as defined experimental conditions including the growth phase or age of treated ciliates are usually lacking so that it is rather difficult to draw any conclusions as to the efficiency of those systems in the study of endosymbiosis.

The present study was initiated in order to clarify the experimental conditions in producing algae-free paramecia. Here, a brief description of a new technique to produce symbiotic algae-free strains of *P. bursaria* is reported.

MATERIALS AND METHODS

Four stocks of *Paramecium bursaria* syngen 1(OK-312, mating type I; H-4, II; HK-48, II; OZ-3, III; OK-1a, IV) were used in this experiment. Stocks OK-312 and H-4 were collected from the pond Okuda-Oike in Higashi-Hiroshima City (Hiroshima, Japan) in 1991 [5] and the lake Hakuryu-ko in Kamo Gun (Hiroshima, Japan) in 1992, respectively. Stock OZ-3 was collected from the river Oze-Gawa in Otake City (Hiroshima, Japan) in 1994. The other 2 stocks (HK-48 and OK-1a) were newly produced by hybridization of stocks H-4×K-8 and K-312×OK-223, respectively, in our laboratory. Stocks K-8 and OK-223 were also collected from a pond in Sera Gun (Hiroshima, Japan) and the pond Okuda-Oike in Higashi-Hiroshima City (Hiroshima, Japan) in 1991 [5], respectively. These stocks were cultivated in lettuce infusion inoculated with *Klebsiella pneumoniae* 24 to 48 hr before use at 23°C. The infusion was prepared by boiling together 0.5 g dried lettuce powder and 2 mg $CaCO_3$ in one litter of distilled water for 5 to 6 min. After filtration and autoclaving, the medium was stocked until use. Dried lettuce powder was prepared as follows: leaves of lettuce were washed and boiled for 30 to 60 sec. After drying at 60 to 80°C, they were powdered and stocked in a desiccator. Artificial illumination of daylight fluorescent lamps (4000 to 5000 lux) was provided to all standard cultures for 12 hr periods each day. Parazet® DC (commercial aqueous solution of 1,1'-Dimethyl-4,4'-bipyridinium, paraquat (24.0%)) was purchased from Otsuka Kagaku Yakuhin Co. Ltd., Osaka, Japan.

Paramecia containing several hundred algae were incubated with a diluted herbicide Parazet® (1/400,000). After 2 days, numbers of algae were examined with a Nikon Nomarski differential interference contrast (DIC) microscope. They were also examined with a Nikon

Accepted August 19, 1995
Received June 6, 1995

[1] Present address: Fukuyama-Iyo High School, Fukuyama, Hiroshima 720, Japan.

[2] Present address: Hagi High School for the Handicapped, Hagi, Yamaguchi 758, Japan.

[3] Present address: 1503-270, Kamimura-cho, Fukuyama, Hiroshima 729-01, Japan.

fluorescence microscope (OPTIPHOT BFD2). A B2 filter was placed between the specimen and the eyepieces to remove ultraviolet light. Because algal chlorophyll in the green paramecia fluoresces red, endosymbiotic algae are clearly observed as red images. Paramecia provisionally scored as "algae-free" on the basis of this fluorescence microscopic examination were washed 3 times with 0.05% (v/v) of van't Hoff artificial sea water to remove the herbicide and separately maintained as one paramecium per one culture. After one week, to examine the absence of endosymbiotic algae, several paramecia proliferated in each culture were observed by the fluorescence microscopy again.

Algae-free paramecia were mixed with symbiotic paramecia of complementary mating type (OZ-3). Conjugating pairs were isolated and then each exconjugant was cultured in a fresh medium. After 2 weeks, survival ratio of algae-free exconjugant was compared with that of normal green exconjugant.

RESULTS

Siegel [10] modified the technique for producing *P. bursaria* without algae in continual darkness reported by Jennings [2]. First we re-examined the technique to produce symbiotic algae-free paramecia. After 12 days of continuous darkness, the numbers of algae per paramecium (stock H-4; stationary phase) decreased from 400 to 150 and thereafter were kept constant (Fig.1). In another stock of *P. bursaria* (stock OK-312; stationary phase), no endosymbiotic algae were removed for up to 10 days in the continuous darkness (Fig. 1). During this period, no algae-free paramecia in both strains were observed in either strain.

