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Efficiency of Chilled and Frozen *Nannochloropsis* sp. (Marine *Chlorella*) for Culture of Rotifer

Shigehisa Yamasaki*[1], Katsunori Tanabe*[2], and Hachiro Hirata*[1]

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Abstract

Tests for food efficiency and characteristics of chilled and frozen *Nannochloropsis* sp. (marine *Chlorella*) were conducted in order to know the applicability of preserved food in massculture of rotifer, *Brachionus plicatilis*, in consideration of the maintenance of the water quality. Chilled alga was prepared through concentration and preservation by chilling at 3°C. While frozen food was prepared by freezing of the concentrated alga at −25°C within the period of 20 minutes. Both types of food were preserved at the respective temperature for a month before the use in the experiment.

As to food efficiency of preserved algae, no significant difference was observed on the population growth of rotifers fed on both fresh and preserved algae. As to activity of preserved alga, chilled alga represented the same growth ability of fresh one under high light intensity. However, at low light intensity of 150 lx, growth was not observed. Frozen alga showed 3 times higher exudation of organic nitrogen from cell, in comparison with chilled alga.

Nannochloropsis sp. is, so far, the best food for rotifer, *Brachionus plicatilis*,[1] and contains several kinds of highly unsaturated fatty acids (HUFA) which are essential for marine fish larvae and are supplied to them through rotifer.[2,3] However, culture of the algae is proved to be troublesome because more space, facility and labor force are required, besides its low tolerance to high temperature during summer. Therefore, in order to utilize the period of off season of fish seed production for the culture of the alga, the efficiencies of the preservation of the alga related to the growth of rotifer and maintenance of the water qualities, were studied.

Materials and methods

Two types of preserved *Nannochloropsis* sp., namely, chilled and frozen, were prepared. The alga was cultured outdoor at 20 ppt salinity, 25°C and supplied with agriculture fertilizer (ammonium sulfate, calcium superphosphate, urea, and trace metals)(Yashima medium).[4] The alga was centrifuged at 5,000 $\times g$. This paste type of concentrated alga was resuspended with artificial seawater and was centrifuged again in order to remove the remaining nutrients in the intercellular water. The concentrated alga obtained from the above step was diluted with artificial

*[1] Laboratory of Fish Cultivation Physiology, Faculty of Fisheries, Kagashima University, 50-20 Shimoarata 4, Kagoshima 890, Japan.
*[2] Yeaster Co. Ltd., Tatsuno, Hyogo 679-41, Japan.

seawater to the density of $3,000\times10^6$ cells/ml. The algae was divided into the two fractions of 3°C chilling and −25°C freezing. Freezing of the alga was completed within 20 minutes using shallow aluminium tray. Both types of the alga were preserved at the respective temperature for a month before experiments on its food qualities were conducted.

a) Feeding test for rotifers

Large size (L type) rotifer, *Brachionus plicatilis*, was inoculated at 100 indiv./ml into 1,000 ml flask containing 600 ml of 20 ppt seawater. Three treatments which referring to the types of food such as chilled, frozen, and fresh algae were used with three flasks for each treatment. Food was supplied daily in every morning at the rate of 300×10^3 cells/indiv./day. Rotifer density was observed before feeding. When rotifer density was above 100 indiv./ml, the density was adjusted to 100 indiv./ml by harvesting. The culture was continued for two weeks without any water exchange. On the final day, PO_4-P concentration of the medium was determined by Strickland & Parsons method.[5] Throughout the culture period, environmental conditions were controlled at 25°C, 600 lx, and 14L:10D photoperiod. Aeration was supplied at 30 ml/min.

b) Activity

Growth ability was tested as an indicator for the activity of preserved algae. Growth experiments were conducted twice. In the first experiment, preserved and fresh algae were inoculated into Yashima medium (20 ppt) at the initial density of 2×10^6 cells/ml, and cultured under light conditions of 2,000 lx and 14L:10D. Triplicate were prepared for each treatment in 1,000 ml flasks. In the second experiment, chilled alga was inoculated at the density of 6×10^6 cells/ml and cultured at two light intensities, 150 and 1,050 lx at 25°C. Five flasks were used in both treatments. In these experiments, the density of alga was counted daily at 10:00 am by using a hemocytometer under a microscope.

c) Exudation of cell contents by preservation

Concentrations of organic nitrogen and ammonium were measured as indicators of exudation of organic matter, by Strickland & Parsons method.[5] Preserved alga was centrifuged at $5,000\times g$ for 10 minutes to get supernatant water used as sample for analysis. In the case of frozen alga, it was centrifuged after thawing under a room temperature.

