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Factors inducing resting-cell formation of *Coscinodiscus wailesii* Gran (Bacillariophyceae) in culture

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Abstract: In a preliminary attempt at resting-cell formation in the centric diatom *Coscinodiscus wailesii* Gran, we found that resting cells were formed only when vegetative cells had been mixed with marine sediments and incubated in darkness. No effects due to nutrient depletion, temperature, temperature shift, L:D cycle, light shift (from light conditions to dark) or growth phase were observed on resting-cell formation of the diatom without marine sediments. The results of screening for factors that induce resting-cell formation in the diatom, with attention to sediment particles, interstitial waters and the bacteria contained in the sediments, and anaerobic conditions, show that a combination of anaerobic conditions and darkness is the most effective combination of factors for inducing resting-cell formation in *C. wailesii*. Additionally, the existence of sediment particles slightly promotes resting-cell formation.

Key words: *Coscinodiscus wailesii*, diatom, marine sediments, rejuvenation, resting cell

Introduction

Diatoms are usually abundant in high latitude regions, temperate coastal areas, and upwelling systems (Garrison 1984). Resting spores are well known in centric diatoms (Garrison 1984), however, they can also be formed by some pennate diatoms (Stosch & Fecher 1979). Diatoms also form resting cells that do not have morphologically distinguishable frustules from the vegetative cells, but appear to have condensed cytoplasm and darker pigmentation (Hargraves & French 1975; Sicko-Goad et al. 1989). Resting spores or resting cells have been confirmed in diatom genera such as *Chaetoceros* (Cupp 1943; Hargraves 1976; Garrison 1981; Stockwell & Hargraves 1986; Imai et al. 1990; Itakura & Imai 1994), *Melosira* (Sicko-Goad et al. 1986, 1989), *Leptocylindrus* (Hargraves 1976; French & Hargraves 1985; Ishizaka et al. 1987; Imai et al. 1990), *Thalassiosira* (Imai et al. 1990; Syvertsen 1979), and *Skeletonema costatum* (Greville) Cleve (Imai et al. 1990; Itakura et al. 1992). Recently, more than 50 diatom species that form resting stages and references to this phenomenon in the literature, have been reviewed by McQuoid & Hobson (1996). The traditional presumption has been that diatom resting-stage cells are formed in response to adverse conditions, and that the spores germinate when conditions again become suitable for vegetative growth (French & Hargraves 1985). In addition, several functions of resting stages in survival and dispersal strategies are stressed by French & Hargraves (1985), Garrison (1981, 1984) and Pitcher (1990). However, the ecological function of diatom resting-stage cells remains unproven (Round et al. 1990).

A considerable amount of information on resting-spore formation, germination, and survival has been reported according to laboratory experiments or to field surveys (e.g. Durbin 1978; Garrison 1981; French & Hargraves 1985; Pitcher 1990). Many factors, such as shifts in temperature, irradiance, and/or photo cycle, and depletion of phosphorus, silicate, and/or iron, have been reported to induce resting-stage-cell formation (e.g. Stosch & Drebes 1964; Anderson 1975; Hollibaugh et al. 1981; Sicko-Goad et al. 1989). Nitrogen depletion is the most effective factor for triggering spore formation (Drebes 1966; Hargraves & French 1983; Itakura et al. 1993). On the other hand, many of various stresses that induce physiological changes can act as a trigger for resting spore formation in *Eunotia Soleirolii* (Kuetzing) Rabenhorst (Stosch & Fecher 1979) and *Chaetoceros pseudocurvisetus* Mangin (Oku & Kamatani 1995).

Nagai et al. (1995a) found resting cells of *Coscinodiscus wailesii* in the sediments of Harima-nada, eastern Seto In-

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land Sea, Japan. Population dynamics of both vegetative cells in the water column and resting cells in the sediments were investigated in Harima-nada in order to clarify the role of resting cells in *C. wailesii* bloom events (Nagai et al. 1996). The results suggest that resting cells play an important role in initiating the blooms of the diatom in Harima-nada. In the present study, attempts were carried out to examine factors that induce resting-cell formation in *C. wailesii* under laboratory conditions.

