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The growth of *Dunaliella* under magnesium hypertonicity

Shuhei FUJII, Mayumi TAKENISHI, Shiro MANTANI, and Hideo TAKADA

Division of Food Science, Tezukayama College, 3-Chome, Gakuene-Minami, Nara, 631 Japan.

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Six species including seven strains of *Dunaliella* were cultured in three hypertonic media containing NaCl, LiCl or $MgSO_4$. All species tested grew in a NaCl-hypertonic medium, but none grew in the LiCl-hypertonic medium. Only *D. tertiolecta* and *D. primolecta* grew in a $MgSO_4$-hypertonic medium, however, $MgSO_4$ could not be replaced with $MgCl_2$. The growth of these two species in a $MgSO_4$-hypertonic medium, compared to that in a NaCl-hypertonic medium, was characterized by their strong dependence on the concentration of CO_2 in the air flow.

Key Index Words: Dunaliella; *growth*; *magnesium hypertonicity*.

Dunaliella is a halotolerant or halophilic volvocalean alga found all over the world. The genus has created considerable research interest because it tolerates high concentrations of sodium chloride. *Dunaliella* may be classified into two groups, the halotolerant organisms which can grow in 0.5 M NaCl and above, and the halophilic which do not grow in media containing less than 2 M NaCl (GINZBURG and GINZBURG 1981). The salt tolerance or preference of this organism may be attributed not only to an osmotic effect but also to a Na^+ ion effect (FUJII *et al.* 1981).

When the salt tolerance of plant species are examined, the effect of salts other than NaCl should be considered. For example, the marine yeast, *Rhodotorula glutinis var. salinalia* grows normally and endures in both NaCl- and $MgCl_2$-hypertonic media (JOHO *et al.* 1969). On the other hand, a halophilic species of *Streptomyces* can grow in only NaCl-hypertonic medium (KAYAMURA *et al.* 1973). Furthermore, two other types of halophytes, chloride and sulphate types, are recognized in herbaceous higher plants (STEINER 1939).

Little is known of the effects of salts other than NaCl. We have examined the specific effect of the Mg ion on the growth of some species of *Dunaliella* and the effects of CO_2 concentration and partial replacement of $MgSO_4$ with $MgCl_2$ on growth of *D. tertiolecta* and/or *D. primolecta*.

Materials and Methods

The six species of *Dunaliella*, including seven strains, were obtained from the Algal Collection of the University of Texas.

Each strain was isotonically grown in hypertonic media with either one of three kinds of salt, NaCl, LiCl and $MgSO_4$, added to basal inorganic medium which (Table 1) was sterilized by autoclaving (120°C, 15 min). Each strain was cultured in a 200 m*l* Erlenmeyer flask containing 100 m*l* medium at 25°C under continuous illumination of fluorescent lamps with the light intensity of 10,000 lux at the surface of the culture flask. Cultures were aerated with 6% CO_2-enriched compressed air except for an experiment on the effect of different CO_2 concentrations.

The cell number was determined by counting formaldehyde-fixed cell suspention aliquots in a microscope using a Thomas cell counting plate. The dry weight of the cells was

Table 1. Composition of basal culture medium*

$MgCl_2 \cdot 6H_2O$	1.5	g
$MgSO_4 \cdot 7H_2O$	0.5	g
KCl	0.2	g
$CaCl_2$	0.2	g
KNO_3	1.0	g
$NaHCO_3$	0.043	g
TRIS	2.45	g
K_2HPO_4	0.045	g
Fe-EDTA	3.64	mg
EDTA-2Na	1.89	mg
$ZnSO_4 \cdot 7H_2O$	0.087	mg
H_3BO_3	0.61	mg
$CoCl_2 \cdot 6H_2O$	0.015	mg
$CuSO_4 \cdot 5H_2O$	0.06	mg
$MnCl_2$	0.23	mg
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.38	mg
H_2O	1.0	*l*

* Medium used by NORO (1978). pH of medium was adjusted to 8 with HCl solution.

determined by filtering 5 m*l* of algal suspension through a weighed membrane filter, which was dried for 6 hours at 110°C. Then the filters were cooled in a desiccator and weighed again. In order to determine the weight of salts, 5 m*l* of the medium was filtered through a separate membrane filter and treated similarly to the filter with algae. The dry weight was obtained by subtracting the weight of the salts from the total weight.

In all cultures, the initial cell density was 0.1 as optical density at 680 nm. Although cultures were not handled under aseptic conditions, no bacteria were observed.

Results and Discussion

a) Growth in NaCl-, LiCl- and $MgSO_4$-hypertonic media.

Several investigators have worked on the NaCl tolerance of *Dunaliella* (FRANK and WEGMANN 1974; BEM-AMOTZ 1975). However, little information has been reported on the effects of salts other than NaCl on growth of *Dunaliella.*

All species tested grew in 0.5 M NaCl-hypertonic medium with some differences in the growth rate (Table 2). *D. bioculata, D. primolecta* and *D. tertiolecta* showed high growth rates. No growth was observed in any strain in LiCl-hypertonic medium. Two species of *D. primolecta* and *D. tertiolecta* showed good growth in $MgSO_4$-hypertonic medium.