Results and discussion

a) Feeding test for rotifers

During the first 2 days, the population growth of rotifer fed on frozen and fresh *Nannochloropsis* sp. were higher than those fed on chilled type (Fig. 1). However, on the second half of the culture period, similar population growth rate of about 1.6 times/day was observed for all treatment. Throughout the culture period, the population growth of rotifer fed on chilled alga was the most stable. Means and standard deviations of population growth rates of rotifer fed on refrigerated, frozen, and fresh alga were 1.48 ± 0.06, 1.56 ± 0.08, and 1.44 ± 0.06 times/day, respectively (Table 1). For the treatment using frozen alga, it can be considered that the mean

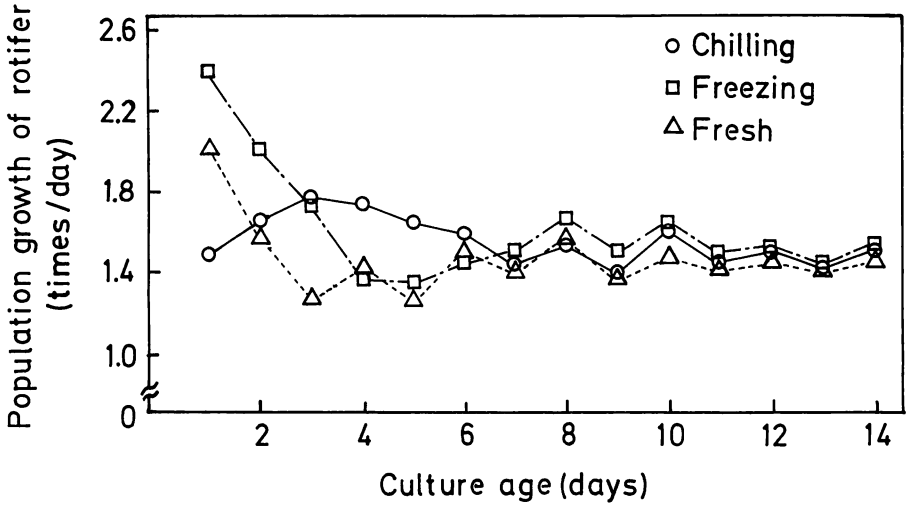

Fig. 1. Population growth rate of rotifer fed on two types of preservation; Chilling (○) and freezing (□) and on fresh (△) algae, *Nannochloropsis* sp..

Table 1. Population growth rate of rotifer during the 14 days of culture period, fed on three types of preservation of *Nannochloropsis* sp.

Type of preservation	Population growth (times/day)	n*
A: Chilling	1.48±0.06	42
B: Freezing	1.56±0.08	42
C: Fresh	1.44±0.06	42

* Number of data.

growth rate of rotifer was elevated by the high growth rate at the begining of the culture period.

Final PO_4-P concentrations in treatments fed on chilled, frozen, and fresh algae were 6.7±0.7, 7.0±0.7, and 4.7±0.4 μg-at/l, respectively. There was no water exchange throughout the experiment, and no significant difference on the population growth of rotifer was observed. Consequently, higher PO_4-P concentration in the medium supplied with chilled and frozen algae as food, must be derived mainly from intercellular waters of those preserved food.

b) Activity

In the first culture test of the algae, both chilled and fresh types showed similar growth rates. The densities increased about 2.5 times from 2×10^6 to 5×10^6 cells/ml in 5 days (Fig. 2). However, density of frozen alga decreased gradually to 1.3×10^6 cells/ml. In the second culture test, the cell density of chilled alga cultured under both light intensities of 150 and 1,050 lx showed no increment during the first 24 hours period (Fig. 3). Thereafter, the density increased from 6×10^6 to 7.6×10^6 cells/ml for light intensity of 1,050 lx in 3 days, while at 150 lx, the algal density was maintained at the initial density during the same period.