Materials and Methods

Preliminary experiments

C. wailesii, axenic clone strain LA2 (Nagai et al. 1995b) was maintained in 50-ml capacity flasks containing 40-ml MP1 culture medium (Nagai & Manabe 1994) at a temperature of 10°C under a photon flux of 35 μmol m^{-2} s^{-1} in a 12:12h L:D cycle. Conditions that induced resting-cell formation in *C. wailesii* were examined with various combinations of nutrient depletion, temperature and light. The procedure for the preliminary study is shown in Fig. 1. For nutrients, the following four media; full MP1 culture medium, the N-depleted, the P-depleted, and the Si-depleted media, were prepared in advance in 200-ml flasks containing 100 ml of each medium. For pre-incubation, some vegetative cells of the diatom were inoculated into each medium and incubated under combinations of temperatures of 10, 15, 20, and 25°C under a photon flux of 45 μmol m^{-2} s^{-1} at two L:D cycles of 16h:8h and 8h:16h, and then incubated for three weeks at 20 and 25°C or four weeks at 10 and 15°C. In the N-, P- and Si-depleted media, 1-ml aliquots of the culture media including 1000–1500 vegetative cells were inoculated into new flasks of each medium and cultivated under the same conditions as the pre-incubation for 1 week. On the other hand, in the full MP1 medium, pre-incubated cultures of 50 cells at 15–25°C and of 200 cells at 10°C were inoculated into new flasks and cultivated under each L:D cycle for 2–3 weeks until the mid–late exponential growth phases occurred. Flasks were transferred into dark conditions at the same temperature for 2 weeks. The flasks cultivated at 10 and 20°C were also placed in darkness at 6 and 10°C, respectively, for the same period (total 48 tests). In addition, to examine the influence of growth phase on resting-stage-cell formation, vegetative cells were incubated until 3 different growth phases, the early (3 cells ml^{-1}), mid (35 cell ml^{-1}), and late (120 cell ml^{-1}) phases, occurred under conditions of 20°C with a photon flux of 45 μmol m^{-2} s^{-1} in a 8:16-h L:D cycle. Flasks were then transferred into dark conditions at the same temperature for 2 weeks (total 3 tests). After the incubation, more than 300 cells were observed in each condition by a conventional light microscopy. The morphological distinction of a resting cell of the diatom was based on the characteristics reported by Nagai et al. (1995a).

An attempt to form resting cells was carried out by inoculation of vegetative cells of *C. wailesii* into marine sedi-

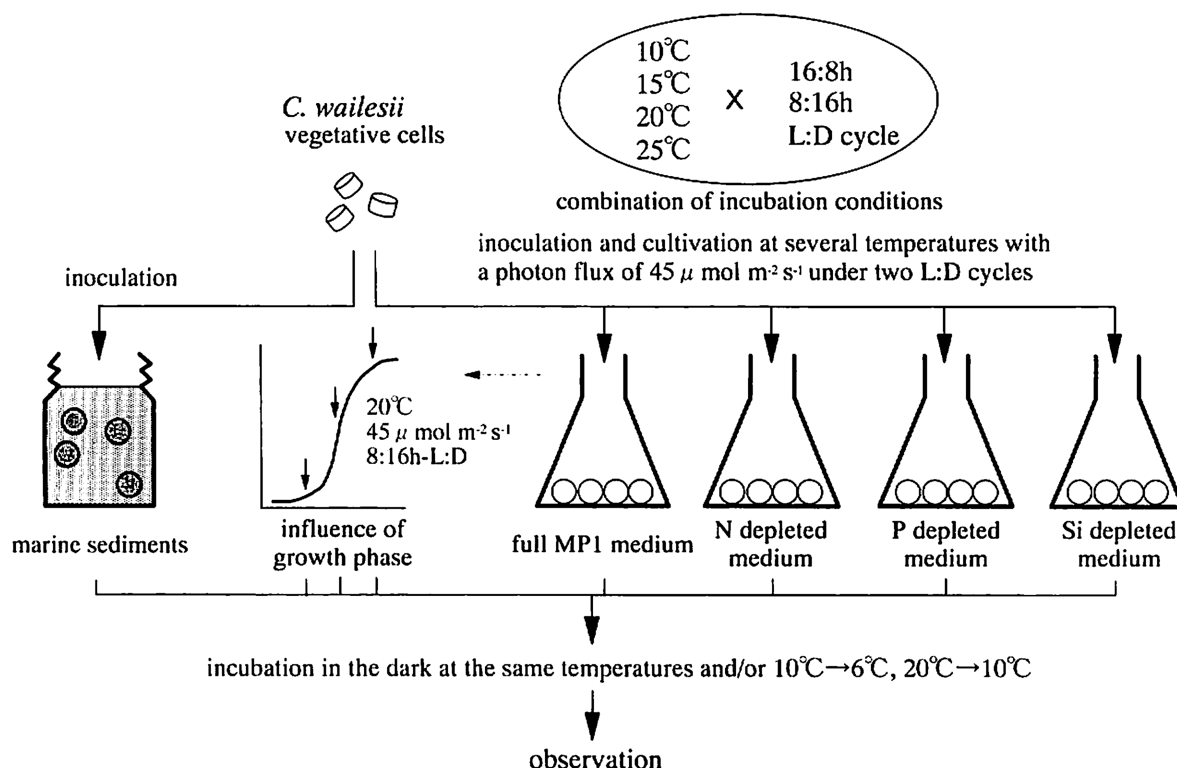

Fig. 1. Procedure for the preliminary study on the induction of resting-cell formation in *Coscinodiscus wailesii* in culture.

