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# Availability of Deep Seawater and Effects of Bacteria Isolated from Deep Seawater on the Mass Culture of Food Microalga *Chaetoceros ceratosporum*

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The possible availability of deep seawater (DSW; seawater under the euphotic layer) and bacteria isolated from DSW (BDS) for the mass culture of food microalga *Chaetoceros ceratosporum* was investigated. Seawater samples pumped up from a depth of 320 m at the kochi Deep Seawater Laboratory in Muroto, Kochi Prefecture, were collected and used for cultivation of *C. ceratosporum*. Growth rate ($\mu$: reciprocal of the dubling time) and maximum cell yields (MCY) of the alga in untreated DSW were 1.25–2.22 (average 1.72)·day$^{-1}$ and 2–11 (average 6)$\times 10^5$ cells/m*l*, respectively. When compared with those obtained by $ASP_6$ medium, the average $\mu$ in DSW was about 90% and the values of MCY were 5–20% of those in $ASP_6$. The values of MCY, however, showed a relatively large variance according to the sampling season of DSW. When some BDS were added to DSW, the growth of *C. ceratosporum* was often stimulated significantly; the $\mu$ values were 1.82–2.86 (average 2.27)·day$^{-1}$ and MCYs were 4–12 (average 8)$\times 10^5$ cells/m*l*. Moreover, the variance of $\mu$ and MCY according to the sampling season tended to decrease when BDS were added. These results suggest that DSW has a high potential for cultivating food microalgae, and it can be made more stable and effective by adding BDS.

Seawater under the euphotic layer, which is defined here as "Deep Seawater (DSW)", has several characteristic properties; the water temperature is constant of below 10°C throughout the year, the water is very clean, and contains less organic matter and few pathogenic microorganisms. Recently, the possible availability of DSW due to these useful properties has been intensively studied by several research groups. In Hawaii, DSW is used for maricultures of abalone and "*nori*" *Porphyra tenera* after it has been used for the air conditioning of housing and electricity generation.*3 DSW of fjords is also utilized for some salmon production in Norway.*4 Mass cultivation of food microalgae using high concentration of inorganic nutrients in DSW is another possibility, which was introduced from high productivities in upwelling areas[1] due to its abundant nutrients. The facility of the Deep Seawater Laboratory of Kochi was founded at Muroto City in Kochi in 1989 and DSW from 320 m depth is pumped up to the laboratory. A large amount of DSW has recently been readily available in Japan as well.

In DSW, on the other hand, is contained a relatively small amount of organic chelators, which resulted in the elongation of lag phases of phytoplankton growth,[2] and also contained are some toxic substances such as copper ions.[3]

*Chaetoceros ceratosporum* is a species of monodispersed diatom which is suitably used as a living food for planktonic larvae of some sea urchins and bivalves.[4,5] It is therefore very important to establish a stable mass-culturing technique for the species, and the potential availability of DSW for this purpose has recently been studied.

It is now widely recognized that bacteria are one of the most effective organisms that influence the growth of microalgae.[6-8] A previous study[9] reported that several bacterial strains isolated from DSW (BDS: bacteria from deep seawater) showed significant growth-promoting effects on

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*C. ceratosporum* and no BDS among ca. 100 isolates did show any inhibitory effects on the growth of this alga. These results suggest the possibility of stimulating the cultivation of *C. ceratosporum* by using the BDS. In that work,[9] however, a nutrient-rich medium was used for incubating the alga, and there is no information available on the potentiality of DSW and the effect of BDS to culture the *C. ceratosporum* in DSW.

In the present study, we investigated the availability of DSW for cultivating a food microalga *C. ceratosporum* and evaluated the possibility to establish the stable mass culture of the alga by using the BDS.