GINZBURG and GINZBURG (1981) reported that *Dunaliella* strains fall naturally into the halotolerant group capable of growing at 0.5 M or above NaCl, and the halophilic group which grow only in media containing more than 2 M NaCl. All of the srtains tested belong to the halotolerant group. Two types of tolerance were recognized in halotolerant group: one which grow in NaCl- and $MgSO_4$-hypertonic media and the other which only grow in NaCl-hypertonic medium.

b) Growth of D. tertiolecta *and* D. primolecta *in Mg-ion hypertonic medium.*

Growth curves of *D. tertiolecta* and *D. primolecta* in 0.84 M $MgSO_4$-hypertonic

Table 2. Results of growth experiments

Species of *Dunaliella*	NaCl	$MgSO_4$	LiCl	$Mg(NO_3)_2$
Dunaliella bieculata (LB 199)	++	−	−	*
D. parva (LB 1983)	+	−	−	*
D. peircei (LB 2192)	+	−	−	*
D. primolecta (LB 1000)	++	++	−	−
D. salina (LB 200)	+	−	−	*
D. salina (LB 1644)	+′	−	−	*
D. tertiolecta (LB 999)	++	++	−	−

* Each strain was cultured for 4 days in 0.5M NaCl, 0.5M LiCl or 0.84M $MgSO_4$ added to the basal culture medium. And only two species of *D. tertiolecta* and *D. primolecta* were cultured for 4 days in 0.36M $Mg(NO_3)_2$ containing the basal culture medium. [++: good; +: weak; −: none]

medium are shown in Fig. 1 and 2. In both speceis, dry weight increased even after the number of cells stabilized, which can be explained by the cells, increasing their weight. The cell volume per 10^6 cells gradually became larger (FUJII *et al.* unpublished data).

The growth of *D. teritolecta* does not depend on the concentration of $MgSO_4$ (Fig. 3). Growth is comparatively constant in spite of the great variation of external concentration of $MgSO_4$.

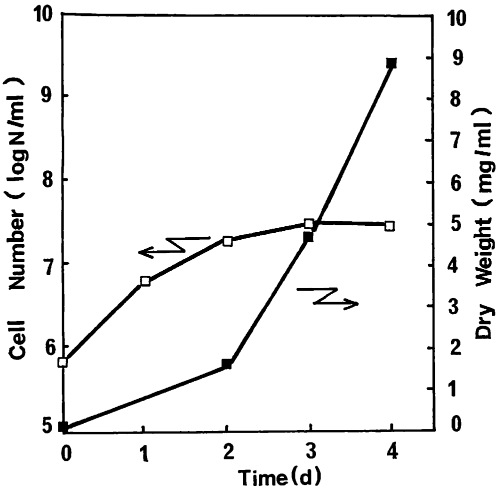

Fig. 1. Growth of *D. tertiolecta* in 0.84M $MgSO_4$ medium.

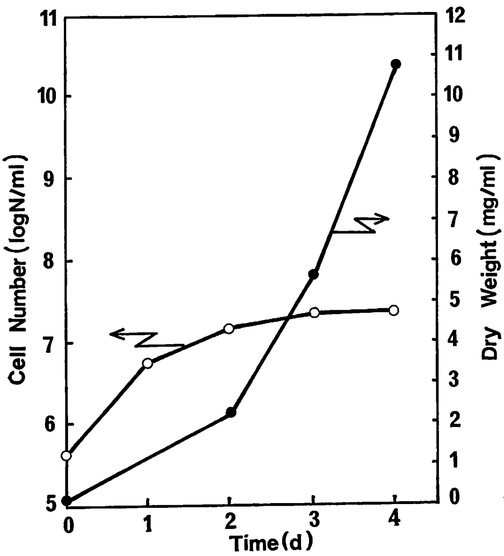

Fig. 2. Growth of *D. primolecta* in 0.84M $MgSO_4$ medium.

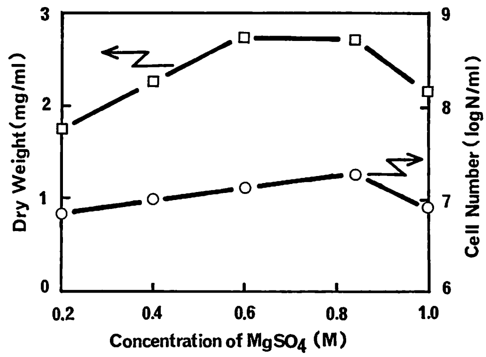

Fig. 3. Growth of *D. tertiolecta* after 3 days culture in media containing various concentrations of $MgSO_4$.

c) Effect of CO_2 concentration in air flow on the growth of D. tertiolecta *in $MgSO_4$-hypertonic medium.*

GINZBURG and GINZBURG (1981) reported that CO_2 acts as a limiting factor for the growth of *D. tertiolecta* in NaCl-hypertonic medium under fixed temperature, light intensity and air flow rate conditions. Figure 4 shows that growth of the alga was promoted by increasing the CO_2 concentration in the air flow in the $MgSO_4$-hypertonic medium as well as the NaCl one. The pH value of the medium did not change during the experimental period irrespective of CO_2 concentration. Thus the CO_2 effect on the growth was not due to the pH change of the medium.