ments and incubation of them under dark conditions as follows. Sediments were collected with a gravity core-sampler (Kimata et al. 1960) from Sta. H2 (134°40.0′E, 34°42.8′N) in Harima-nada, eastern Seto Inland Sea, Japan in July 1994 and stored at 10°C for several months (moisture content of the sediment, 27.6%; median particle diameter, 218 μm; sulfide concentration, 0.07 mg g^{-1} dry sediment; see Nagai 1995). No resting cells of *C. wailesii* were reported in the sediment under observation with an inverted epifluorescence microscope (Nagai et al. 1996). Ten-ml glass vials filled with 15 g of the sediment were then prepared. The vegetative cells were cultivated until the middle exponential growth phase in MP1 medium at each of the temperatures and photo cycles mentioned above. The cells were collected on a nylon sieve (mesh size 100 μm), and ca. 1000 cells concentrated in 400 μl of the MP1 medium were inoculated into the glass vials. The vials were tightly sealed and placed in the dark for 2 weeks at each of temperatures mentioned above (total 12 tests). After the incubation under dark conditions, the sediment suspensions were sieved through nylon meshes with pore sizes between 100 and 500 μm in order to obtain *C. wailesii* cells, and more than 200 resting cells were then observed by the conventional light microscopy.

The rejuvenation process of a resting cell formed in this experiment was observed by incubation of this resting cell in a well of a 24-well microplate (Costar) inoculated with 1.5 ml of MP1 medium at 20°C under continuous illumination with a photon flux of 65 μmol m^{-2} s^{-1}. Sequential observations were carried out at 2, 6, 11, 12, 13, 14, and 16 h into the incubation.

Effect of dilution of sediments with MP1 medium on resting-cell formation

In the above preliminary study of resting-cell formation in *C. wailesii*, no effects due to nutrient depletion, temperature, temperature shift, L : D cycle, light shift (from lighted conditions to darkness) and/or growth phase were observed on resting-cell formation in this diatom. However, we found that the resting cells were only formed when vegetative cells had been inoculated into marine sediments and incubated in darkness.

In this study, the influence of dilution of sediments with MP1 medium was examined to clarify the effect of sediments on resting-cell formation in this diatom. The same sediments as mentioned above were diluted with MP1 medium to several concentrations: 100 (no dilution), 50, 35, 20, 10, 5, 2, 1 and 0.5% (w/w). The 15 g of sediment (100%) and 9-ml aliquots of each mixture were then inoculated into 10-ml glass vials in triplicate. The MP1 medium used for the dilution was previously adjusted to pH 7.5 because this was the pH of the sediment. *C. wailesii* vegetative cells were incubated at 20°C under a photon flux of 45 μmol m^{-2} s^{-1} with an 8 : 16-h L : D cycle until the mid-late exponential growth phases occurred (50–100 cells ml^{-1}). The cells were washed through a nylon sieve submerged in MP1 medium at pH 7.5. About 1000 cells concentrated in 400 μl of the MP1 medium were inoculated into each glass vial. After sealing the vials with caps, the vials were then kept under dark conditions at 20°C for 20 d. The number of resting cells and vegetative cells were thereafter enumerated to estimate the incidence of resting-cell formation in each mixture.

Screening of factors influencing resting cell formation

According to the effects of sediment dilution with MP1 medium on resting-cell formation, we tried to do a screening of factors actually stimulating resting-cell formation of the diatom with attention to particle size, interstitial waters, bacteria in sediments and anaerobic conditions. Sediments were collected from Sta. H2 in Harima-nada in August 1994 and stored at 10°C in the dark for several months. These sediments were used in the experiments.

Sediments were fractionated to the size ranges of <63, 63–125, 125–250 and 250–500 μm through sieves, and washed well with distilled water before desiccation at 110°C for 3 d. Fourteen grams of each size class of sediments were mixed with 7 ml of MP1 medium at pH 7.5 and the mixtures were then poured into triplicate 10 ml glass vials. *C. wailesii* vegetative cells were inoculated as outlined above. The vials were placed at 20°C for 20 d under dark conditions. After the incubation the number of resting cells, vegetative cells, and dead cells were enumerated to estimate the incidence of resting cell formation in each vial. The percentage value for the number of surviving cells divided by that of total cells (S/T), the percentage value for the number of resting cells divided by that of the surviving cells (R/S), and the percentage value for the number of resting cells divided by that of total cells (R/T) was then calculated. The S/T was 96.6±2.3 (mean±SD) % when the diatom cells were inoculated into the fractionated sediments.

