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Characteristics of ciliated protozoa inhabiting colonies of pelagic blue-green algae*

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Abstract: Ciliated protozoa were isolated from the blue green alga *Trichodesmium thiebautii* which is ubiquitous and often abundant in surface seawater of the pelagic sea. Through protargol impregnation, nuclear staining and observations of the living cells, the morphological characteristics of the protist were determined and this ciliate was identified as *Holosticha diademata* (REES, 1884) KAHL, 1932. This ciliate was frequently found in the coastal seawater which suggests the wide distribution of a certain species of protozoa in the coastal and pelagic sea. This species preferentially fed on bacterial strains of *Pseudomonas* spp. when several genera of bacteria were offered as feed.

1. Introduction

In the course of our research on bacterial biomass in seawater, bacterial carbon was determined to occupy several tens of per cent of particulate organic carbon even within the euphotic zone of the seawater column (MAEDA and TAGA, 1979; MAEDA, 1982). As a result of this research, bacterial carbon is now considered to be one of the largest energy sources in the sea. WILLIAMS (1981) also mentioned the significantly large biomass of bacteria in the sea. There have been several reports describing small animals which feed on bacteria (PAFFENHÖFER and STRICKLAND, 1970; HEINLE *et al.*, 1977; KING *et al.*, 1980; MAEDA, 1989). A large portion of ciliated protozoa are also known to be bacteria feeders (WEBB, 1956; FENCHEL, 1968; TAYLOR and BERGER, 1976; ALONSO *et al.*, 1981; MAEDA and CAREY, 1985; MAEDA, 1986) and the existence of energy transfer from bacteria to animals through ciliates was conclusively established in the laboratory (SEKI, 1966; TEZUKA, 1974). Thus the role of bacteria as feed seems to be substantial and ciliates are probably one of the key animals in the process of food transfer in the marine ecosystem. From this point of view we have been interested in investigating the ecological aspects of ciliates in the marine environment.

In this report we describe the taxonomical characteristics of ciliated protozoa attached to suspended colonies of the blue green alga *Trichodesmium thiebautii* in the South China Sea. Bacterial strains which coexisted in the ciliate culture were also identified and their availability as feed for the ciliates was determined.

2. Materials and methods

Sampling

Trichodesmium colonies were collected using a plankton net with a mesh size of 330 μm in the southern area of the South China Sea during the cruise of R/V Hakuhomaru

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in November, 1981. Coastal seawater were collected with a small glass bottle at Aburatsubo Inlet, Japan. *Trichodesmium* colonies were picked up with sterilized pipettes and kept in sterilized seawater for subsequent isolation or examination of the ciliates attached to them.

Isolation of ciliated protozoa

Cililates attached to *Trichodesmium thiebautii* were isolated using a micropipette under a binocular microscope (magnification, ×45). Isolated ciliates were placed into a medium which contained the following components ($g \cdot l^{-1}$ seawater): Proteose peptone (Difco), 0.02; Tripticase (Sigma), 0.02; Bacto yeast extract (Difco), 0.02; ribonucleic acid (Sigma), 0.002; Bacto agar (Difco), 15 and extract of cerophyl leaves (Sigma), 2 ml/l (that is, 5 g of cerophyl leaves was boiled with 1 litre of distilled water for 5 min. and the supernatant after filtration with Toyo filter paper (Type 1) was used.). Ten ml of this agar medium was put into a 200 ml of flask and 10 ml of sterilized seawater was placed on top of the agar after solidification. The ciliate cultures were kept at 25 ℃.

Identification of ciliates

The species of ciliate was identified by the protargol impregnation technique (TUFFRAU, 1967) and the Feulgen nuclear reaction for examining cirri and nuclei, respectively.

Isolation and identification of bacteria

Bacteria were isolated from seawater *in situ* and from the cultivation bottle for ciliates and were cultured on the same agar medium described above. The isolated bacteria were identified according to the scheme of SHEWAN *et al.* (1960). The vibriostatic compound 0/129 (2, 4-diamino-6, 7-diisopropylpteridine) was not used to distinguish *Vibrio* from *Aeromonas*. Their discrimination was made on the basis of gas production from glucose. A few Gram-negative, oxidase-negative rods with polar flagella were assigned to *Vibrio* or *Aeromonas* but not to Enterobacteriaceae. The mode of glucose metabolism of the isolates was determined using the Hugh-Leifson's medium (HUGH and LEIFSON, 1953) made with artificial seawater instead of freshwater.

