EXCRETION OF A DIATOM-INHIBITORY SUBSTANCE BY PROROCENTRUM MICANS EHRENBERG1,2)

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Prorocentrum micans Ehrenberg による珪藻阻害物質の分泌について
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Synopsis


The growth of Skeletonema costatum and Chaetoceros didymus was measured in bialgal cultures with Prorocentrum micans and in Prorocentrum-conditioned media, and it has been found that P. micans excretes a diatom-inhibitory substance which may play an important role in maturing of Prorocentrum-blooms. This substance was considered to have a high molecular weight because it was non-dialyzable by a cellulose tube and its inhibitory effect disappeared on autoclaving.

Introduction

In Muroran harbor, Hokkaido, diatom flowering from spring to summer was observed and it was followed by a Prorocentrum micans-bloom from late summer to autumn every two years. To analyze this succession from diatoms to flagellates some culture experiments have been conducted with a view to seeing if there is a predictable interaction between them. The present study was carried out to determine if Prorocentrum micans produces a substance toxic to Skeletonema costatum and Chaetoceros didymus.

Materials and Methods

Skeletonema costatum (Greville) Grunow and Chaetoceros didymus Ehrenberg were used as assay organisms because each species has been observed to appear every year and to be a member of the spring-summer diatom flowering, especially the former species which has been found to be a major constituent (Fig. 1). The strains of P. micans, S. costatum and Ch. didymus used in the present study were isolated axenically from Muroran harbor seawater in October, 1973, June, 1974, and July, 1974, respectively. BSW 5 was used as a standard medium (Table 1). All cultures were grown at 20±1°C in a 14:10 hr light-dark cycle and illumination by cool white fluorescent lamps regulated at 2,000-3,000 lux. Growth was measured by counting cells with a haemocytometer or by counting all the cells in a 0.1 ml sample.

Results

The growth of Skeletonema and Chaetoceros in bialgal cultures with Prorocentrum was measured. The inoculum of Prorocentrum was adjusted to 1,000-2,000 cells/ml.

The results are shown in Figs. 2 and 3. In an early stage no differences were observed between growth in bialgal cultures and that in controls for both

Fig. 1. Abundance of three phytoplankton species at the surface in Muroran harbor.

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Table 1. BSW 5 medium.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Amount/liter</th>
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<tbody>
<tr>
<td>Seawater</td>
<td>900 ml</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>8 mg</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>10 mg</td>
</tr>
<tr>
<td>Na₂SiO₃ *9H₂O</td>
<td>100 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0.05 μg</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.2 mg</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.5 μg</td>
</tr>
<tr>
<td>P II metals*</td>
<td>2 ml</td>
</tr>
<tr>
<td>Tris</td>
<td>1 g</td>
</tr>
<tr>
<td>pH</td>
<td>8.0-8.3</td>
</tr>
</tbody>
</table>

* Provasoli et al. 1957.

Fig. 2. Growth of Skeletonema in bialgal cultures with Prorocentrum.

species. However, 3 days after inoculum the growth of both species in bialgal culture was suppressed as compared to controls. These results show that both diatom species are inhibited in their growth by the presence of Prorocentrum, but it is still unknown by what agent their growth is suppressed when cultured with this flagellate.

Experiments were undertaken to find out whether a toxic substance mediated this competitive phenomenon or not. For this purpose each diatom species was grown in Prorocentrum-conditioned medium. Prorocentrum cells were grown in medium BSW 5 for about 10 days; then they were filtered with a millipore filter (0.22μ, GS) and the filtrates were enriched in the same way as BSW 5. As shown in Table 2, the growth of each diatom was suppressed in the conditioned medium as compared to that in the control. Evidently, Prorocentrum-conditioned medium has an inhibitory effect on both diatom species. These results imply that Prorocentrum micans excretes a substance inhibitory to these diatoms. However, amounts of nutrients in the conditioned medium may exceed those in the standard medium because unconsumed nutrients by Prorocentrum are considered to remain. Accordingly, it may be possible that excess nutrients inhibit the diatom growth in conditioned media. However, the following experiments showed that this assumption could not be supported. The growth of these diatom species was grown in media containing different concentrations of nutrients from 1/1-2.5/1 times as much as in the standard medium. No unusual inhibition was observed in either species up to 2/1 times the concentration of the standard medium (Fig. 4).