Interstitial water was obtained by the centrifugation of 200 g of sediment in a 350-ml polypropylene centrifugation tube at 5000 rpm for 15 min. and filtrated through a 0.22-μm-pore filter (Millipore). Nine-ml aliquots of the interstitial water, of MP1 medium (pH 7.5), of 15 g of the untreated sediment and of 15 g of the autoclaved sediment (121°C for 15 min) were poured in triplicate into 10-ml glass vials. All vials were aseptically prepared except for those containing the untreated sediment. *C. wailesii* vegetative cells were then inoculated as described above. Two sets of these vials for each of the experimental conditions were prepared to examine the effect of anaerobic conditions on resting-cell formation in the diatom. For one set, the vials were sealed with a cap and then placed in the dark. For the other set, the vials were lightly sealed with a cap and then put into a plastic jar with a GasPak anaerobic system (BBL). After checking for anaerobic conditions in the jar with a dry anaerobic indicator (BBL), the jar was placed in the dark. Both sets were kept at 20°C for 20 d in the dark.

After the incubation the number of resting cells, vegetative cells, and dead cells were measured to estimate the incidence of resting-cell formation for each of the experimental conditions.

Results

Resting-cell formation of *C. wailesii*

In the preliminary attempt at resting-cell formation in *C. wailesii*, no effects due to nutrient depletion, incubation temperature or temperature shift, light shift and/or L:D cycle were observed. In full MP1 medium, no resting cells were formed at any growth phase. However, we found that resting cells morphologically similar to those observed in natural sediments (Nagai et al. 1995a) were only formed when vegetative cells had been mixed with sediments and incubated under dark conditions (Fig. 2). Figure 3 shows resting cells (A) formed in the sediment, and vegetative cells that did not turn into resting cells in full MP1 medium (B). The resting cells are distinct from vegetative cells, in that the cytoplasm of resting cells is partially separated from the frustules and is concentrated towards the center of the cell.

Figure 4 shows the sequential rejuvenation process of an artificially formed resting cell. The resting cell rapidly begins to rejuvenate (A, B) and after 6 h it is difficult to distinguish it from common vegetative cells (C). The rejuvenated cell then turns into a vegetative cell without forming new valves for vegetative cells (D). After 11 h the cell slightly elongates (D), and forms a cell disk necessary for cell division after 12 h (E). After 13–14 h the first cell division begins (F, G) and after 16 h the cell has finished the division (H).

Culture medium	Temperature (℃) 10→10	10→6	15→15	20→20	20→10	25→25	L:D cycle
full MP1	−	−	−	−	−	−	8hL
	−	−	−	−	−	−	16hL
MP1 (N depletion)	−	−	−	−	−	−	8hL
	−	−	−	−	−	−	16hL
MP1 (P depletion)	−	−	−	−	−	−	8hL
	−	−	−	−	−	−	16hL
MP1 (Si depletion)	−	−	−	−	−	−	8hL
	−	−	−	−	−	−	16hL
sediment with MP1	−	−	+	+	+	−	8hL
	+	−	−	+	−	−	16hL

+ : formation of resting cells, − : no formation

Fig. 2. Induction of resting cells of *Coscinodiscus wailesii* in culture. Effects of nutrients (N, P, or Si depleted media of MP1 culture medium), temperature (10, 15, 20, and 25°C), temperature shift (20°C→10°C, 10°C→6°C) and L:D cycle (8 h:16 h and 16 h:8 h L:D), light shift (from light conditions to dark), and growth phase, on the resting-cell formation of the diatom were investigated. In addition, an attempt to induce the formation of resting cells was carried out by the inoculation of vegetative cells of *C. wailesii* into marine sediments and incubation of them in the dark. The numerals at the left of the arrows show the incubation temperatures of vegetative cells and those on the right show the storage temperatures in darkness.

Effect of dilution of sediments with MP1 medium on resting-cell formation

In the 0.5% diluted sediment, the percentage of the number of resting cells formed divided by that of the surviving cells (R/S) was 6.7%, with the R/S of the undiluted sediment (100%) being 71.7% (Fig. 5). The proportion of cells forming resting cell gradually decreased as the sediment was diluted. This result clearly indicates that resting cell formation of the diatom is induced by the addition of sediments.

Screening of factors influencing resting-cell formation

Table 1 shows the effect of the particle size of sediments on resting-cell formation in *C. wailesii*. The percentage of the number of surviving cells to that of total cells (S/T) in

Fig. 3. Light microphotographs of resting cells of *Coscinodiscus wailesii* (*arrows*) formed in culture with sediments (**A**) and vegetative cells that did not turn into resting cells in full MP1 medium (**B**). The resting cells (**A**) are distinct from the vegetative cells (**B**) because the cytoplasm of resting cells is partially separated from the frustules and is concentrated towards the center of the cell.

