Growth of Conchocelis in Artificial Medium in Relation to Carbon Dioxide and Calcium Metabolism*

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Since the discovery of Conchocelis-phase in the life history of *Porphyra umbilicalis* by DREW (1949), the technique for aquiculture of *Porphyra tenera* or *Porphyra yezoensis* (Japanese laver) has developed rapidly depending on her theory of Conchocelis. This technical advancement in the laver aquiculture has played an important role in the laver industry in Japan, so consequently the year's crop of laver in Japan has come to be harvested more plentifully than before. But the fundamental knowledge concerning the growth of Conchocelis has not yet far advanced. It seems, therefore, to be important to know the fundamental theories on the growth of Conchocelis in order to establish the technique for aquiculture of Japanese laver and Conchocelis.

Three-dimensional aspects of the growth of Conchocelis in pearl oyster shell matrix were successfully visualized in the author's earlier report (1961) accompanying the many other morphological observations achieved. Also a physiological survey was made by him concerning the growth of Conchocelis affected by such conditions as light, salinity, pH, some growth substances, anaerobic environment, etc. Among these results obtained, the most attractive fact was the influence of anaerobic conditions. That is, shell-inhabiting Conchocelis was able to grow to some extent even when the whole surrounding sea water was replaced completely with liquid paraffin under adequate conditions of temperature and illumination. Morphologically observable aspects of Conchocelis which grew under such peculiar conditions were somewhat abnormal; namely, they had a slenderer and less branched form than that of normal one. Despite of these morphological deviations, the growth rates of Conchocelis in both media, sea water and liquid paraffin were quite identical.

In general, ordinary marine plants require much carbon source dissolved in medium for photosynthesis and soon exhaust it when the volume of culture medium is limited.

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So this growth of Conchocelis under the anaerobic conditions appears to be rather exceptional.

In order to explain this fact, some studies were made using the Conchocelis-phase of *Porphyra tenera* inhabiting in shell matrix and artificial sea water in relation to carbon dioxide and calcium metabolism from January, 1964 in the Shimonoseki area in Japan.

Materials and Methods

Conchocelis-phase used as material was prepared by infecting the carpospores liberated from mother thalli on the surface of shell matrices on January 21, 1964. Matured thalli as mother plants were collected from the laver farm near the laboratory, and pearl oyster shell was used as shell matrix. Carpospore-infected shell matrices were precultured in natural sea water for 5 days. Several pieces were cut out of these shell matrices which harbored the young Conchocelis germlings. These shell pieces were immersed in the prepared artificial media and cultured under the day and night illumination of 2000 lux and the room temperature without any agitation or aeration. The sizes of young Conchocelis were 145.7 μ in long axis and 82.0 μ in short axis on the average at the initiation of examination. Quantitative measurements of growth were made by means of microscopy averaging the sizes of 10 individuals.

The composition of standard medium (A) and supplementary medium (B) are shown in Table 1.

Table 1. Composition of medium

A		B	
NaCl	23.5 g/l	EDTA・2Na	3.0 g/l
Na_2SO_4	4.0 〃	$FeCl_3 \cdot 6H_2O$	0.386 〃
$MgCl_2 \cdot 6H_2O$	11.0 〃	$MnCl_2 \cdot 4H_2O$	0.432 〃
KCl	660.0 mg/l	$ZnCl_2$	0.031 〃
H_3BO_3	30.0 〃	$CoCl_2 \cdot 6H_2O$	0.012 〃
$CaCl_2 \cdot 2H_2O$	700.0 〃	$CuSO_4 \cdot 5H_2O$	0.0047 〃
$NaNO_3$	20.0 〃		
$Na_2HPO_4 \cdot 12H_2O$	10.0 〃		
$NaHCO_3$	200.0 〃		
$SrCl_2 \cdot 6H_2O$	40.0 〃		
KBr	100.0 〃		
NaF	3.0 〃		
$LiNO_3$	0.7 〃		
$Na_2MoO_4 \cdot 2H_2O$	0.05 〃		

Sodium bicarbonate-free medium is represented as A-$NaHCO_3$, calcium chloride-free medium is A-Ca, calcium chloride and magnesium chloride-free medium is A-Ca-Mg.

