Effects of an alginate oligosaccharide mixture (AOM) on *Nannochloropsis oculata*, a unicellular marine microalga, were investigated. The growth of *N. oculata* was significantly promoted by AOM in a concentration-dependent manner. The maximum effect was attained at 20 mg/ml, at which the growth rate of the alga became nearly 5 times higher than that of control without AOM. The growth-promoting effect of AOM decreased slightly at 40 mg/ml. Furthermore, the algicidal effect of Cu$^{2+}$ was profoundly alleviated by the addition of AOM. These results suggest that AOM is useful for promoting and/or improving the growth of *N. oculata*.

**Key words:** alginate oligosaccharide; *Nannochloropsis oculata*; marine microalgae; growth-promoting activity; Cu$^{2+}$-stress

Alginate, a natural acidic linear polysaccharide derived from seaweed, is constituted of α-L-guluronate (G) and β-d-mannuronate (M) (uronic acids). The residues are arranged in a block structure of a homopolymer (polyguluronate or polymannuronate) or heteropolymer (a mixed sequence of these residues). These block structures are called G-blocks, M-blocks, and MG-blocks, respectively. Bulk alginate is manufactured from seaweed, and is widely used in the food industry and for some medical purposes due to its physicochemical characteristics, such as high viscosity in aqueous solution and its gel-forming property in the presence of calcium ions. Besides the various biological activities of alginate in the mammalian system, there have been few reports of the effects of alginate on the physiological activities of plant cells. Furthermore, over the past few years, the oligosaccharides derived from alginate have been investigated from a wide variety of viewpoints. For instance, it has been reported that enzymatically depolymerized alginate oligomers promoted the growth of bifidobacteria, whereas the original alginate polysaccharide had no effect. Alginate oligomers with an average molecular weight of 1800, prepared with bacterial alginate lyase, increased shoot elongation after germination of *komatsuna* (*Brassica rapa var. pervidis*) seeds.

Iwasaki *et al.* have reported that an alginate oligosaccharide mixture had promoting effects on the root growth of lettuce seedlings. Root growth-promoting activities of alginate oligomer on carrot and rice plants has also been reported. These findings suggest that alginate oligosaccharides may act on certain plant cells as growth promoting agents.

In the aquaculture industry, microalgae are indispensable food sources at all growth stages of bivalves and for the larvae of some crustacean and fish species. They are also consumed by zooplankton, which are used in the feeding of larvae and juveniles of marine fish as well as some crustaceans. *Nannochloropsis oculata* is a marine unicellular microalga classified into the Eustigmatophyceae class, and is frequently used in fish seedling production and rearing firms. Through the food chain based on microalgae, important nutrients, such as vitamin E, from microalgae are transferred to higher trophic levels via intermediate zooplankton. It has been reported that *N. oculata* has a superior nutritional value, with relatively high levels of vitamin E. Thus *N. oculata* is a highly important organism not only as a food source for many aquatic animals, but also as a feeding organism for zooplankton rotifers, which are used as secondly effective organism in seedling production of marine fishes and crustaceans. Although microalgal culture is a primary important step in seedling production in hatcheries, this activity is still far from being optimized, and stable and efficient culture procedures for microalgae have not been established yet. In particular, sudden mass mortality of microalgae due to unknown mechanisms is a serious problem in the establishment of successful seedling production of commercially valuable marine species. Therefore, it is urgently required to establish a stable and efficient mass-culturing technique for microalgae in the aquaculture industry. Since *N. oculata* is a eukaryotic plant cell with biological systems similar to higher plant cells, it is considered that the growth of *N. oculata* is also influenced by alginate oligomers. Under these circumstances, as a pilot study, we investigated the effects of an AOM on the growth of *N. oculata* under normal and Cu$^{2+}$-induced stress conditions.
Alginate polymer (IL-6M) was obtained from Kimika (Tokyo). The AOM was prepared by digestion of alginate polymer with bacterial alginate lyase, as described previously. Gel-filtration analysis of the AOM indicated that the AOM used in this study was mainly composed of dimer, trimer, and tetramer. N. oculata was supplied by the microalgae collection of National Research Institute of Aquaculture Fisheries Research Agency. N. oculata was cultured at 17 °C in 100 ml of modified Guillard's f medium, in which Na₂SiO₃·9H₂O, vitamin B₁₂, biotin, and thiamine HCl were omitted from the originally reported constituents. The culture was kept under illumination by fluorescent lamps at 30 μmol/m²/s under a cycle of 12 h light and 12 h dark. All cultivation was done using sterilized instruments, with continuous stirring with a magnetic stirrer. Exponentially growing algal cells were used throughout the experiments. The initial cell density of the algal cells was adjusted to about 10⁵ cells/ml, and the culture was started in a medium containing the indicated concentration of AOM under the conditions described above. To examine the effects of Cu²⁺ on the growth of N. oculata, the indicated final concentrations of CuSO₄·5H₂O were added to the medium, and this was cultured for a few days, as described above. The algal cell densities were determined by hemocytometer counts, and the growth conditions were also monitored microscopically every day.