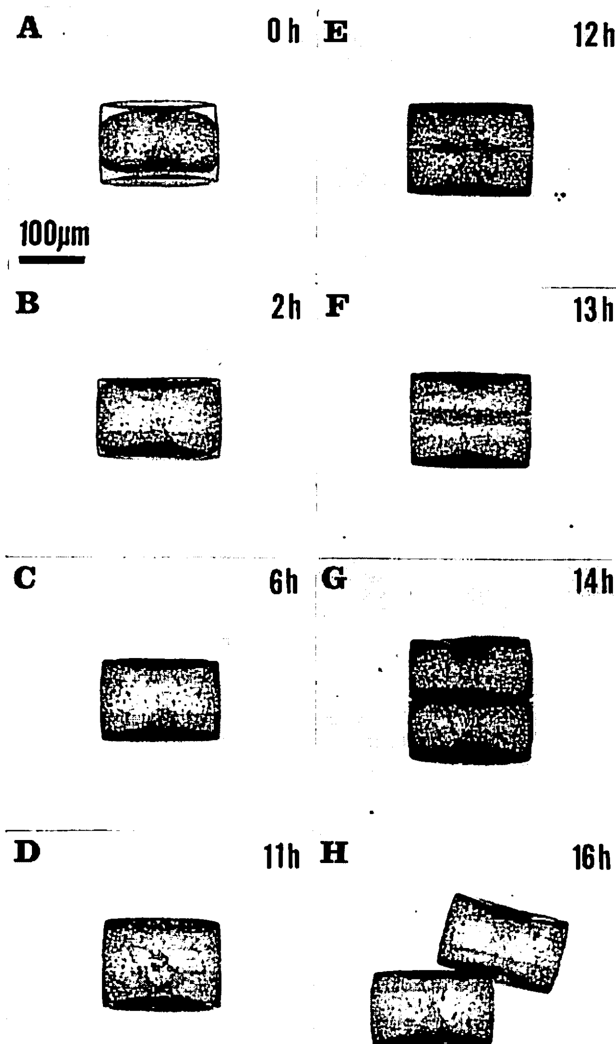

Fig. 4. Sequential observation of the rejuvenation process in a resting cell of *Coscinodiscus wailesii* artificially formed in sediments in culture. **A.** A resting cell of the diatom at the onset of incubation. **B.** The onset of rejuvenation. **C.** A resting cell indistinguishable from a normal vegetative cell. **D, E.** Elongation of the cell and formation of a cell disk. **F, G.** Growth of each daughter cell. **H.** The first cell division. Incubation time is indicated at the upper right of each figure.

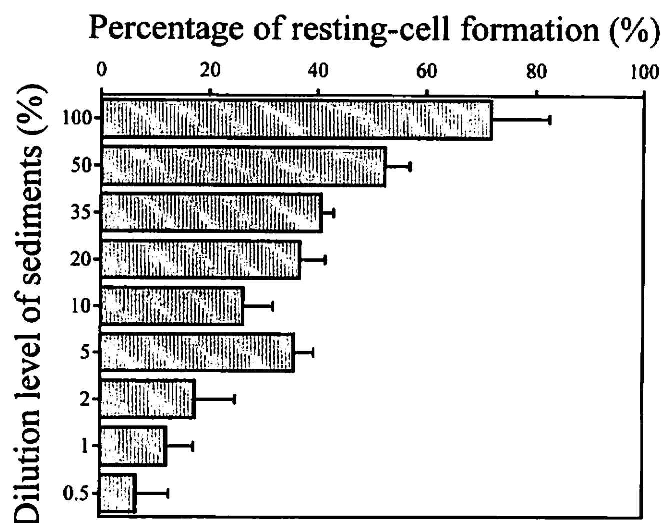

Fig. 5. Effects of dilution of sediments with MP1 medium on resting-cell formation of *Coscinodiscus wailesii*. The sediment was diluted with MP1 medium to several concentrations: 100 (no dilution of the sediment), 50, 35, 20, 10, 5, 2, 1 and 0.5% (w/w).

Table 1. Effects of marine sediment particles of different size fractions on the resting-cell formation of *Coscinodiscus wailesii* in culture.

