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The Influences of Extracts from *Ceratium* spp. on the Growth of Harmful Microalgae

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The growth response of the fish-killing dinoflagellate *Cochlodinium polykrikoides* was studied in cultures, using the treatment of *Ceratium* extracts by a methanol, a water-soluble, and a cell-free medium. The cell-free medium had the most increasing on the growth of *C. polykrikoides* cultures, enriched with ≥25% *Ceratium*, whereas the methanol and water-souble fractions did not affect the growth of *C. polykrikoides* exposed to even higher concentration. In particular, the cell-free medium also increased the growth of *Gyrodinium impudicum* and *Chaetoceros* sp., similar species to *C. polykrikoides*. In contrast to *C. polykrikoides*, *G. impudicum* and *Chaetoceros* sp., the growth of *Alexandrium tamarense* was inhibited significantly, and there was no great effect on the growth of *Prorocentrum minimum*. These results imply that *Ceratium* extracts may play an important role in the stimulatory effect of *C. polykrikoides*, and they have to affect the interaction between *C. polykrikoides* and *Ceratium* when co-existing.

Key words – *Cochlodinium polykrioides*, extracts, growth, red tide

"Red tide" algae can bloom and then die very rapidly. To date, the mechanism of this growth and decline has not been determined. Generally, the rapid development of red tides has been linked to various environmental factors. Red tides themselves have been thought to "condition" the waters, leading to a change in the dominant microalgae [4]. It is necessary to understand red tide organisms that can effect on the growth and decline of microalgae. Some researchers have demonstrated such effects, and recently an autoinhibitor was isolated from *Skeletonema costatum* (Greville) Cleve [3,4,7]. Bacteria have also been shown to effect phytoplankton growth and to cause the sudden collapse of blooms, by the production of inhibitory or stimulatory substances [9]. A specific bacterium [5], fungus [9] and virus [10] have been shown to infect and finally kill microalgae. Some researchers suggest that extracellular organic matter from *Heterosigma akashiwo* (Hada) Hada ex Hara et Chihara can promote or inhibit the growth of other microalgae, demonstrating a relationship between the extract and phytoplankton communities [13].

The harmful dinoflagellate *Cochlodinium polykrikoides* Margalef has occurred since 1982 and associated with heavy-cultured fish kills; it has been regarded as the main red tide phytoplankton among other harmful microalgae isolated from Korean coastal waters[6]. However, little investigation has been carried out on the changes in succession of the phytoplankton community when the blooms of dominant organisms occur, and how much effect other phytoplankton populations have on bloom promotion or decline. This present study aims to determine the response of *C. polykrikoides* to different concentrations of extracts from *Ceratium* spp., in order to assess the relative importance of extracts on the growth of other red tide microalgae.

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Materials and Methods

Microalgae

A clonal culture of *C. polykrikoides*, established during the mid-1997 outbreaks in the coastal waters of Tongyong, was maintained in an f/2 medium [2]. Other microalgae (*Prorocentrum minimum* (Pavillard) Schiller, *Alexandrium tamarense* (Lebour) Balech, *Gyrodinium impudicum* Fraga et Bravo and *Chaetoceros* sp.) were isolated from Korean coastal waters during period 1996-2000. All algae were grown at 20℃ with cool-white fluorescent light (100 μmol m^{-2} s^{-1}) under a 12:12 h light:dark cycle.

Extracts

The sample of *Ceratium* was collected from Changsun, Namhae, where an outbreak of *Ceratium* spp. occurred in June, 1999. The sample was harvested on a Whatman GF/C membrane filter (0.45 μm) and the filtrate was frozen until required. For each sample, 1 ml of 100% methanol was used to extract at rotary for 1 day. The methanol fraction was transferred to a new tube, and then determined the weight after completely evaporating the methanol under vacuum

for 4 hrs at 30℃. For a stock solution of methanol-soluble fraction, 1 ml of methanol was added to the dried extract at a ratio of 50%. After dissolving the stock solution, it was diluted 100 fold and filtered by a syringe filter (0.22 μm) before use.