Actually used media in this study were as follows: 1) A+B, 2) A, 3) A-$NaHCO_3$, 4) A-Ca, 5) A-Ca-Mg. In the case of medium A+B, one part of B solution was added to 500 parts of A solution.

Quantitative analyses of calcium production were made by means of the chelatometric titration method and for those of total carbon dioxide the alkalinity determination method was adopted in this study. Alkalinity determination was done using methylorange (0.1%) and aniline-blue (0.1%) mixed indicator after MACHIDA (1953). Blank test was made about each medium containing sterile shell pieces, respectively.

Results

The growth of Conchocelis in each medium: During a 10 days' cultivation, Conchocelis in each medium grew up to considerable sizes. Hereafter it came to be impossible to measure the growth of Conchocelis owing to the dense distribution of germlings and the anastomotic growth of branches.

So far as observed the results of a 10 days' cultivation, Conchocelis had most rapidly grown in the medium A and next in the medium A+B, then A-$NaHCO_3$, A-Ca-Mg, and A-Ca in this order. In the medium A, for example, it grew from 145.7 μ up to 465 μ in the size of long axis and from 82.0 μ up to 370 μ in short axis. And in the medium A-Ca, from 145.7 μ up to 280 μ in length and from 82.0 μ up to 165 μ in width. The results obtained in the other media were all plotted within these two sets of the uppermost and the lowermost values.

Although some differences were observed in the growth rate of Conchocelis in each medium, conclusively inhibiting effects by the absence of these important elements were not observed. This fact seems to mean that the antecedent presence of bicarbonate or calcium and magnesium in the medium never be indispensable for the growth of Conchocelis whenever it inhabits in a shell matrix. This fact will conflict with the fact that *Porphyra* bud or thallus can neither grow nor survive in either bicarbonate-free or calcium-free media. Conchocelis, therefore, may have somewhat peculiar mechanisms in its carbon dioxide or calcium metabolism. Of course, it has become clear that the presence of bicarbonate or calcium is somewhat favourable for the growth of Conchocelis. Fig. 1 represents these results. After 1 or 2 months, the surface of Conchocelis-inhabiting shell came to be markedly tinted with the original color of Conchocelis in every medium.

Carbon dioxide: Total carbon dioxide increased from 0 up to 3.62 milliequivalent per liter in the bicarbonate deficient medium containing Conchocelis-inhabiting shell pieces and also did from 0 up to 2.64 meq/1 in the same medium containing sterile shell pieces during a 14 days' cultivation. That is, total carbon dioxide increased more rapidly in the bicarbonate deficient medium containing Conchocelis-inhabiting shell pieces than in the same medium containing sterile shell pieces. As the weight of both Conchocelis-inhabiting and sterile shell pieces was equalized, it is supposed that the excess may be due to the metabolism by Conchocelis. That is, shell-inhabiting Conchocelis seems to

derive some form of carbon sources from calcareous matrix over the exhaustion of it for photosynthesis. Perhaps some parts of carbon sources would be derived directly from the