		Size fraction of sediment particles (µm)			
		<63	63–125	125–250	250–500
S/T (%)[a]	Mean	8.5	21.8	22.6	15.6
	S.D.	0.6	4.3	3.5	3.5
R/S (%)[b]	Mean	72.4	77.8	33.0	33.0
	S.D.	7.0	6.8	8.6	10.5
R/T (%)[c]	Mean	6.2	16.7	7.6	4.9
	S.D.	1.0	1.9	2.6	1.2

[a] Percentage of the number of surviving cells to that of total cells.
[b] Percentage of the number of resting cells to that of surviving cells.
[c] Percentage of the number of resting cells to that of total cells.

the <63 µm size fraction was 8.5% and was markedly lower than the others. The R/S of <63 and 63–125 µm size fractions were 72.4 and 77.8%, respectively, and were higher compared with those of the 125–250 and 250–500 µm size fractions (both were 33.0%). In the 63–125 µm fraction, the percentage of the number of resting cells formed to that of total cells (R/T) was 16.7% and was the highest of the group. Accordingly, the size of sediment particles is an important factor influencing resting-cell formation in this diatom.

Figure 6 shows the effects of interstitial water and bacteria in the sediment on resting-cell formation in the diatom under both anaerobic and aerobic conditions. Under aerobic conditions with untreated sediment, resting cells were frequently formed and the R/S was 92.1%. In the autoclaved sediment, the R/S was 33.5% and lower than that in the untreated sediment ($p<0.001$). In addition, the S/T of the autoclaved sediment was about 20% lower than that in the untreated sediment. Accordingly, the R/T of the autoclaved sediment was remarkably lower than that of the untreated sediment ($p<0.001$). No resting cell formation was observed in MP1 medium under aerobic conditions, although a few resting cells were formed in the interstitial water. On the other hand, under anaerobic conditions, the R/S of the interstitial water, MP1 medium, the untreated sediment, and the autoclaved sediment was 83.3, 82.4, 86.2, and 86.9%,

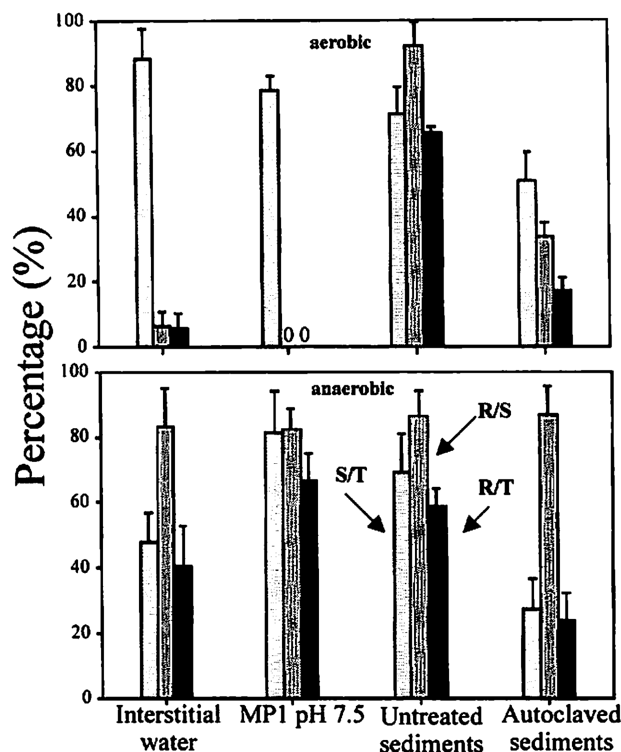

Fig. 6. Effects of interstitial waters from a marine sediment, the untreated sediment, the autoclaved sediment and MP1 culture medium adjusted to pH 7.5, on resting-cell formation of *Coscinodiscus wailesii* under aerobic and anaerobic conditions in culture.

respectively. In particular, the R/S value of the interstitial water and MP1 medium under anaerobic conditions were much higher than those under aerobic conditions. This finding indicates that an anaerobic atmosphere is a crucial factor inducing resting-cell formation in this diatom. The S/T values of the interstitial water and the autoclaved sediment under anaerobic conditions were markedly lower than those under aerobic conditions. The R/T of the autoclaved sediment was significantly lower than that of the untreated sediment under both aerobic and anaerobic conditions ($p<0.005$).

Discussion

Natural resting cells of *C. wailesii* can survive in darkness for long periods of at least 15 months at a temperature of 10°C and can rejuvenate rapidly under adequate incubation conditions (Nagai et al. 1995a). We have also confirmed that resting cells of the diatom artificially formed in culture survive and rejuvenate after at least 15 months preservation at 10°C in darkness, although vegetative cells can survive for only 3 months in the dark.