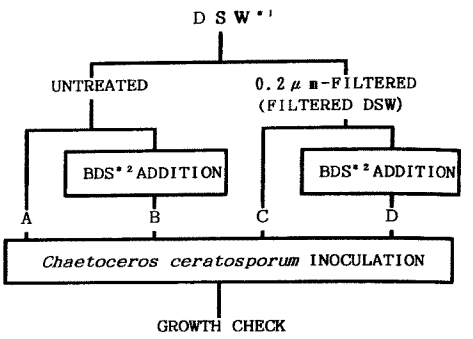


**Fig. 1.** Scheme of the experimental procedure. DSW,*1 deep seawater; BDS,*2 bacteria isolated from deep seawater.

## Materials and Methods

*C. ceratosporum* was kindly provided from Japan Marine Science and Technology Center. An axenic clone culture of the alga was obtained by successive rinsing with capillaries in a mixture of 5 antibiotics (streptomycin, penicillin G potassium salt, polymyxin, chloramphenicol, and erythromycin; 20 μg/m*l* for each).[9] DSW of 320 m depth was collected with sterile glass bottles at Deep Seawater Laboratory of Kochi from April to December in 1990. To subsamples of either untreated DSW or those filtered through sterile 0.2 μm Millipore filters ("filtered DSW") were inoculated an axenic culture of *C. ceratosporum* previously incubated in fiter-sterilized DSW for 4 days. The initial density of *C. ceratosporum* was *ca.* $5\times10^3$ cells/m*l*. During incubation for 1 week at 20°C with the L: D cycle of 12: 12 under 10,000 lux, the numbers of the alga were counted and growth patterns were compared with those in $ASP_6$ medium to evaluate the potentiality of DSW for culturing of *C. ceratosporum* (Fig. 1, A and C).

Eight strains of BDS (DM-6, DM-10, DO-5, DO-7, DN-8, DN-9, DN-10, and DN-17), which showed significant growth-promoting effects on *C. ceratoporum* in $ASP_6$ medium as described previously,[9] were used for the experiment. Each BDS was incubated at 15°C for 2 or 3 days in a FeTY medium containing 0.5 g trypticase peptone, 0.05 g yeast extract, and 0.01 g ferric citrate in 80% aged seawater, and both bacterial cells and *C. ceratosporum* in the exponential growth phases were added simultaneously to other subsamples of both untreated and filtered DSW. These samples were incubated in the same way as described as above, and the effects of BDS on the growth of the alga in DSW were determined (Fig. 1, B and D). Initial densities of the alga and the bacteria were about $5\times10^3$ and $1\times10^5$ cells/m*l*,

**Table 1.** Seasonal fluctuations of the potentiality of untreated or 0.2 μm-filtered (Filtered) Deep Seawater (DSW) for the growth of *Chaetoceros ceratosporum*

| Sampling Date | Untreated DSW | | Filtered DSW | |
|---|---|---|---|---|
| | $\mu$*1 day$^{-1}$ | MCY*2 $\times10^5$ cells/m*l* | $\mu$*1 day$^{-1}$ | MCY*2 $\times10^5$ cells/m*l* |
| Apr. 20 | 2.22 | 9.1 | 2.00 | 6.7 |
| Jun. 28 | 2.00 | 7.7 | 2.22 | 7.7 |
| Aug. 28 | 1.67 | 2.2 | 1.67 | 2.4 |
| Sep. 28 | 1.67 | 2.6 | 1.67 | 5.9 |
| Oct. 15 | 1.25 | 5.3 | 1.33 | 8.1 |
| Dec. 19 | 1.53 | 11.0 | 1.67 | 12.5 |
| Average (±SD) | 1.72 (±0.31) | 6.3 ( ±3.3) | 1.7 (±0.28) | 7.2 (± 3.0) |

*1 Growth rate as reciprocal of the dubling time (d).
*2 Maximum cell yield.

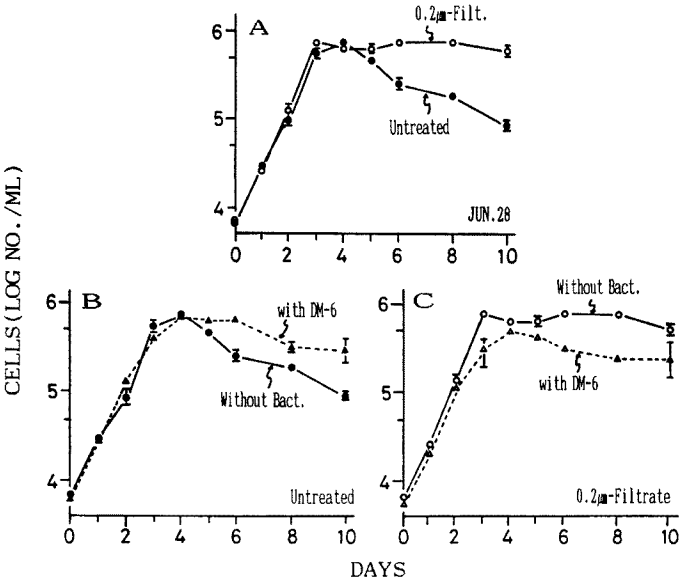


**Fig. 2.** Growth curves of *Chaetoceros ceratosporum* in untreated or 0.2 μm-filtered Deep Seawater (DSW) (A), and with or without a bacterium (DM-6 strain) isolated from DSW in untreated (B) or filtered (C) DSW collected on June 28, 1990.