Response of ciliates to bacteria

Ten freshly cultured ciliates were washed three times in sterilized seawater for 24 hrs and placed in a small Petri dishes (27 mm diameter) with seawater. The bacterial strains used as feed for the ciliates were washed with sterilized seawater by centrifugation after cultivating for two days and were added at the concentration of 10^8 cells/ml to the Petri dishes containing the ciliate. The Petri dishes were placed in the wet chamber at 25 ℃ and ciliate numbers were determined under the binocular microscope.

3. Results

Fig. 1 shows a colony of *Trichodesmium thiebautii*. Ciliates of the order Hypotrichida were found among the *Trichodesmium* colony and these ciliates were isolated and investigated in this work.

Protargol staining shows the arrangement of cirri in the adoral, ventral and caudal zones of the ciliate cell (Fig. 2). Three frontal cirri and pairs of ventral cirri with zig-zag shape were the characteristic features

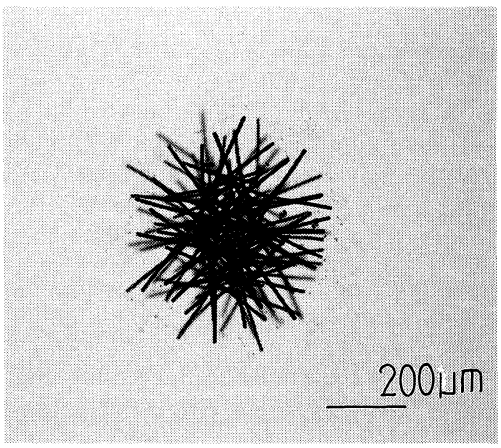

Fig. 1. A colony of the blue green algae *Trichodesmium thiebautii*.

of this ciliate. Nuclear staining revealed two macronuclei and 4 micro-nuclei (Fig. 3). Based on these observations and direct observations of the living cells, taxonomical characteristics of the ciliate are summarized diagrammatically in Fig. 4. Further characteristics of this protist are as follows. Size around 80 × 30 μm. Body flat dorsoventrally, rounded at ends. Membranelles of adoral zone (AZM) 19-26, 2 frontal cirri, about 11 left and 10 right marginal cirri. Transverse cirri around 7 and pairs of ventral cirri with zig-zag shape about 8. Bend of the anterior end of the left marginal cirrus row and a marine habitat. We identified this ciliate as *Holosticha diademata* (REES, 1884) KAHL, 1932, although the number of AZM was slightly different from that reported by BOR ROR (1963). The same species was also found frequently on zooplankton detritus in coastal waters of Japan, such as Aburatsubo Inlet.

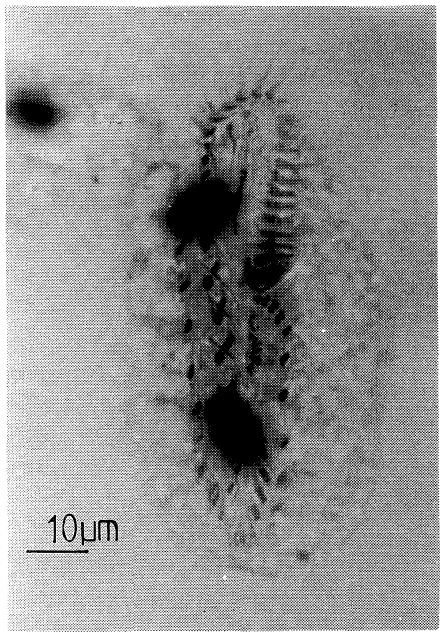

Fig. 2. Protargol impregnation of the ciliate.

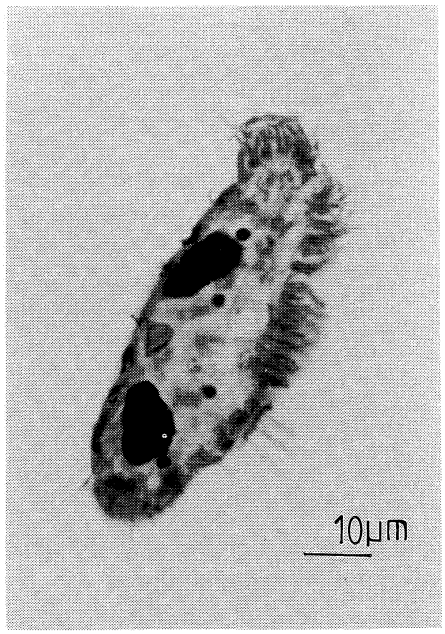

Fig. 3. Nuclear staining of the ciliate.