Culture conditions

The cultures were grown in a culture cabinet at 20℃ under 12 : 12h light-dark cycles of white fluorescence illumination, providing a photon flux density of about 150 μmol m^{-2} s^{-1}. Growth was measured by directly counting cells under an inverted Carl Zeiss MC80 light microscope in Multiwell tissue culture plates (Becton Dickinson) in quadruplicate for 17 days. The tissue chambers were sealed to reduce the evaporation of the cell medium. Extracts of methanol-soluble, water-soluble and cell-free fractions were treated at concentrations of 1, 5, 10, 25, and 100% to the culture medium. The initial cell density was *ca.* 20-50 cells per well, inoculated into a filtered medium (0.2 μm AS020). Growth rates for the exponential phase were calculated as doubling per day (K). The growth data was analyzed by one-way factor analysis of the variance in a completely random factorial design, and was run using the ANOVA routine t-test.

Results and Discussion

When the methanol extracts from *Ceratium* spp. were added to *C. polykrikoides* at concentrations of 1-100%, the growth rate showed a similar cell yield compared with the control. In the water-soluble extracts, similar growth by the treatment of methanol was exhibited, as shown in Fig. 1. In contrast to the methanol and water-soluble extracts, the addition of the cell-free medium obtained the growth of *C. polykrikoides* increased substantially at elevated concentrations. In particular, higher concentrations ($\geq$25%) caused major differences in growth (p<0.05 by student t-test) compared with the control. Figure 2 showed the effect of the cell-free medium (100%) on the growth of other microalgae. In *P. minimum*, the growth response was similar to that in the control; however, *A. tamarense* was suppressed significantly (p<0.05) by addition of the cell-free medium. Both *G. impudicum* and *Chaetoceros* sp. treated with the 100% cell-free medium, obtained a higher growth rate (p<0.05) than that of the control.

Our results assure us that the increased amounts of the

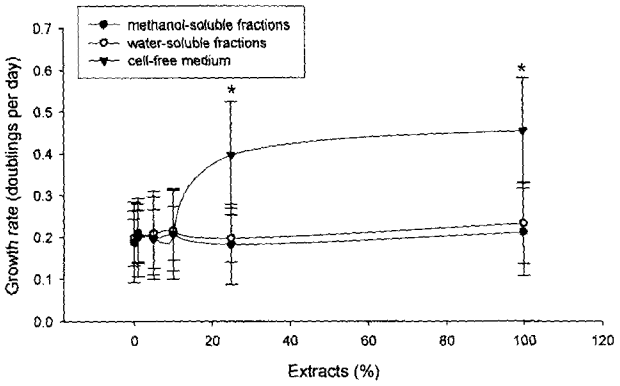

Fig. 1. Response of the growth rate of *Cochlodinium polykrikoides* as a function of fractions from *Ceratium* spp. after 17 days. Error bars indicate S.E. *p<0.05 by t-test, compared with the control.

cell-free medium not only contributed to a strong stimulative growth of *C. polykrikoides*, but also impacted the growth of *G. impudicum* and *Chaetoceros* sp. However, the methanol and water-soluble media did not play an important role in the inhibitory or stimulatory effect of *C. polykrikoides* (Fig. 1) or other microalgae (*P. minimum*, *A. tamarense*, *G. impudicum*, *Chaetoceros* sp., data not shown), indicating that unknown organic substances of lower molecular weight than that of higher molecular weight may be associated with a critical stimulatory effect of *C. polykrikoides* and that of strains of microalgae used in this study.

The blooms caused by *C. polykrikoides* usually begin in the early September and persist for just one month in Korean coastal waters [6]. Several species of *Prorocentrum* and *Ceratium* before *Cochlodinium* red tide have been observed [6]. As can been seen in Fig. 1, increased amounts of cell-free medium play an important role in the stimulatory effect on the growth of *C. polykrikoides*, indicating that the interaction between *C. polykrikoides* and *Ceratium* may be shown in a higher positive correlation: extracellular products produced by massive blooms of *Ceratium* cause the growth of *C. polykrikoides*. Accordingly, this realize that outbreaks of *C. polykrikoides* are associated with environmental conditions, nutrients and possibly extracellular organic matter from *Ceratium*.