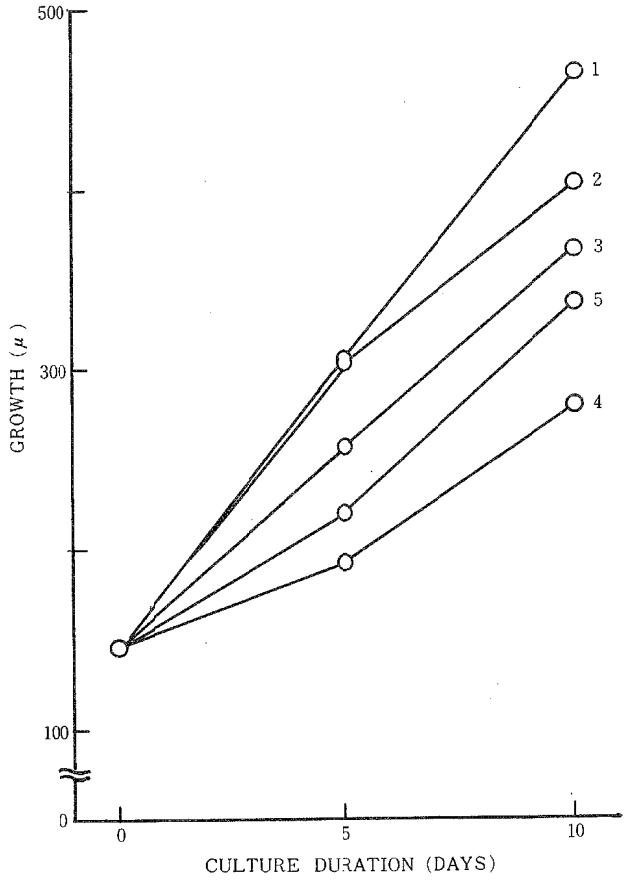

Fig. 1. Growth of Conchocelis in some important elements deficient media under 2000 lux and room temperature. 1 : standard medium, 2 : standard medium supplemented with chelating agent, 3 : sodium bicarbonate-free medium, 4 : calcium-free medium, 5 : calcium and magnesium-free medium.

shell matrix itself. In the standard medium A, total carbon dioxide was rather high from the start owing to prior addition of bicarbonate. Moreover, it increased higher than before the cultivation. After a 14 days' cultivation, each medium was changed to new one and the cultivations continued hereafter for 26 days, and the trends thus obtained were quite the same as that of preceding examination described above. These trends are represented in Fig. 2.

Calcium: Analyses of calcium showed that the quantities of calcium increased from 0 up to 64.9 mg per liter in the calcium deficient medium containing Conchocelis-inhabiting shell pieces and did from 0 up to 56.5 mg/1 in the same medium containing sterile shell pieces during a 15 days' cultivation. That is, calcium increased more rapidly in the medium containing Conchocelis-inhabiting shell pieces than in the same medium containing sterile

shell pieces. This trend was quite identical with the results obtained in both calcium and magnesium deficient media with or without Conchocelis. On the excessive production

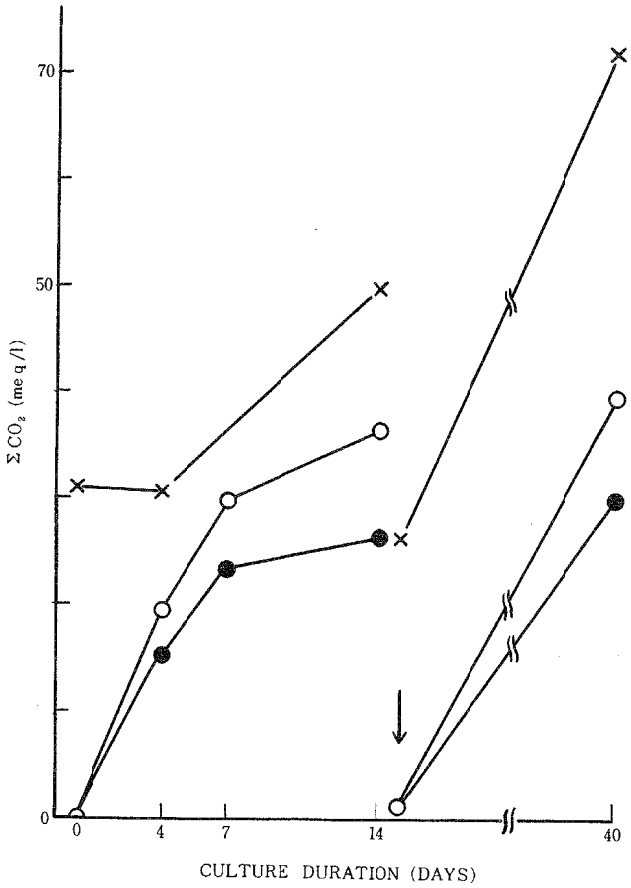

Fig. 2. Increase of total carbon dioxide during the cultivation of Conchocelis. Open circles : bicarbonate-free medium containing Conchocelis-inhabiting shell pieces, solid circles : the same medium containing sterile shell pieces, crosses : bicarbonate-rich medium containing Conchocelis-inhabiting shell pieces. The period of medium change is shown by the arrow.

of calcium in the medium containing Conchocelis-inhabiting shell pieces, the same reason as the case of bicarbonate metabolism by Conchocelis is most proper for explanation. That is, Conchocelis seems to derive some calcium salts from calcareous matrix in which it inhabits and at the same time Conchocelis uses calcium salts for calcium requirement.