Bacterial strains isolated from the agar-seawater medium bottle of *Holosticha diademata* were all *Pseudomonas* spp. Among 9 bacteria isolated, *H. diademata* could be grown with 3 of these bacterial strains as feed, and amongst these strains the Strain No. 7 supported the maximum growth of ciliates during 5 days incubation (Table 1). Among the strains of *Pseudomonas, Vibrio, Acinetobacter* and *Flavobac-*

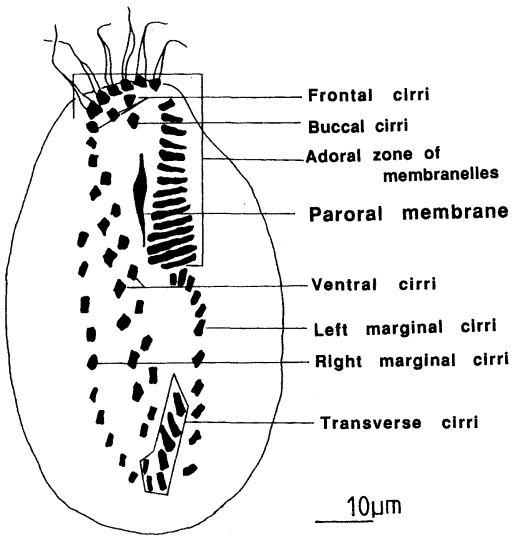

Fig. 4. Diagram summarizing the taxonomical characteristics of the ciliate, *Holosticha diademata* REES, 1884.

terium isolated from coastal seawater of Aburatsubo Inlet, *H. diademata* grew well when the animal was kept with *Pseudomonas* spp. (Table 2).

4. Discussion

The blue green alga *Trichodesmium* is ubiquitous and frequently abundant in sub-equatorial sea areas. It occurs at and/or slightly below the surface water layer and cells (size; 5-10 μm) are linked to each other in strings. The strings form coagulates resulting in colonies which are often large enough to be visible. *Trichodesmium* frequently blooms in the South China Sea and this prominent productive algae which occurs in oligotrophic environments provides a habitat for various microorganisms.

BORROR (1963) reported the distribution of *Holosticha diademata* in the sediment of a salt marsh. We found this species frequently on detritus in coastal seawater in

Table 1. Bacteria isolated from culture liquid of *Holosticha diademata* and growth of the ciliate in their presence

Strain No.	Bacterial genus	Growth of *Holosticha*
HPP 4	*Pseudomonas*	+
HPP 5	*Pseudomonas*	++
HPP 7	*Pseudomonas*	++
HPP 8	*Pseudomonas*	−
HPP 13	*Pseudomonas*	−
HPP 15	*Pseudomonas*	−
HPP 20	*Pseudomonas*	−

\+ : numbers of the ciliate less than 50 cells/cm^{2*}
++ : numbers of the ciliate more than 50 cells/cm^{2*}
± : little identifiable growth of the ciliate
− : no growth of the ciliate
(*: Numbers of *H. diademata* was expressed as the unit of cells/cm^{2*} because this ciliate tended to stay on the bottom of the culture container.)

Table 2. Bacteria isolated form coastal seawater and the growth of *Holosticha diademata* in their presence

Strain No.	Bacterial genus	Growth of *Holosticha*
HPO 1	*Vibrio*	−
HPO 2	*Vibrio*	−
HPO 3	*Pseudomonas*	−
HPO 6	*Pseudomonas*	+
HPO 7	*Vibrio*	−
HPO 8	*Pseudomonas*	±
HPO 15	*Pseudomonas*	++
HPO 16	*Acinetobacter*	±
HPO 17	*Acinetobacter*	−
HPO 19	*Pseudomonas*	−
HPO 62	*Flavobacterium*	−

(Notations are same as those in Table 1.)

eutropic areas, as well as in pelagic environments. The occurrence of the same species of ciliate in both pelagic and coastal areas seems to suggest that even in oligotrophic areas the potential extent of biological productivity might be high in microbial communities. Thes high productivity of ciliates was attributed by the bloom of *Trichodesmium*.

H. diademata fed preferentially on *Pseudomonas* strains of bacteria. Although the reasons for the unsuitability of some bacteria as ciliate feed are still not clear from this study, feeding specificity may also provide a mechanism for niche partitioning among cohabiting bactivorous ciliates in the natural environment, as spatial distribution and temporal succession of ciliates can be explained by prey specificity (NOLAND, 1925; COLER and GUNNER, 1969; TAYLOR and BERGER, 1976).

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References

ALONSO, P., I. GIL and D. RODRIGUEZ (1981): Nutritional efficiency of different bacterial species on the growth of *Tetrahymena pyriformis* W. Protistol., **17**, 199–201.

BORROR, A. C. (1963): Morphology and ecology of the benthic ciliated protozoa of Alligator Harbor, Florida. Arch. Protistenk., **106**, 465–534.

COLER, R. A. and H. B. GUNNER (1969): Microbial populations as determinants in protozoa succession. Water Res., **3**, 149–156.

