

Organic ligands regulating growth of microalgae and the regulation mechanisms

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Summary

(Introduction) The growth of harmful microalgae causes red tides in Japanese embayments, and has given severe damage to both natural and cultured fish and shellfish. Fishery society requires powerful strategies reducing the occurrences of red tide. The ligands limiting the iron uptake of microalgae and reducing the algal growth were useful tool for regulating the microalgal biomass. In this study, the four organic ligands were evaluated for preventing the microalgae from uptaking iron and growing under iron limitation. Furthermore, for the molecular mechanism of microalgal cells adapting to iron deficiency, the functional genes induced the transcription by iron deficiency were cloned using differential display analysis.

(Materials and Methods) *Prymnesium* sp. causing red tide was incubated in modified f/2 medium including 2,2'-Bipyridy, FerroZine, BPDS (bathophenanthroline disulphonate), or DFB (defferrioxamine B). After incubation, the growth yields were determined using absorbance of 550 nm at every two days. The algal culture in f/2 culture medium including iron at the concentrations of 0 μ M, 0.05 μ M and 1 μ M and the algal culture to which chelate was added at 7 day incubation were used for differential display analysis. Total RNA was obtained from each culture, and cDNA was synthesized from total RNA. The PCR products of cDNA were observed using the agarose gel electrophoresis. The specific bands to iron limited culture were purified and cloned. Nucleotide sequences of the clones were determined and investigated using FAST research on genbank database.

(Results and Discussion) The two ligands, 2,2'-Bipyridy and DFB, inhibited the growth of *Prymnesium* sp., while the other two ligands, FerroZine and BPDS, showed no effects on the algal growth. The 2,2'-Bipyridy and DFB which bind Fe (III) would prevent *Prymnesium* sp from uptaking iron and lead to iron deficiency. At the differential display analysis, 17 specific bands to iron limited culture appeared on the agarose gel, and nucleotide sequences of 83 clones of the specific bands were determined. The 83 clones were classified into 17 genetic types, of which 8 types similar to the known functional genes and 9 types indicated unknown sequences. Especially, the 3 types of the known functional genes, such as eucaryotic release factor, enolase, and heat shock protein, occupied 24 clones of all 83 clones, suggesting that the algal cells under the stress of iron deficiency induce to transcript these functional genes to adapt the environmental stress.