Good Growth of *Chlorella saccharophila*, on the Basis of Dry Weight, under NaCl Hypertonic Condition

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In salt-free and saline media containing NaCl at the concentrations up to 1.0M, on the dry weight basis, the growth of a strain MY701 of *Chlorella saccharophila*, grown autotrophically, was promoted more significantly in 0.5 M NaCl medium than in salt-free medium at 72 hr-incubation. The highest growth rate was observed in 0.5 M NaCl medium for during 24 hrs from 48 to 72 hr.

LiCl at 0.5 M concentration did not stimulate significantly the growth rate as NaCl did. The promotion of the growth rate of this strain in 0.5 M NaCl medium resulted from the enhanced photosynthetic rate associated with the increase in the respiratory rate.

When a strain TK66 of *Chlorella vulgaris* was used instead of the present material, this fact was not the case.

Studies on the productivity of autotrophic microorganisms in the sea and the substances which were obtained from them, have received considerable attention during the last decade. If autotrophic microorganisms which originally were habituated in fresh water, grow well in saline medium and produce an organic matter at higher level than originally marine-habituated ones do, they will afford an interesting ecological and economical situation. For example it has been reported by TAKADA *et al.*¹⁾ that a strain MY701 of *Chlorella saccharophila* can grow well heterotrophically in saline medium and the growth-promoting effect of extract of this *Chlorella* cell was observed substantially on yeast and a protozoa, *Tetrahymena pyriformis* strain W.

If the cells of MY701 used here were autotrophically mass-cultured, according to rough estimation of the present experimental data, reasonable productivity will be supported to reach at least 0.5 ton dry matter per ha per day by saline culture method.

This communication deals with the autotrophic growth of a strain MY701 of *Chlorella saccharophila* in medium hypertonicity added with NaCl, when the growth was expressed as dry weight.

Materials and Methods

A strain MY701 of *Chlorella* was isolated from salt-farm at Yashima along the Seto Inland Sea in Japan in 1970 and was identified as *Chlorella saccharophila* (KRÜGER) MIGNOLA var. *saccharophila* by Dr. Yuichi YONEDA. A strain TK66 of *Chlorella vulgaris*

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Table 1. Composition of M4N medium used in this experiment

KNO ₃	5.0 g
KH ₂ PO ₄	1.25 g
MgSO ₄ · 7H ₂ O	2.5 g
FeSO ₄ · 7H ₂ O	0.003g
A ₅	1.0 ml
Dist. Water	1000 ml
A ₅	
H ₃ BO ₃	2.86 g
MnCl ₂ · 4H ₂ O	1.81 g
ZnSO ₄ · 7H ₂ O	0.22 g
CuSO ₄ · 5H ₂ O	0.08 g
Na ₂ MoO ₄	0.021g
Dist. Water	1000 ml
Conc. H ₂ SO ₄	1 drop

was also used as an experimental material.

Cells were grown in medium whose composition was given in Table 1 which was basically similar to that of M4N medium (TAMIYA *et al.*²¹). Culture tubes were 250 ml test tubes and each was fitted with a bubbling tube extending down into the culture solution. Each test tube contains 100 ml of culture solution. The cotton plug was forced into the top end of the test tube. Filtered air and CO₂ gas in air were passed vigorously through culture solution in each test tube. The cells have been precultured heterotrophically in M4N medium containing 1% glucose for 5–6 days at the light intensity of 8,000 lux at 25°C under stationary condition. Hereafter the cells which were starved in 0.01 M KH₂PO₄ solution for 48 hrs at 25°C in dark, were inoculated in salt-free and saline media, respectively. The inoculated test tubes were placed in a temperature-controlled room at 25°C and were illuminated from both side by fluorescent lamps. The distance from the surface of the test tube to lamps was about 3.5 cm. Light intensity was about 10,000 lux at the surface of the test tube.

The culture reached the cell density of 2.2×10^8 cells per ml culture solution at the stationary phase of growth under salt-free, normal condition. The dry weight of the cells was determined by drying the cells for 48 hrs at 98°C after washing with distilled water three times and the data were expressed as mg dry weight per ml culture solution.

Respiratory rate was estimated from the decrease in dry weight of cells per ml culture solution during subsequent 24 hr dark-incubation after light-growth for different periods in media and also photosynthetic rate was estimated from adding respiratory rate to growth rate during the same incubation.

Results

Data of the growth of the present material, *Chlorella saccharophila* MY701 strain, during 72 hr-incubation were shown in Fig. 1. Under saline condition with NaCl at the

Fig. 1

Fig. 1. Time course of growth of a strain MY701 of *Chlorella saccharophila*, expressed as mg dry weight per ml culture solution, in autotrophic media with NaCl at various concentrations. Open circles: salt-free medium; open triangles: 0.3 M NaCl medium; closed circles: 0.5 M NaCl medium; closed triangles: 1.0 M NaCl medium.