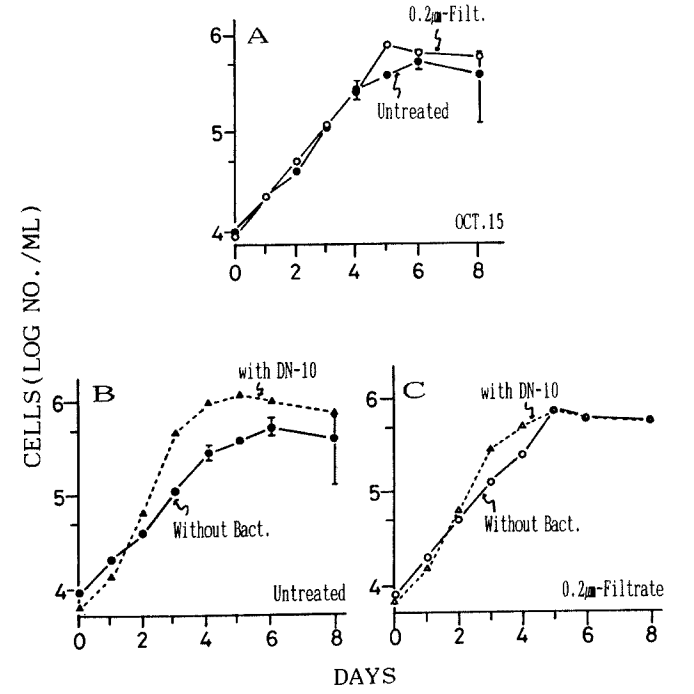


**Fig. 3.** Growth curves of *Chaetoceros ceratosporum* in untreated or 0.2 μm-filtered Deep Seawater (DSW) (A), and with or without a bacterium (DN-10 strain) isolated from DSW in untreated (B) or filtered (C) DSW collected on October 15, 1990.

respectively.

## Results and Discussion

Two examples of the growth pattern of *C. ceratosporum* in untreated DSW were illustrated in Figs. 2A and 3A. Growth rates ($\mu$) calculated as reciprocals of the dubling time and maximum cell yields (MCY) of the alga in untreated DSW were 1.25–2.22 (average 1.72)·day$^{-1}$ and 2–11 (average 6)$\times 10^5$cells/m*l*, respectively (Table 1). Since the $\mu$ and MCY of *C. ceratosporum* growing in $ASP_6$ medium were 1.92·day$^{-1}$ and $6\times 10^6$ cells/m*l*, respectively,[9] the average $\mu$ in DSW was about 90% and values of MCY were 5–20% of those in $ASP_6$. It was previously reported that the minimal cell density of *C. ceratosporum* to support the mass culture of sea urchin and bivalve larvae was about $1\times 10^4$ cells/m*l*.[5] The MCY in this study was always more than one order of magnitude greater than the minimal value, suggesting that DSW has high AGP (algal growth potential) enough to support the cultivation of *C. ceratosporum* as food diatom, probably due to its high nutrient concentration (initial values in Fig. 4).

The values of MCY, however, showed a rellatively large variance with the sampling season of DSW (Table 1). The fluctuation did not coincide with that of any inorganic nutrient.* There is apparently a tendency that AGP of DSW for *C. ceratosporum* based on MCY was high in winter and low in summer (Table 1). These

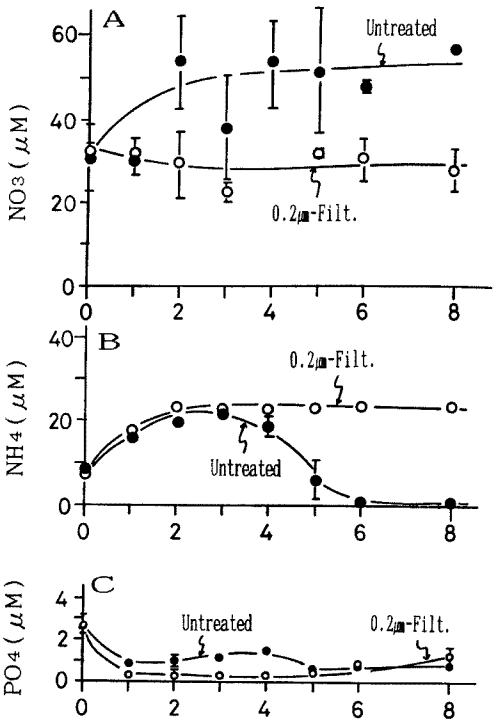


**Fig. 4.** Fluctuations of $NO_3$-N (A), $NH_4$-N (B), and $PO_4$-P (C) concentrations in untreated or 0.2 $\mu$m-filtered DSW collected on December 19, 1990, during incubation with bacteria isolated from DSW.