The results seem to confirm that P. micans ex-
cretes a substance inhibitory to both diatom species.

This substance was subjected to dialysis and autoclaving (120°C, 15 min.). The conditioned medium was dialyzed using cellulose tubes in seawater at 14°C for 20 hrs. Both the outer and inner waters from such treated tubes were assayed with *Skeletonema*. The results are shown in Fig. 5 together with the results of growth in autoclaved conditioned medium. The inhibitory substance produced by *Prorocentrum* could not pass through the cellulose membrane and its inhibitory effect disappeared on autoclaving.

**Discussion**

In bialgal experiments no inhibitory effect was observed for the first 3 days, though in later stage the growth of each diatom species was suppressed. This is consistent with the findings of PRATT (1966) in his bialgal experiments using *Skeletonema costatum* and *Olisthodiscus luteus*. Furthermore, he showed the existence of a *Skeletonema*-inhibitory substance produced by *O. luteus*. He did not examine the nature of this substance, but suggested that it has a tannoid nature, judging by previous results reported by McLACHLAN & CRAIGIE (1964). AUBERT (1971) also reported that *Prorocentrum micans* excretes a protein which inhibits the synthesis of antibiotics by *Asterionella japonica*, though he did not refer to its effect on diatom growth. In the present study, it was demonstrated that *Prorocentrum micans* excretes a diatom-inhibitory substance and that this substance is a relatively large molecule because it could not pass through a cellulose membrane and its inhibitory effect disappeared on autoclaving.

Such inhibitory substances are considered to play important roles in phytoplankton ecology. The competition between *O. luteus* and *S. costatum* demonstrated in culture has also been observed in nature (PRATT, 1966). According to ADACHI (1972), *Prorocentrum micans* sometimes mingle with *Skeletonema costatum* red tides in Ise Bay. On the other hand, the red tides of this species occurring in Muroran harbor and its vicinity are usually almost monospecific, although a few diatoms were sometimes observed when the density of the blooms was comparatively low (IZUKA & KOMAI, 1974; UCHIDA, unpublished). The inhibitory substance examined in the present study may play a part in the ecological behavior of this species.

![Fig. 4. Growth of Skeletonema and Chaetoceros in different concentrations of nutrients (Growth after 5 days).](image)

![Fig. 5. Growth of Skeletonema in dialyzed conditioned medium and in autoclaved conditioned medium (Growth after 5 days).](image)

**Summary**

1. Each diatom species, *Skeletonema costatum* and *Chaetoceros didymus* was grown in bialgal cultures with *Prorocentrum micans*, and in *Prorocentrum*-conditioned media. Under such conditions their growth was inhibited.

2. Nutrients in conditioned media were considered to exceed those in controls, but excess nutrients were shown to have no inhibitory effect on the growth of either diatom species.

3. From the results obtained it was concluded that *Prorocentrum micans* excretes a substance inhibitory to both diatom species.

4. This substance was found to be non-dialyzable, and its inhibitory effect disappeared on autoclaving.

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References


摘 要

Procentrum micans 分泌物の2種珪藻プランクトンに及ぼす影響を調べた。P. micans と Chaetoceros didymus 及び P. micans と Skeletonema costatum 組合わせによる2重培養の結果と、P. micans を一定期間培養して得られたConditioned medium におけるこれら珪藻プランクトンの生長度合から P. micans が生長阻害物質を分泌していることが明らかとなった。この物質は透析膜を通らず、オートクレープ処理によって失活することが判明した。