Here, green paramecia (OK-312) in logarithmic phase were incubated with the herbicide Parazet®. As the broad-leaf herbicide Parazet® acts on plants by diversion of electron flow from illuminated chloroplast photosystem 1, preferentially competing with oxidized ferredoxin [4, 14], the herbicide may affect electron flow in the chloroplast of symbiotic algae and damage it. After 2 days of incubation with Parazet®, the Nomarski DIC images showed that the treated samples had no endosymbiotic algae (data not shown).

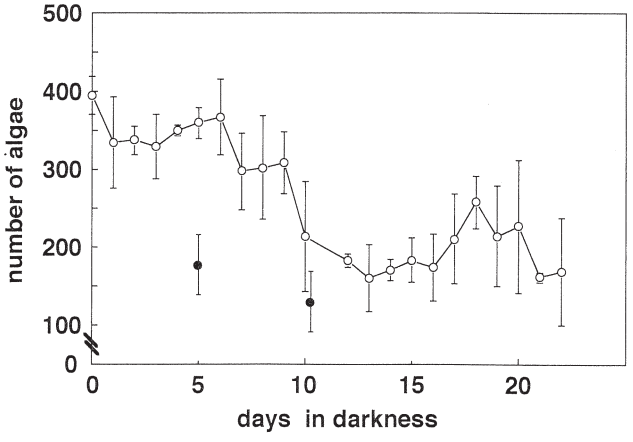

FIG. 1. Number of symbiotic algae per paramecium (2 stocks; OK-312 (●) and H-4 (○)) which were cultured under the condition of prolonged starvation in continuous darkness. *Abscissa*: days in continuous darkness; *ordinate*: mean number of symbiotic algae in green paramecium. Vertical bars denote one standard error. The average number of symbiotic algae per paramecium (in both 2 strains) gradually decreased until 10 days in darkness and thereafter kept constant. Results were expressed as the mean of 2 to 4 paramecia.

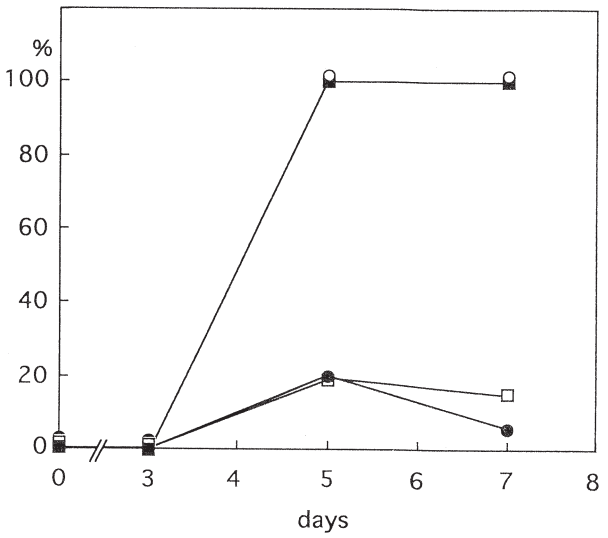

FIG. 2. Effect of paraquat on the symbiotic algae in *P. bursaria* (OK-312). Paramecia were incubated with paraquat (■; 100 μg/ml, ○; 1 μg/ml, □; 10^{-2} μg/ml, ●;10^{-4} μg/ml) and algae-free paramecia were counted. *Abscissa*; days after addition of paraquat. *Ordinate*, (number of algae-free paramecia)/(total number of paramecia) (%).

Next we cultivated green paramecia in the logarithmic phase (OK-312) with various concentrations (10^{-4} to 10^{2} μg/ml) of paraquat, which was a main component of Parazet®. DIC images also showed that the paramecia which have no algae can be seen in the paraquat-treated samples 5 days after addition of paraquat (Fig. 2). In the presence of paraquat more than 1 μg/ml, all of treated paramecia had no algae.