**Table 2.** Effects of the addition of bacteria isolated from Deep Seawater (DSW) on the growth of *Chaetoceros ceratosporum* in untreated DSW

| Sampling Date | DSW | | DSW with bacteria | | |
|---|---|---|---|---|---|
| | $\mu$*1 day$^{-1}$ | MCY*2 $\times 10^5$ cells/m*l* | $\mu$*1 day$^{-1}$ | MCY*2 $\times 10^5$ cells/m*l* | strain*3 added |
| Apr. 20 | 2.22 | 9.1 | 2.22 | 7.2 | DN-17 |
| Jun. 28 | 2.00 | 7.7 | 2.22 | 7.2 | DM- 6 |
| Aug. 28 | 1.67 | 2.2 | 2.50 | 4.1 | DN-17 |
| Sep. 28 | 1.67 | 2.6 | 2.00 | 5.9 | DO- 5 |
| Oct. 15 | 1.25 | 5.3 | 2.86 | 12.0 | DN-10 |
| Dec. 19 | 1.53 | 11.0 | 1.82 | 11.0 | 6 Mixture |
| Average (±SD) | 1.72 (±0.31) | 6.3 (±3.3) | 2.27 (±0.34) | 7.9 (±2.8) | |

*1 Growth rate as reciprocal of the dubling time (d).
*2 Maximum cell yield.
*3 The most stimulative bacterium among 8 strains added were described.

* T. Toyota, T. Nakashima, T. Fujita, and S. Ishii: The deep seawater delivery system in Kochi. Summary of Lectures, "The international forum on deep sea water," 1991, pp. 125–127.

results strongly suggest that water quality of DSW fluctuated seasonaly in terms of not only inorganic nutrient concentration but also some growth-promoting and/or -inhibiting factors present in DSW for the alga.

An influence of filtration on the AGP of DSW for *C. ceratosporum* was investigated (Table 1). MCY of *C. ceratosporum* in 0.2 $\mu$m-filtered DSW was sometimes higher than that in untreated DSW while the difference of $\mu$ values between the two was insignificant. On some occasions, although MCY was similar in both untreated and filtered DSW, algal cell density after reaching stationary growth phase decreased quickly in untreated DSW, whereas the cell number in filtered DSW was kept nearly constant until the 10th day (Fig. 2A). These results indicate that some inhibitory factors against the growth of *C. ceratosporum* in DSW were present as particulate materials. Such inhibiting factors could possibly be speculated as micrograzers including protozoa.

Effects of the addition of BDS on the growth of *C. ceratosporum* in DSW were shown in Figs. 2 (B, C) and 3 (B, C). The effect of BDS was not so obvious, or sometimes was rather suppressive (Fig. 2C) when 0.2 $\mu$m-filtered DSW was used for cultivation. In untreated DSW, however, the growth was often significantly stimulated by the addition of some strains of BDS (Fig. 3B). In Table 2 were described the values of $\mu$ and MCY of *C. ceratosporum* stimulated by the addition of the most effective bacterium among 8 strains at each sampling occasion. The $\mu$ values of *C. ceratosporum* in untreated DSW usually increased significantly to 1.82–2.86 (average 2.27)·day.$^{-1}$ The MCY occasionally increased, too, and ranged between 4–12 (average 8) $\times 10^5$ cells/m*l* (Table 2). Even though both $\mu$ and MCY were not stimulated, BDS maintained a nearly constant algal density for a longer period after reaching the stationary phase (Fig. 2B). Bacterial promoting effects on MCY of *C. ceratosporum* were more evident when AGP of DSW were lower. Moreover, variance of $\mu$ and MCY along with the sampling season tended to decrease with BDS added. Untreated DSW, of course, contains bacteria originally living in DSW. Isolates of BDS added to *C. ceratosporum* cultures grew actively and showed dense turbidity, while untreated DSW without the addition of BDS isolates gave just slight turbidity after incubation with *C. ceratosporum*. These results strongly suggest that some strains of BDS affect the growth of *C. ceratosporum* by supplying growth-promoting factors which were in shortage in DSW and/or by eliminating or inactivating the growth-inhibiting factors in DSW. These BDS, in return, recieved growth-substrates from *C. ceratosporum* indicating that they had syntrophic relationships.