Resting spores are morphologically distinct from vegetative cells because they are heavily silicified by additional cell divisions that differ from normal vegetative cell divisions (Hargraves 1976). In the formation of resting cells, alterations in the frustules are not accompanied by additional cell divisions and it is difficult to distinguish them from vegetative cells through the external morphology (Lund 1954; Anderson 1975). The resting cells (Figs. 3A, 4A) show no sign of alterations in the frustules through additional cell divisions, and the morphological differences are less distinct than those between typical resting spores and vegetative cells. However, resting cells differ from vegetative cells in the alteration of cytoplasmic characteristics such as condensed cytoplasmic mass, darker pigmentation, or unusual plastid structure (Anderson 1975; Sicko-Goad et al. 1986; Itakura et al. 1992). In addition, the accumulation of lipid granules have been observed in the bodies of resting cells of diatoms such as *Amphora coffaeformis* (Ag.) Kütz (Anderson 1975), and several *Melosira* and *Fragilaria* species (Sicko-Goad et al. 1986, 1989). Greenish granules observed in natural resting cells of *C. wailesii* (Nagai et al. 1995a) also appear to be composed of a kind of lipid. The morphology of the resting cells of *C. wailesii* is in accordance with the characteristics of typical resting cells as reported above.

A contrasting view might be that the cells shown in Figs. 3A and 4A are not resting cells but are in fact plasmolysed cells as demonstrated in *Ditylum brightwellii* (West.) by Gross (1939). In our experience, which includes the maintenance of more than 100 clone cultures of *C. wailesii* and the undertaking of laboratory experiments such as induction of sexuality and vegetative cell enlargement (Nagai & Manabe 1994; Nagai et al. 1995b; Nagai & Imai 1998), however, plasmolysed cells have hardly ever been observed, suggesting that this is indeed a difference in the physiological characteristics of these two diatoms. Once plasmolysis of the vegetative cells occurs and the plasmolysed cells are extruded from their frustules, only a few cells are able to survive through regenerative methods such as (1) pseudo-auxospore formation directly from the protoplasts, and (2) new valve formation with much size reduction (Stosch 1965; Nagai et al., unpublished data). However, the resting cells use their frustules whenever they rejuvenate (Fig. 4). In addition, resting cells can survive for long periods in marine sediments (15 months at 10°C). Accordingly, it is obvious that the cells shown in Figs 3A and 4A are typical diatom resting cells.

The results shown in Table 1 suggest that the particle size of sediments has a profound effect on resting-cell formation. It seems likely that there is an optimum size range for sediment particles to promote resting-cell formation. It is assumed that the existence of sediment particles causes the formation of an anaerobic micro space around the diatom cells through the respiration of the diatom itself in darkness. This would explain why resting cells are formed when sediment particles are incubated in the dark. However, though the experiment used sediment particles, the S/T values for all size fractions (Table 1) were markedly lower than those for untreated (containing all size classes) sediment (Fig. 6). It may be necessary to examine the effect of combinations of different particle size classes to fully under-

Table 2. Variables inducing resting-spore or resting-cell formation.

Species	N	P	Si	Fe	pH	Tem-perature	Salinity	Light	Photo cycle	Source
Amphora coffaeformis						+		+		Anderson (1975)
Chaetoceros sp.	+							(+)		French & Hargraves (1980)
C. anastomosans	+						(+)			Oku & Kamatani (1995)
C. compressus	+	+								Oku & Kamatani (1995)
C. debilis	+									Garrison (1981)
C. diadema	+							(+)		French & Hargraves (1980, 1985)
C. diadema									+	Hollibaugh et al. (1981)
C. didymus									+	Hollibaugh et al. (1981)
C. didymus	+									Itakura et al. (1993)
C. pseudocurvisetus	+									Kuwata & Takahashi (1990)
C. pseudocurvisetus	+	+								Oku & Kamatani (1995)
C. socialis	+									French & Hargraves (1980)
C. teres	+									French & Hargraves (1980)
C. vanheurckii	+									Garrison (1981)
C. vanheurckii									+	Hollibaugh et al. (1981)
Ditylum brightwelli	+									Hargraves (1984)
Detonula confervacea	+					(+)				Durbin (1978)
D. confervacea	+	+								Syvertsen (1979)
Eunotia soleirolii	+	+	+	+	+	+				Stosch & Fecher (1979)
Leptocylindrus danicus	+									Davis et al. (1980)
L. danicus	+					(+)				French & Hargraves (1980, 1985)
Melosira granulata						+		+		Sicko-Goad et al. (1989)
Stephanopyxis palmeriana									+	Steele (1965)
S. palmeriana	+	+								Drebes (1966)
S. turris						+		+		Stosch & Drebes (1964)
S. turris	+									French & Hargraves (1980)
Thalassiosira antarctica	+	+								Syvertsen (1979)
T. nordenskioeldii	+					(+)				Durbin (1978)
T. nordenskioeldii	+	+								Syvertsen (1979)

+, effective; (+), has effect when coupled with primary factor. The table was modified from Table 1 in Hargraves & French (1983).

stand the effect of sediment particles on resting-cell formation.

