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Organic Ligands Regulating Growth for Microalgal Species and the Iron Regulation Mechanisms of *Prymnesium parvum*

Teruya Maki

Kanazawa university , Graduate School of Natural Science and Technology

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 lignads limiting the iron uptake of microalgae and reducing the algal growth were useful tool for regulating the microalgal biomass. In this study, the three organic ligands preventing the algal growth were evaluated for the specificity to microalgal species. Furthermore, for the molecular mechanism of algal cells adapting to iron deficiency, the functional genes, which induced the transcription by iron deficiency, were cloned using differential display analysis.

(Materials and Methods) After two or three species of the red tide algae *Prymnesium parvum*, *Heterocapsa circularisquama* and *Heterosigma akashiwo* were inoculated into modified f/2 medium including Bipyridine, Ferrozine, or DFB (defferrioxamine B), the growth yields were determined using microscopy observation. For differential display analysis, the culture of *P. parvum* in the culture medium was added DFB at the concentrations of 0 μM, 10 μM and 100 μM at the 0th day, the 1st day, the 5th day and the 9th day. The PCR products of cDNA synthesized from total algal RNA were observed using the polyacrylamide gel electrophoresis. The specific bands to iron limited culture were purified and cloned. Nucleotide sequences of the clones were determined and analyzed.

(Results and Discussion) In the culture medium including chelators, the survival rates of three algal species were changed comparing the culture medium without chelator addition. Under iron deficiency, *H. circularisquama* and *H. akashiwo* increased the survival rates, while *P. parvum* decreased the survival rates. Presumably, the two algal species can effectively adapt the iron deficient condition and survive under iron deficiency. At the differential display analysis, 20 specific bands to iron limited culture appeared on the polyacrylamide gel, and nucleotide sequences of 69 clones of the specific bands were determined. The 63 clones were classified into 56 genetic types, of which 27 types similar to the known functional genes, 8 types similar to the sequences of hypothetical protein, and 21 types indicated unknown sequences. Some genes were relate to iron uptake, energy production, repair and production of protein, and change of cell structure, whicih would be induced under the stress of iron deficiency.

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