The effects of AOM at various concentrations on the cell growth of N. oculata are shown in Fig. 1. From the growth curve profiles, it is obvious that algal growth was dramatically promoted by AOM in a concentration-dependent manner in a concentration range of 1 to 20 mg/ml. Even at 1 mg/ml of AOM, a slight increase in the growth was observed. The maximum effect was attained at 20 mg/ml, at which the cell number reached a plateau nearly 5 times higher than that of the control without AOM. However, the growth-promoting effect of AOM was slightly decreased at 40 mg/ml, to a level similar to that observed at 5 mg/ml. Thus it appears likely that AOM has an optimum concentration to promote the growth of N. oculata, at least under the conditions used. As judged by the patterns of growth of N. oculata in the presence of effective concentrations of AOM, the rate of cell division during the exponential growth phase also tended to be accelerated by AOM (Table 1). Based on these data, it is likely that AOM is capable of promoting the growth rate of N. oculata during the exponential phase and of increasing maximum cell density during the stationary phase.

The culture of microalgae often suffers from various environmental stresses even under highly controlled conditions. Especially, trace amounts of contamination with heavy metals are known to cause suppression of the growth of microalgae. Although Cu²⁺ is often used to exterminate parasites attached to cultured fish in aquaculture, this metal is also known to be toxic to microalgae. Hence Cu²⁺ was used as a representative heavy metal stressor for N. oculata. As shown in Fig. 2, the addition of 2.5 mg/ml of Cu²⁺ to the medium resulted in complete inhibition of the growth of N. oculata. Even after 24 h of incubation with Cu²⁺, almost all the cells were decolorized and became incapable of subsequent cell division. The potent toxic effect of Cu²⁺ on N. oculata almost completely disappeared with the addition of a semioptimal concentration of AOM (10 mg/ml), and the growth curve in the presence of AOM and Cu²⁺ together was similar to that of the control. These results suggest that AOM is able not only to promote the growth of N. oculata under normal conditions, but also to exert a beneficial effect on algal cells under stress-induced suppressive conditions.

Several previous studies have demonstrated that alginate oligomers show growth promoting activity in various higher plant systems. Since N. oculata is a unicellular marine phytoplankton of eukaryotic plant cell type, it is possible that it has many functional and structural similarities to higher plant cells at the cellular level and is influenced by alginate oligomers. In agreement with this, our results clearly indicated that the AOM prepared in this study showed a growth-promoting effect on N. oculata (Fig. 1 and Table 1). In addition, Cu²⁺-induced growth suppression of N. oculata was profoundly mitigated by AOM. Since alginate and biologically active galacturonic acid oligomers are known to form complexes with Ca²⁺ through their carboxyl groups, one can speculate that the mitigation...
Furthermore, Farmer et al. have suggested that sodium alginate oligosaccharides exert antagonist activity towards the calcium channels, especially voltage-operated calcium channels. Since alginate consists of guluronic acid and mannnuronic acid, AOM might act on *N. oculata* through the formation of a complex with Ca\(^{2+}\) which can trigger certain signaling pathways. Further studies of the action mechanism of AOM on *N. oculata* might provide a new view of oligosaccharin-mediated signal transduction pathways.

In conclusion, our results indicate that AOM not only promotes the growth of *N. oculata* but also overcomes Cu\(^{2+}\)-induced growth suppression of the alga. Thus it might prove to be a useful agent for the establishment of stable and efficient mass culture of *N. oculata*.

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**Fig. 2.** Effects of AOM on the Growth of *Nannochloropsis oculata* in the Presence of Cu\(^{2+}\). *N. oculata* cells were inoculated into a medium (100 ml) containing 2.5 mg/ml of Cu\(^{2+}\) and 10 mg/ml of AOM (●), 2.5 mg/ml of Cu\(^{2+}\) (○), or one without these agents as a control (■), and cultured for the indicated periods of time under the conditions, as described in the text. The number of the algal cells in each culture was counted daily, as described in the text. During the incubation period, the algal cells were observed microscopically. Each value represents the average of quadruplicate measurements. The pictures show the algal cells after 2 d the incubation with 2.5 mg/ml of Cu\(^{2+}\) and 10 mg/ml of AOM (a), 2.5 mg/ml of Cu\(^{2+}\) (b), or without these agents as a control (c).