To confirm that the treated paramecia contained no algae, we further observed them by fluorescence microscopy. When the cell is illuminated with a hydrogen lamp, the chlorophyll in the algae absorbs radiation, becomes excited and re-emits radiation at a longer wave-length. Then, the algal chlorophyll in the green paramecia fluoresces red. Fluorescence microscopic observations showed that no algae were present in the paramecia after 3 days of incubation with paraquat (Fig. 3).

Such algae-free strains became obligate bacterial feeders and entered into conjugation when mixed with symbiotic green paramecia of complementary mating type (OZ-3). Conjugating pairs were isolated and, after 2 weeks, the survival ratio of each exconjugant was examined. The survival ratio of green paramecia and algae-free paramecia was 94.0 to 98.5% (4 experiments).

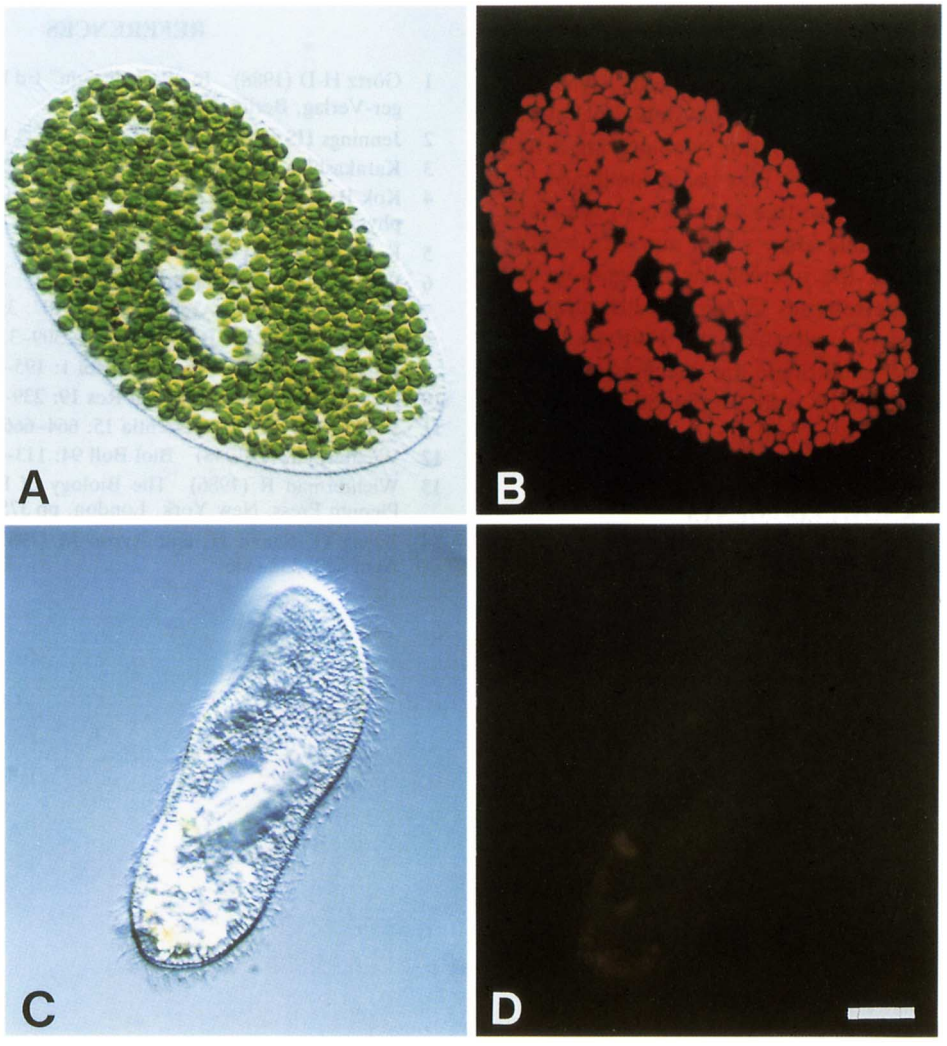

FIG. 3. Nomarski differential interference contrast images (A and C) and their fluorescence images (B and D) of symbiotic (A and B) and algae-free (C and D) *P. bursaria* (strain OK-312). These algae-free paramecia were produced 5 days after addition of 1 μg/ml of paraquat. The cell is illuminated with a ultraviolet light source. A filter (B2) is placed between the specimen and the eyepieces to remove the ultraviolet light, so that just the fluorescent colours are seen. Here, the algal chlorophyll fluoresces red, as shown in (B). The cell treated with paraquat does not fluoresce red (D). Scale bar, 20 μm.

DISCUSSION

The results presented here indicate that the appropriate exposure to a herbicide produces symbiotic algae-free strains of *P. bursaria* in the logarithmic phase. Although the animal toxicology of the herbicide is still obscure, our observations clearly show that the algae are more sensitive to paraquat than their hosts. In our preliminary experimenrs, treated green paramecia in the stationary phase also had no symbiotic algae. These findings suggest that the algae have remained susceptible to paraquat throughout the stage of the growth cycle of the host.

In our stocks used here, prolonged growth in the dark (Fig. 1) or exposure to an inhibitor of photosynthesis (DCMU) (our unpublished data) did not eliminate the symbiotic algae from the green paramecia in spite of other successful reports [3, 6, 7, 11, 12]. However, the endosymbiotic algae from green paramecia was eliminated 5 days after addition of the herbicide in this report. Perhaps, the explanation lies in the fact that other authors used different criteria for defining succesful elimination of algae from the green paramecia than those employed here. The susceptibility to continuous darkness or an inhibitor of photosynthesis may differ among strains of paramecia or algae used in experiments. Further studies are required to elucidate this discrepancy.

