No. 0714

Elucidation of Halotolerant Mechanism of Seaweeds, with Special Reference to the Search of Halotolerant Genes of *Ulva*

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Summary

The green macroalgal genus *Ulva* (Ulvales, Ulvophyceae, Chlorophyta) is well known for its wide distribution from marine to brackish water all over the world. Freshwater macroalgal species, *Ulva limnetica* Ichihara et Shimada (Ulvales, Ulvophyceae) showed same level of DGR (Daily Growth Rate) during 10 days between 30, 5 and 0 PSU cultured conditions. Sequence analyses of the nuclear encoded 18S rDNA and chloroplast encoded *rbc*L gene strongly support the independent status of *U. limnetica*, and this species was included in the *Ulva* clade with high bootstrap values other genera of Ulvaceae usually distributed in marine were recognized as the earliest diverging lineage within the order. This results indicate that *U. limnetica* might be derived from a marine ancestor.

Ulva limnetica was investigated to understand molecular mechanism of its tolerance or adaptation to seawater. A 19 kDa protein was detected by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which was accumulated in freshwater-cultured samples than seawater-cultured samples. The band was excised and the partial amino acid sequence was determined by Edoman degradation. Based on the sequences, the corresponding cDNA was isolated by rapid amplification of cDNA ends (RACE) technique. The protein encoded by the cDNA showed 30% identity to lectin isolated from *Ulva pertusa* Kjellman. Northern blot analysis demonstrated that the expression level of the gene in the freshwater-cultured sample was higher than in the seawater-cultured sample. Differential display analysis indicated that some genes accumulated in seawater-cultured samples than freshwater-cultured samples, and the cDNAs showed similarity to ubiquitin carboxyl-terminal hydrolase isolated from *Oryza sativa* L., or glucan endo-1,3-beta-D-glucosidase isolated from *Hordeum vulgare* L.