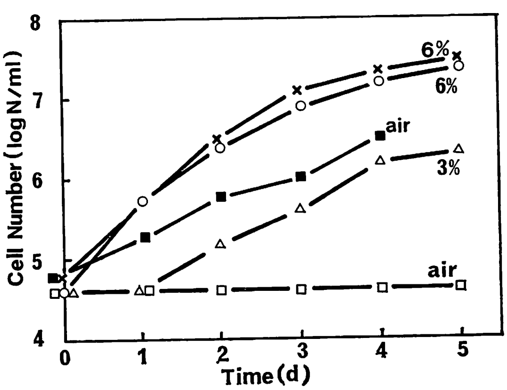

Fig. 4. Effect of CO_2 concentration on the growth of *D. tertiolecta*. (○), (△) and (□) indicate the growth curves in 0.84M $MgSO_4$ medium, and (×) and (■) those in 0.5M NaCl medium.

Moreover, in 0.84 M $MgSO_4$ the appearance of the exponential phase was significantly delayed by lowering the CO_2 concentration compared to 0.5 M NaCl. This suggests that the growth in 0.84 M $MgSO_4$ medium is more sensitive to CO_2 in the atmospher than that in 0.5 M NaCl medium.

d) Effect of partial replacement of $MgSO_4$ *with* $MgCl_2$ *on the growth of* D. tertiolecta *and* D. primolecta.

The effect of the sulphate anion on *D. tertiolecta* and *D. primolecta* in $MgSO_4$ is shown in Figs. 5 and 6. Neither species grew in medium containing only $MgCl_2$, but the addition of $MgSO_4$ in concentration greater than 0.17 M promoted growth. Furthermore, $Mg(NO_3)_2$ did not support growth

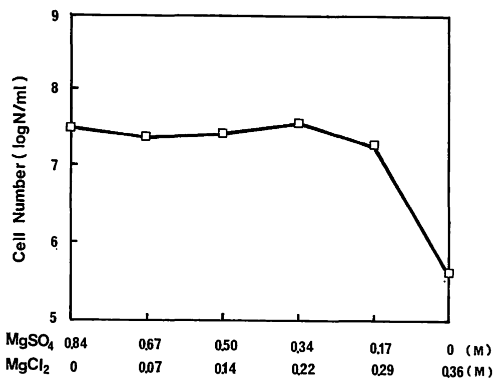

Fig. 5. Effect of different ratios of $MgSO_4$ and $MgCl_2$ on the growth of *D. tertiolecta* after 3 days culture.

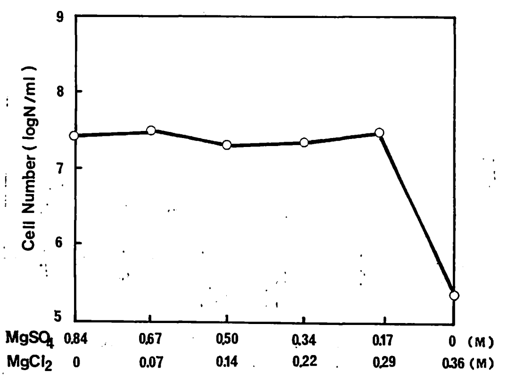

Fig. 6. Effect of different ratios of $MgSO_4$ and $MgCl_2$ on the growth of *D. primolecta* after 3 days culture.

(Table 2). Therefore, it appears that tolerance to $MgSO_4$-hypertonicity is particularly characteristic of *D. tertiolecta* and *D. primolecta* among the species tested. STEINER (1939) classified herbaceous higher plant halophytes into chloride- and sulphate- types. Although no such classification has been applied to the algae, *D. tertiolecta* and *D. primolecta* would belong to the sulphate-type according to Steiner's scheme.

It is not clear why *D. tertiolecta* and *D. primolecta* can grow in $MgSO_4$-hypertonic medium. We plan to continue the research into the mechanisms of this phenomenon.

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藤井修平・竹西真弓・万谷司郎・高田英夫：数種の *Dunaliella* の Mg イオン高張環境下における生長

Dunaliella の食塩耐性についての報告は多くみられるが，食塩以外の塩耐性に関する知見はない。6種7株の *Dunaliella* について，NaCl, LiCl, $MgSO_4$ 塩高張液で，それぞれ生長実験を行なった。すべての株は NaCl 高張液で増殖したが，LiCl では増殖しなかった。*D. tertiolecta* と *D. primolecta* の2種のみが，NaCl 塩以外に $MgSO_4$ 塩高張下で生長することがわかった。これら両種の $MgSO_4$ 塩高張下での生長は，NaCl 塩高張下での生長に較べ CO_2 濃度に強く依存することが示唆された。又，他の Mg 塩，$MgCl_2$ や $Mg(NO_3)_2$ 塩高張下では生長を示さず，何故，$MgSO_4$ 塩高張下で生長可能なのか，現在検討中である。(631 奈良県奈良市学園南3丁目，帝塚山短期大学)