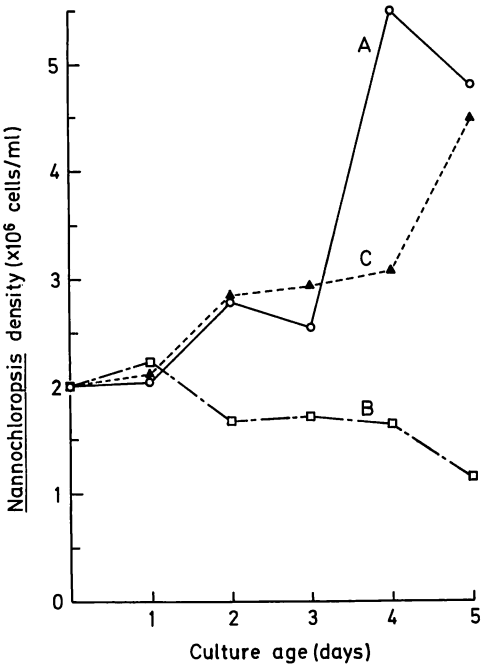

Fig. 2. Growth ability of three types of preservation of *Nannochloropsis* sp. under the light intensity of 2,000 lx. A: Chilling, B: freezing, C: fresh.

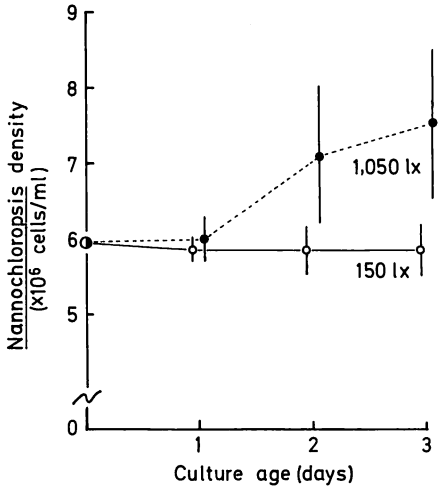

Fig. 3. Growth of chilled *Nannochloropsis* sp. under light intensities of 1,050 and 150 lx.

It could be concluded that chilled alga retains the same growth ability as that of the fresh alga even after one month of storage. Chilled alga grows normally at higher light intensity (1,050 lx), however, no growth at dim light (150 lx), which is probably lower than compensation light intensity for the alga.[6] Furthermore, it can be considered that chilling preservation of the alga at low temperature and dark conditions inhibited its growth ability. The culture of this alga under high light intensity induce its growth ability back to normal.

c) Exudation of cell contents during preservation

Concentrations of dissolved organic nitrogen in the intercellular water for chilled and frozen algae were 896 and 2,814 μg-at/l, respectively. On the other hand, ammonium contents were 16 and 17 μg-at/l in chilled and frozen algae, respectively (Table 2). High concentration of the organic nitrogen recorded in medium containing frozen alga can be considered as due to the exudation of the materials from damaged cells during the process of freezing and thawing. This exudated nitrogen becomes one of materials for pollution.

During the fish seeds production period, facilities and labors have to be concentrated on the rearing of fish larvae. On the other hand, fall and winter seasons, which are off season for fish seed production in Japan, higher quality of *Nannochloropsis* sp. on HUFA content can be produced

Table 2. Concentrations of dissolved organic nitrogen and ammonium in the intercellular water of two preservation types of *Nannochloropsis* sp. after one month of preservation period.

Type of preservation	Dissolved organic N ($\times 10^{-3}$ μg-at /10^9 cells)	Ammonium N ($\times 10^{-3}$ μg-at /10^9 cells)	n*
A: Chilling	896± 42	16±7	3
B: Freezing	2,814±134	17±7	3

* Number of samples.

under lower temperature.[7] The alga produced during this off season can be utilized efficiently in few months of storage. In addition, chilled alga maintains a better water quality, which is important for continuously harvesting of rotifer for a long period.[8-10] It produces low water pollution and has high growth ability, which could induce high nutrient absorption from the culture medium of rotifer through photosynthesis under strong light condition. Consequently, chilled alga is considered to be more useful preserved food for rotifer culture, in comparison with frozen type. However, chilled alga, which was preserved for long period, should be analyzed chemically on nutritional quality, i.e., HUFA. It was reported that HUFA contents of frozen rotifer fed on several types of food material; marine *Chlorella,* baker's yeast, and fatty acid enriched yeast; were reduced after long preservation period of about 4 months.[11] Chilled alga is also appropriate as food for culture experiments of rotifer in laboratory. For example, a study on the effect of food density on the growth of rotifer could be conducted, since level of the algal food is controllable as the chilled alga will not grow in the culture medium during the experiment if light intensity is set at low levels (below 150 lx).

During the storage even at low temperature of 3°C, the alga require oxygen through metabolism. Therefore, periodical supply of oxygen is essential during storage. In the case of frozen alga, 20 minutes were required for the completion of freezing process in this experiment. The quality of alga could be improved if it could be frozen within a shorter period using a lower temperature than that used in this experiment.

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