Fig. 2

Fig. 2. Time course of changes in growth rate of a strain MY701 of *Chlorella saccharophila*, expressed as mg dry weight per ml culture solution per 24 hr, in autotrophic media with NaCl at various concentrations. Open circles: salt-free medium; open triangles: 0.3 M NaCl medium; closed circles: 0.5 M NaCl medium; closed triangles: 1.0 M NaCl medium.

concentration from 0.3 to 0.5 M, a good growth could be obtained at 72 hr-incubation, while under saline condition with 1.0 M NaCl a growth was reduced.

The data in Fig. 2 expressed the growth rates which were calculated from the data in Fig. 1. The growth rates in salt-free and saline media were almost the same during early stage of incubation, but hereafter the rates varied from each another for 24 hr-incubation from 48 to 72 hr. Among them the highest growth rate was observed in 0.5 M NaCl medium, but the lowest one was done in 1.0 M NaCl medium.

On the other hand when NaCl was substituted for LiCl in the medium, the growth rate, expressed as increase in dry weight of cells per unit culture solution, in 0.5 M LiCl medium for 24 hrs from 48 to 72 hr was only 25 per cent of that in 0.5 M NaCl medium (Table 2).

The increase in dry weight of cells per unit culture solution may be resulted from following five different patterns between two rates of both photosynthesis and respiration; 1) the increase in photosynthetic rate without the change in the respiratory rate, 2) the decrease in the latter without the change in the former, 3) the increase in the former together with the decrease in the latter, 4) the decrease in the latter at the higher level com-

Table 2. Growth rate of a strain MY701 of *Chlorella saccharophila* in autotrophic media with 0.5 M NaCl or 0.5 M LiCl for 24 hr-incubation time from 48 to 72 hr. Data were expressed as mg dry weight per ml culture solution per 24 hr

	0.5 M NaCl	0.5 M LiCl
Growth rate	0.8	0.2

pared with the decrease in the former, 5) the increase in the former at the higher level compared with the increase in the latter. Our understanding penetrates to the approach to salt-tolerant processes with the increasing complexity in both photosynthetic and respiration chains because both pathways seemed to be different responses of utilizations of minerals and carbon dioxide in saline medium from those in salt-free medium. However the process of coupling between photosynthesis and respiration in the increase in dry weight of cells is an interesting problem.

The data of both photosynthetic and respiratory rates were shown in Fig. 3 and 4, respectively. The both rates increased in the course of incubation time. The photo-

Fig. 3

Fig. 3. Time course of changes in rate of increase in dry weight by photosynthesis of a strain MY701 of *Chlorella saccharophila*, expressed as mg dry weight per ml culture solution per 24 hr, in autotrophic media with NaCl at various concentrations. Open circles: salt-free medium; open triangles: 0.3 M NaCl medium; closed circles: 0.5 M NaCl medium; closed triangles: 1.0 M NaCl medium.

synthetic rate was promoted by the hypertonicity with NaCl and the highest rate was observed in 0.5 M NaCl medium for 24 hrs from 48 to 72 hr, but the lowest one was done in 1.0 M NaCl medium. The respiratory rate was not changed so much as the photosynthetic rate in the range from zero to 0.5 M NaCl, while the rate in 1.0 M NaCl medium

Fig. 4

Fig. 4. Time course of changes in rate of decrease in dry weight by respiration of a strain MY701 of *Chlorella saccharophila*, expressed as mg dry weight per ml culture solution per 24 hr, in autotrophic media with NaCl at various concentrations. Open circles: salt-free medium; open triangles: 0.3 M NaCl medium; closed circles: 0.5 M NaCl medium; closed triangles: 1.0 M NaCl medium.

was low significantly. Accordingly the process of the increase in dry weight of cells of MY701 in 0.5 M NaCl medium for 24 hrs from 48 to 72 hr may be attributed to the last pattern among the five ones. The same results were observed in 0.3 and 1.0 M NaCl media for 24 hrs from 48 to 72 hr, although the photosynthetic rates were lower than that in 0.5 M NaCl medium, but the process of increase in dry weight of cells per unit culture solution in salt-free medium for 24 hrs from 48 to 72 hr was attributed to the reverse of the last pattern. These facts show that the decrease in growth rate at the higher level from 0.5 to 1.0 M NaCl medium than that from 0.5 M NaCl to salt-free medium may be due to some different responses of mechanisms of photosynthesis and respiration to the hypertonicity with NaCl, even if a same difference of 0.5 M NaCl. Furthermore the processes of increase in dry weight of cells per unit culture solution in 0.5M NaCl medium during each 24 hr-incubation were attributed to the last pattern, but the highest rates of photosynthesis and respiration were observed for 24 hrs from 48 to 72 hr and the process of increase in the photosynthetic rate during 72 hr-incubation was different from that in the respiratory rate.