FENCHEL, T. (1968): The ecology of marine microbenthos II. The food of marine benthic ciliates. Ophelia, **5**, 73–121.

HEINLE, D. R., R. P. HARRIS, J. F. V STACH and D. A. FLEMER (1977): Detritus as food for estuarine copepods. Mar. Biol., **40**, 341–353.

HUGH, R. and E. LEIFSON (1953): The taxonomic significance of fermentative versus Gram negatiave bacteria. J. Bacteriol., **66**, 24–26.

KAHL, A. (1932): Urtiere oder Protozoa I. Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha, *In* Dahl, F.(Ed.) Die Tierwelt Deutschlands und der angrenzenden Meeres-teile. Gustav Fisher, Jena. **25**, 399–650.

KING, K. R., J. T. HOLLIBAUGH and F. AZAM (1980): Predator-prey interactions between the larvacean *Oikopleura dioica* and bacterioplankton in enclosed water columns. Mar. Biol., **56**, 49–57.

MAEDA, M. (1982): Determination of bacterial standing crop in seawater and its significance. Ecol. Microorganisms, Gakkai Shu-ppan, No. **10**, 33–44. (in Japanese)

MAEDA, M. (1986): An illustrated guide to the species of the Families Halteriidae and Strobilidiidae (Oligotrichida, Ciliophora), free swimming protozoa common in the aquatic environment. Bull. Ocean Res. Inst., Univ. Tokyo, No. **21**, 67 pp.

MAEDA, M. (1989): Microorganisms and protozoa as feed in mariculture. Prog. Oceanogr., **21**, 201–206.

MAEDA, M. and P. G. CAREY (1985): An illustrated guide to the species of the Family Strombidiidae (Oligotrichida, Ciliophora), free swimming protozoa common in the aquatic environment. Bull. Ocean Res. Inst., Univ. Tokyo, No. **19**, 68 pp.

MAEDA, M. and N. TAGA (1979): Chromogenic assay method of lipopolysaccharide (LPS) for evaluating bacterial standing crop in seawater. J. Appl. Bacteriol., **47**, 175–182.

NOLAND, L. E. (1925): Factors influencing the distribution of fresh water ciliates. Ecol., **6**, 437–452.

PAFFENHÖFER, G.A. and J.D. H. STRICKLAND (1970):A note on the feeding of *Calanus helgolandicus* on detritus. Mar. Biol., **5**, 97–99.

PARKER, J. G. (1976): Cultural characteristics of the marine ciliated protozoa, *Uronema marinum* Dujardin. J. Exp. Mar. Biol.

Ecol., **24**, 213-226.

REES, E. (1884): Protozoaires de l'escaut de l'est. Tijdschr. Ned. Dierk. Vereen. Suppl. D, **1(2)**, 592-673.

SEKI, H. (1966): Studies on microbial participation to food cycle in the sea Ⅲ. Trial cultivation of brine shrimp to adult in a chemostat (1). J. Oceanogr. Soc. Japan, **22**, 27-32.

SHEWAN, J. M., G. HOBBS and W. HODGKISS (1960): A determinative scheme for the identification of certain genera of Gram-negative bacteria with special reference to the Pseudomonadaceae. J. Appl. bacteriol., **23**, 379-390.

TAYLOR, W. D. and J. BERGER (1976): Growth responses of cohabiting ciliate protozoa to various prey bacteria. Can. J. Zool., **54**, 1111-1114.

TEZUKA, Y. (1974): An experimental study on the food chain among bacteria, *Paramecium* and *Daphnia*. Int. Rev. Gesamten Hydrobiol., **59**, 31-39.

TUFFRAU, M. (1967): Perfectionnements et pratique de la technique d'imprégnation au protargol des infusoires ciliés. Protistol., **3**, 91-98.

WEBB, M. G. (1956): An ecological study of brackish water ciliates. J. Anim. Ecol., **25**, 148-175.

WILLIAMS, P. J. LEB. (1981): Incorporation of microheterotrophic processes into the classical paradigm of the plankton food web. Kiel Meeresforsch., **5**, 1-28.

外洋水域の藍藻より分離した原生動物繊毛虫の特徴

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南シナ海より採集した藍藻，*Trichodesmium thiebautii* に付着している原生動物繊毛虫を分離した。プロタルゴール染色，核染色，および生体観察の結果，この繊毛虫は*Horosticha diademata* (REES, 1884) KAHL, 1932と同定された。この種類は沿岸域海水中にも多く，今回の分離により，海洋における原生動物同一種の広範な分布が示唆された。餌料として，いくつかの細菌種を投与したところ，本繊毛虫は，*Pseudomonas* 属の細菌の存在下において，よく増殖することが明かとなった。