After a 15 days' cultivation, each medium was changed to new ones and then surveys were made in succession hereafter for 26 days but whole trends were identical with those of preceding examination. All trends are shown in Fig. 3.

Summary

This report describes the growth of Conchocelis in relation to the increase in total carbon dioxide and calcium in artificial media during the cultivation. In measuring Conchocelis quantitatively, shell-inhabiting Conchocelis showed rather normal but somewhat slower growth in both bicarbonate-free and calcium-free media than in complete one. But this was not serious even in these deficient media. Measurements on the carbon dioxide and calcium contents in such media were made in an attempt to find how Conchocelis secure its growth under these circumstances. Both total carbon dioxide and calcium increased more rapidly in either of deficient media containing Conchocelis-inhabiting shell pieces than in the same media containing sterile shell pieces, respectively. So the shell-inhabiting Conchocelis seems to derive some forms of carbon sources and

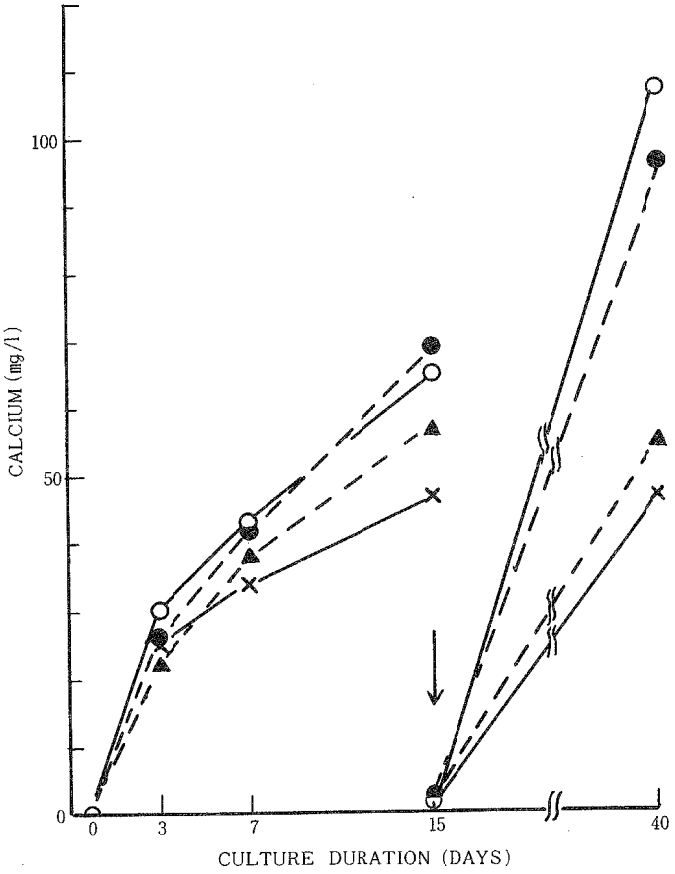

Fig. 3. Increase of calcium during the cultivation of Conchocelis. Open circles : calcium-free medium containing Conchocelis-inhabiting shell pieces, crosses : calcium-free medium containing sterile shell pieces, solid circles : calcium and magnesium-free medium containing Conchocelis-inhabiting shell pieces, triangles : calcium and magnesium-free medium containing sterile shell pieces. The period of medium change is shown by the arrow.

calcium salt from calcareous matrix and uses the former for photosynthesis and the latter for calcium requirement. The fact that *Porphyra* thallus can neither grow nor survive in either bicarbonate-free or calcium-free medium also supports this view on Conchocelis. Further studies on this problem are now under way.

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