Since the most effective strain among 8 BDS isolated changed from time to time, mixture of 6 strains (DM-6, DO-5, DN-8, DN-9, DN-10, and DN-17) excluding 2 strains (DM-10 and DO-7), which were not so effective, was added to the culture. The result (data not shown) showed that even a mixture of 6 strains showed a stimulative effect on the $\mu$ nearly as similar to that by the most effective strain in each occasion. This result showed that there were no interfering effects among BDS added nor would they be competitive or cooperative one another. Therefore, the addition of mixture of 6 strains is the best way to obtain a constant growth-promoting effect from BDS.

As the growth-promoting effect of BDS was more significant in the untreated DSW, the following experiment was carried out to clarify the growth-promoting mechanisms. Into both untreated and filtered DSW collected on December 19 in 1990, were inoculated cell suspensions of BDS solely (without *C. ceratosporum*) and these were incubated in similar light conditions to the usual cultivation of *C. ceratosporum* for several days. During the incubation, fluctuations of the concentration of $NH_4$-N, $NO_3$-N, and $PO_4$-P were measured. The results (Fig. 4) showed that nutrient concentration increased significantly in untreated DSW. The growth of microalgae originally present in DSW was confirmed after the 4th day in untreated DSW, resulting in the consequent decrease of ammonium. This result indicates that an increase in inorganic nutrients derived from the decomposition of suspended materials due to the bacterial activity was one of the growth-promoting effects for *C. ceratosporum*.

From results of the present study, it was suggested that DSW was a promising resource, and that untreated "raw" DSW, which would be practically used in aquaculture plant, could be utilized for the stable and effective mass culture of *C. ceratosporum* by adding some useful BDS isolated in the present study.

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## References

1) M. Takahashi, I. Koike, T. Ishimaru, T. Saino, K. Furuya, A. Hattori, and S. Ichimura: Upwelling plumes in Sagami Bay and adjacent water around the Izu Islands, Japan. *J. Oceanogr. Soc. Japan*, **36**, 209–216 (1980).
2) T. Nakashima: Effects of deep sea water on the growth of a marine diatom species, *Skeletonema costatum*. *Bull. Plankton Soc. Japan*, **35**, 45–55 (1988). (in Japanese with English abstract).
3) E. Steeman Nielsen and S. Wium-Andersen: Copper ions as poison in the sea and freshwater. *Mar. Biol.*, **6**, 93–97 (1970).
4) Y. Tanaka: The thermotolerant unicellular diatom, *Chaetoceros ceratosporum* OSTENFELD, as a useful feed to bivalve larvae. *Bull. Natl. Res. Inst. Aquaculture*, **3**, 31–36 (1982). (in Japanese with English abstract).
5) A. Tenjin and T. Ishii: Studies on the living food for planktonic larvae of sea urchin and bivalve. *Bull. Fukushima Pref. Fish Farm. Exp. Stat.*, **1**, 29–34 (1984). (in Japanese).
6) C. E. Riquelme, K. Fukami, and Y. Ishida: Effects of bacteria on the growth of a marine diatom, *Asterionella glacialis*. *Bull. Japan. Soc. Microb. Ecol.*, **3**, 29–34 (1988).
7) K. Fukami, T. Sakami, Y. Ishida, and the late N. Tanaka: Effect of bacterial film on the growth of the attached diatom, *Nitzschia* sp. in "Current Topics in Marine Biotechnology" (eds. by S. Miyachi, I. Karube, and Y. Ishida) Fuji, Technology Press, Tokyo, 1989, pp. 415–418.
8) K. Fukami, T. Nishijima, H. Murata, S. Doi, and Y. Hata: Distribution of bacteria influential on the development and the decay of *Gymnodinium nagasakiense* red tide and their effects on algal growth. *Nippon Suisan Gakkaishi*, **57**, 2321–2326 (1991).
9) K. Fukami, T. Nishijima, and Y. Hata: Isolation of bacteria promoting the growth of microalgae from deep seawater and its effects. *Kuroshio* (Rep. Inst. Kuroshio Sphere., Kochi Univ.), special issue **5**, 17–21 (1991). (in Japanese).