In the autoclaved sediment, a significantly lower S/T was obtained than for untreated sediment under both aerobic and anaerobic conditions (Fig. 6). Although we expected to be able to clarify the effect of bacteria on diatom resting-cell formation by comparing untreated sediments and autoclaved sediments, no clear evidence of bacterial effects was obtained. There is a possibility that the cells were damaged by toxic matter released from the sediment through autoclaving. Therefore, we were unable to accurately evaluate bacterial effects in this study. However, since bacterial metabolites are known to bring about oxygen deficiency in marine sediments, it is certain that bacteria play at least an indirect role in regulating resting-cell formation in *C. wailesii*.

Resting cells formed in MP1 medium under anaerobic conditions were morphologically identical to resting cells formed in the sediments (data not shown). That resting cells were frequently formed in the interstitial water and MP1 medium under dark anaerobic conditions (Fig. 6) indicates that as long as the vegetative cells are subjected to anaerobic conditions and darkness, they are fully able to change into resting cells even without the presence of sediment particles. Nagai et al. (1995a) clarified that when this diatom is isolated from sediments in nature resting cells are formed regardless of the cell division cycle.

Nagai et al. (1996) found that even under suitable conditions of temperature, nutrients, and light, natural resting cells of the diatom were unable to rejuvenate under oxygen deficient conditions in culture. In artificially formed resting cells cultured anaerobically, no rejuvenation was observed when incubations was under illuminated conditions. In addition, almost all cells died after 1 week of anaerobic incubation (data not shown). Therefore, it is assumed that a combination of anaerobic conditions and darkness is indispensable in the formation of resting cells of *C. wailesii*. When a spring bloom of this diatom declined in the water column and the number of resting cells increased in the sediments, a few vegetative cells were found in the sediments of the Harima-nada (Nagai et al. 1996). This suggests that the vegetative cells changed into resting cells upon sinking into the bottom layer or after sedimentation. The authors also proposed that the autumn bloom of *C. wailesii* can be triggered by the resuspension of resting cells through vertical mixing. At the end of summer when vertical mixing occurs, resting cells are released from oxygen deficient conditions and are able to remain for a longer period in the euphotic zone, allowing rejuvenation and vegetative growth. The seasonal fluctuation of natural resting cells in sediments (i.e. formation and rejuvenation) can be well explained by the interaction of two factors: dissolved oxygen levels (anaerobic or aerobic) and light conditions (light or dark).

A summary of conditions that induce the formation of resting-stage cells in diatoms was gleaned from the literature and is shown in Table 2. In planktonic diatoms, nitrogen depletion is the most effective factor triggering resting spore formation (Drebes 1966; Hargraves & French 1983; Itakura et al. 1993). For many planktonic species the formation of resting spores is not induced by darkness (Hargraves & French 1975). On the other hand, a combination of slightly reduced temperatures and darkness was most efficient for producing resting cells of the fresh water diatoms *Amphora coffaeformis* and *Melosira granulata* (Ehr.) Ralfs (Anderson 1975; Sicko-Goad et al. 1989). Incubation in the dark is necessary to induce resting-cell formation in *C. wailesii* (present study). Accordingly, the effect of light on resting-cell formation may be different from that for resting spores (French & Hargraves 1980). On the conditions for cyst formation in *Heterosigma akashiwo* (Hada) Hada (Raphidophyceae), a similarity to that for resting-cell formation in *C. wailesii* has been reported by Itakura et al. (1996). *H. akashiwo* cysts were formed by the addition of 5 g of autoclaved marine sediment in a 500-ml aliquot of sample water containing natural vegetative cells and by culture under dark conditions.

In the present study, we defined the factors inducing or triggering resting-cell formation in *C. wailesii*. The survival periods for resting cells in *C. wailesii* formed by mixing vegetative cells with sediments depend on the incubation temperature in the dark, i.e. the periods were longer in the order of 10, 6, 15, 20, and 25°C (Nagai et al. 1999). In general, temperature strongly influences the dark survival periods of diatoms (Smayda & Mitchell-Innes 1974; Hargraves & French 1975; Antia 1976; Yamochi 1989; Itakura et al. 1997). Therefore, temperature may be one of the most important factors influencing the dark survival of resting cells in *C. wailesii*. Lund (1954) reported that resting cells of *Melosira italica* (Ehr.) Kütz were able to survive dark, anaerobic conditions for up to three years. Viable resting cells of *M. granulata* were obtained from anoxic lake sediments, although Davis et al. (1980) and Hollibaugh et al. (1981) reported that anoxia was considered to be an adverse environmental factor that reduces spore viability (Sicko-Goad et al. 1986). It remains necessary to further clarify how important anaerobic conditions and darkness are for the dark survival of diatom resting cells.

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