Some researchers have suggested that *Prorocentrum* red tide is responsible for the abundant occurrence of *S. costatum*, which suggests in turn that extract from *S. costatum* strongly stimulates the growth of *P. minimum* [7]. Previously, there were some reports that the occurrence of *S. costatum* impacts on the growth of other phytoplankton [3,4]. Organic

substances excreted by *S. costatum* have been shown to stimulate the growth of *P. minimum* [7] and to inhibit the growth of *Scrippsiella trochoidea* (Stein) Loeblich III [8]. However, *Ceratium* extracts by methanol and water-soluble media do not play an important role in inhibiting or stimulating the growth of *P. minimum* (data not shown), and neither do they appear to impact *P. minimum* exposed to even *Ceratium* by the cell-free medium (100%, Fig. 2). It is thought that organic substances excreted during the blooms of *Ceratium* and *S. costatum* are remarkably different in their effect on the growth of *P. minimum*.

Researchers have identified 15-hydroxyeicosapentaenoic acid from *S. costatum* and have suggested that this extract might play a role as an autoinhibitor or effect the growth of other phytoplankton [1,3,4]. In addition, a variety of dissolved extracellular organic products produced by phytoplankton were shown to be important stimuli or inhibitors of feeding by zooplankton on phytoplankton [11,12], and might also have an effect on the growth of bacteria[5]. Extract from phytoplankton is important in elucidating the growth or decline of blooms because, these extracellular products might effect the pre-predator relation. As shown in Fig. 2, the cell-free medium plays a role in inhibiting or stimulating the growth of *A. tamarense*, *G. impudicum* and *Chaetoceros* sp. This suggests that *Ceratium* extract is similar to *S. costatum*, as an inhibitor or stimulator. In this respect, I need to identify and purify the organic substances excreted by *Ceratium*.

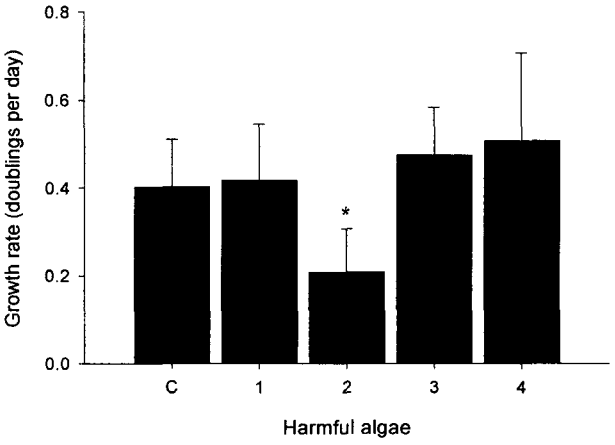

Fig. 2. Response of the growth rate of other microalgae as a function of 100% the cell-free medium. after 17 days. C, *Cochlodinium polykrikoides*, 1, *Prorocentrium minimum*, 2, *Alexandrium tamarense*, 3, *Gyrodinium impudicum*, 4, *Chaetoceros* sp. Error bars indicate S.E. *$p<0.05$ by t-test, compared with *C. polykrikoides*.

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초록 : 유해성 적조생물에 대한 *Ceratium* 추출물 영향 평가

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Ceratium 적조생물을 메타놀이나 수용성 물질을 분리하거나 cell-free medium 추출물을 *Cochlodinium polykrikioides* 성장에 미치는 영향을 조사했다. cell-free medium을 25% 이상 첨가한 시험구에서 가장 우수한 *C. polykrikoides* 성장을 가져왔다. 그러나 메타놀이나 수용성으로 처리한 추출물에서는 고농도로 배지에 첨가시켜도 *C. polykrikoides* 성장에는 큰 영향이 없었다. 특히 cell-free medium은 *Gyrodinium impudicum*이나 *Chaetoceros* 종에서도 *C. polykrikoides*와 비슷한 양상을 보였으나, *Alxendrium* 적조생물에 대해서는 현저히 성장을 저해시키는 결과를 나타내었고, *Prorocentrium minimum*에서도 성장을 촉진시키지는 못했다. 이러한 결과로 보와서 *Ceratium* 추출물은 *C. polykrikoides* 성장을 촉진시킬 수 있는 주요한 역할을 하는 것으로 보여서 두 적조생물이 공존하게 되면 상호간에 영향을 미칠 수 있을 것으로 판단된다.