Jennings reported that the endosymbiotic algae in the green paramecia can be removed entirely from the green paramecia by providing an excess of food enabled them to reproduce more rapidly than their algae [2]. The resulting symbiotic algae-free paramecia may be extremely aged by such a series of rapid fissions. However, in our experiments, symbiotic algae-free strains can be established within several days without aging. Furthermore, these established algae-

free strains showed the same survival ratio as that of normal green paramecia. These results suggest that the herbicide produced symbiotic algae-free strains without significant damaging. A detailed comparison of physiological, ultrastructural and behavioral features of our symbiotic algae-free strains and naturally green ones is now being conducted.

Infection of symbiotic algae-free paramecia by symbiotic algae is potentially a reliable assay which may be useful in studies on the nature of the intercellular recognition and communication in endocytobioses in animal cells, although we are only beginning to understand it. Further experiments are now progress to elucidate the infection process in paramecia endocytobioses by using symbiotic algae-free strains of *P. bursaria* presented in this report.

ACKNOWLEDGMENTS

This work was supported by grants for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1 Görtz H-D (1988) In "Paramecium" Ed by H-D Görtz, Springer-Verlag, Berlin, pp 393–405
2 Jennings HS (1938) Proc Natl Acad Sci USA 24: 112–120
3 Karakashian MW (1963) Physiol Zool 36: 52–68
4 Kok B, Rurainski HJ, and Owens OVK (1965) Biochim Biophys Acta 109: 347–356
5 Kosaka T (1994) Zool Sci 11: 517–526
6 Pado R (1965) Folia Biol (Krakow) 13: 173–182
7 Reisser W (1976) Arch Microbiol 107: 357–360
8 Reisser W (1984) Br Phycol J 19: 309–318
9 Reisser W (1986) Progr Protistol 1: 195–214
10 Siegel RW (1960) Exp Cell Res 19: 239–252
11 Weis DS (1969) Experientia 15: 664–666
12 Wichterman R (1948) Biol Bull 94: 113–127
13 Wichterman R (1986) The Biology of Paramecium, 2nd ed, Plenum Press, New York, London, pp 375–420
14 Zweig G, Shavit N. and Avron M (1965) Biochim Biophys Acta 109: 332–346