Table 3. The photosynthetic and respiratory rates of a strain MY701 of *Chlorella saccharophila* and a strain TK66 of *Chlorella vulgaris*, on the basis of change in dry weight, in autotrophic medium with 0.3 M NaCl for 24 hr-incubation time from 24 to 48 hr. Data were expressed as mg dry weight per ml culture solution per 24 hr

	<i>Ch. saccharophila</i>	<i>Ch. vulgaris</i>
Photosynthetic rate	0.8	0.6
Respiratory rate	0.1	0.1

On the other hand in 0.5 M NaCl medium A strain TK66 of *Ch. vulgaris* did not grow at all for the same incubation time. The process of increase in dry weight of cells of TK66 in 0.3 M NaCl medium for 24 hrs from 24 to 48 hr was attributed to the last pattern among the five ones. The results were shown in Table 3. In 0.3 M NaCl medium there was a significant difference between two kinds of *Chlorella* cells in the photosynthetic rate rather than the respiratory one. This fact showed that photosynthesis played an important role for the mechanism of salt-tolerance of cells of MY701

Discussion

Numerous investigations have established the fact that the hypertonicity bring about the inhibition of growth in fresh water, unicellular green algae (WETHERELL,³⁾ INOUE *et al.*⁴⁾). The significant difference between halophilic green algae and mesophilic, fresh water ones was also recognized in the degree of photosynthetic rate and respiratory rate when exposed to saline environment (CHIMIKLIS *et al.*⁵⁾, GESSNER,⁶⁾ OGATA *et al.*,⁷⁻⁸⁾ NAKA NISHI *et al.*⁹⁾).

A strain MY701 of *Ch. saccharophila* as the present material is considered as a facul-

tative halophilic *Chlorella* with the good growth on the dry weight basis in salt-free as well as saline media. Therefore the marked divergence in salt-tolerance provides us with a different mechanism in the physiological characteristics from those of fresh water algae as well as obligatory marine algae.

The basic consideration is the necessity for the different mechanisms of photosynthesis and respiration to grow in either saline or salt-free medium. Such comparative biochemistry of photosynthesis and respiration must be significantly assembled in the present materials.

As shown in results mentioned above, basically to increase in dry weight of cells of *Chlorella*, five different patterns were proposed. The highest growth rate in a strain MY701 of *Ch. saccharophila* was observed in 0.5 M NaCl medium for 24 hrs from 48 to 72 hr and the process of increase in dry weight of cells was attributed to the last pattern. The same results were observed in 0.3 and 1.0 M NaCl media for 24 hrs from 48 to 72 hr, while the process of increase in dry weight of cells in salt-free medium for the same incubation time was attributed to the reverse of the last pattern. These facts show that the decrease in growth rate at the higher level from 0.5 to 1.0 M NaCl medium than that from 0.5 M NaCl to salt-free medium may be due to some different responses of mechanisms of photosynthesis and respiration to the hypertonicity with NaCl, even if a same difference of 0.5 M NaCl. Furthermore in 0.5 M NaCl medium the process of increase in the photosynthetic rate during 72 hr-incubation was different from that in respiratory rate. This shows that the response of the mechanism of photosynthesis to the hypertonicity with NaCl is different from that of respiration.

On the other hand LiCl could not substituted for NaCl under the same hypertonic condition, because the increase in dry weight of cells of MY701 in 0.5 M LiCl medium was approximately equal to that in 1.0 M NaCl medium. The role of Na ion on the photosynthetic activity appears to differ from that of Li ion in which Cl ion fail to contribute to the activity.

A significant difference was also recognized in the change of photosynthetic rate in comparison with that of the mesophilic, fresh water alga *Ch. vulgaris*.

Conclusively the nature of the specificity of Na ion under hypertonic condition had a profound influence on the photosynthetic rate. Therefore some mechanisms of enhanced photosynthetic activity by the hypertonicity with NaCl must be investigated in future. The substances which especially were synthesized by such NaCl-enhanced photosynthetic metabolism must be incorporated effectively to dry matter of the cells to increase dry weight of cells per unit culture solution. Changes in dry matter per unit autotrophic culture solution during experimental period must related to changes in cell number and further in daughter cells within a single cell. Some informations concerned with cell number and formation of daughter cell were obtained recently by the authors under the exactly

same condition as the present case. Such evidences will be reported by the authors in the near future.

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