Growth Inhibition of *Heterosigma akashiwo* and *Prorocentrum minimum* by the substances released from *Ulva* sp.

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Summary

Overgrowth of microalgae in the eutrophicated coastal waters causes well-known "red tide". In many relatively closed coastal waters, shallow lagoons and estuaries, mass development of macroalgal biomass has been observed. The most characteristic species of these macroalgae in the eutrophic Japanese coasts are the green algae *Ulva* spp. The mass occurrences of *Ulva* spp. result in the formation of so called "green tide". We are interested in the reasons why *Ulva* spp. become the predominant species in these eutrophicated waters.

Two important factors of mass development of *Ulva* spp. are topographical elements and biological characteristics of these algae. While, a growth of microalgae is not observed in the coast where the mass of *Ulva* spp. is developed. We, therefore, assumed that *Ulva* spp. inhibit the growth of microalgae by chemical means. This speculation was proved by the following experiments. When *Ulva* sp. was cultured with *Heterosigma akashiwo* or *Prorocentrum minimum*, isolated from a red tide, the growth of microalgae was significantly inhibited. The same inhibition of microalgal growth was observed by the broth or the ethyl acetate extract of the broth cultured *Ulva* sp.

For the isolation of bioactive substances, the assay method using 24-well plastic plates were developed. The active fractions cause the morphological change of algal cells within 30 min, and after a short time, destruction of cells and leak of protoplasm were detected. The broth of *Ulva* sp. was extracted with ethyl acetate followed by *n*-butanol. The butanol extract showed the strongest activity and was subjected to bioassay-guided separation by high-performance liquid chromatography (HPLC). The inhibition of microalgal growth is revealed to be initiated by several compounds, since the activity was detected in the different fractions separated by HPLC. One of these active compounds was isolated and measured the proton nuclear magnetic resonance spectrum. Unfortunately, the spectrum was not good enough to elucidate the structure because of insufficient amounts isolated. The analysis of the structure is now in progress.