Lipid formation by Arctic microalga *Chlamydomonas* sp. KNF0008 at low temperature

Eun Jae Kim[1], Woongsic Jung[1,2], Suyoun Lim[1,3], Sanghee Kim[1], Han-Gu Choi[1], Se Jong Han[1,4*]

[1] Division of Polar Life Sciences, Korea Polar Research Institute, KIOST, Incheon, South Korea
[2] Research and Development, GDE, Gyeonggi-do, South Korea
[3] Functional Genomics R&D Team, Syntekabio, Daejeon, South Korea
[4] Department of Polar Sciences, University of Science and Technology, Daejeon, South Korea
*Corresponding author's Tel: +82-32-760-5521, E-mail: hansj@kopri.re.kr

Abstract

In the present study, we introduce a new lipid-producing microalga, *Chlamydomonas* sp. KNF0008, collected from the Arctic. This strain was capable of growing at temperatures ranging from 4 °C to 20 °C, and the highest cell density was measured at 15 °C and 100 µmol photons m^{-2} s^{-1} light intensity under continuous shaking and external aeration. KNF0008 showed the elevated accumulation of lipid bodies under nitrogen-deficient conditions, rather than under nitrogen-sufficient conditions. Fatty acid production of KNF0008 was 4.2-fold (104 mg L^{-1}) higher than that of *C. reinhardtii* CC-125 at 15 °C in Bold's Basal Medium. The dominant fatty acids were C16:0, C16:4, C18:1, and C18:3, and unsaturated fatty acids (65.69%) were higher than saturated fatty acids (13.65%) at 15 °C. These results suggested that Arctic *Chlamydomonas* sp. KNF0008 could possibly be utilized for production of biodiesel during periods of cold weather because of its psychrophilic characteristics.

Materials and Methods

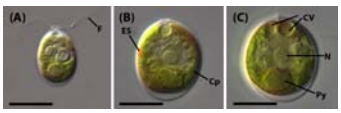
The microalga strain, *Chlamydomonas* sp. KNF0008, was collected from a freshwater lake near the Dasan, Korean Arctic Station located at 78°55´ N, 11°56´ E.

The nucleotide sequences were aligned with the Genetic Data Environment (GDE 2.2) program and MODELTEST v. 3.7 to determine the best fit model for the data. Molecular identification was carried out with both Bayesian and RAxML (Randomized Axelerated Maximum Likelihood) methods.

Cells were cultured in a 500 mL flask containing 250 mL of BBM (Bold's Basal Medium) or nitrogen-deficient BBM (BBM-N) under a light intensity of 100 µmol photons m^{-2} s^{-1} at 15 °C. The cultures of KNF0008 were supplied with air bubbles (~5 mL/min) from a compressor at the bottom of the flask. Pure industrial CO_2 gas (~0.1 mL/min) was added to the cultures to investigate the effect of CO_2 on KNF0008 growth.

Total lipids were extracted from 20 mg of freeze-dried cells as described by Sasser. Fatty acids in the organic phase were analyzed by gas chromatography (YL-6100GC, Young Lin Science, Korea) with a flame-ionized detector (FID) and HP-FFAP capillary column (30 m × 0.32 mm × 0.25 µm, Agilent, USA). Fatty acid methyl ester (FAME) analysis was performed under the following conditions: (1) constant flow mode of 3 mL min^{-1}, (2) temperature of 100 °C for 5 min, then changed at 4 °C min^{-1} to 240 °C, at which the program was held for 20 min, and (3) detector temperature of 250 °C. Each FAME component was identified and quantified by using the standard material in Supelco 37 Component FAME Mix (Sigma, USA).

Results



The components of the cell are as follows: flagella (F), chloroplast (Cp), contractile vacuole (CV), eyespot (ES), nucleus (N), and pyrenoid (Py). Scale bars = 10 µm.

Fig. 1. Normarski interference micrographs of vegetative cells of the Arctic green alga, *Chlamydomonas* sp. KNF0008. (A) Optical section of a vegetative cell with two flagella. (B) Surface view of a cell demonstrating the chloroplast and eyespot. (C) Surface view of a cell showing contractile vacuoles, the nucleus, and the pyrenoid.

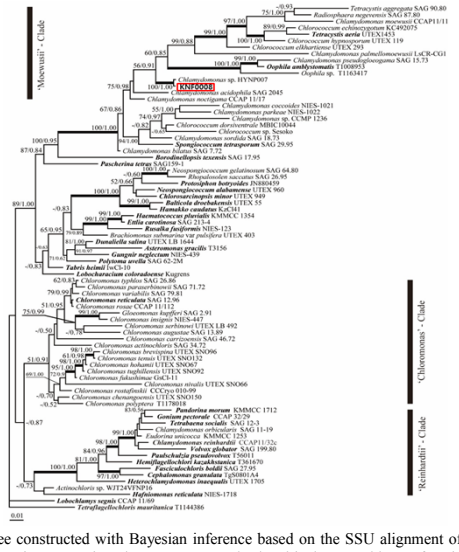


Fig. 2. Tree constructed with Bayesian inference based on the SSU alignment of sequences from the Arctic green microalga KNF0008 and related isolates. Bold type face indicates the isolate represents the type species of the genus. Values at branch nodes represent 1000 bootstrap replicates for ML analysis (left value) and Bayesian posterior probabilities (right value). Branches marked with bold lines demonstrated strong values of support (>95%) in all analyses, whereas those lacking values received less than 50% support. Scale bar = 0.01 substitutions/site.

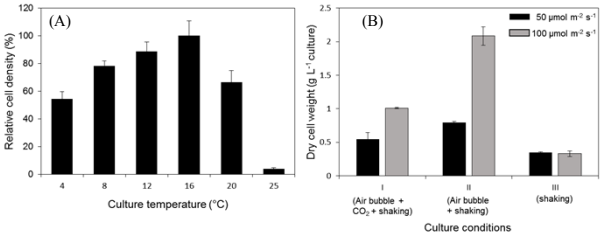


Fig. 3 Effects of culture conditions on the growth of KNF0008. (A) Effect of temperature. Cells were grown at 4 °C, 8 °C, 12 °C, 16 °C, 20 °C, and 25 °C under the same culture conditions with relative cell densities measured after 2 weeks of cultivation. (B) Effects of light intensity, shaking and aeration. Cells were cultivated in different culture conditions of light intensity (50 and 100 µmol photons m^{-2} s^{-1}) and aeration (no air, air-only, air and CO_2) with shaking at 15 °C.

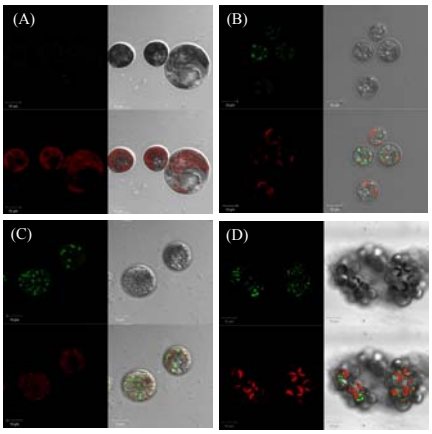


Fig. 4. Confocal microscopy image of *Chlamydomonas* sp. KNF0008 (A, B, C) and *Botryococcus braunii* (D). (A) KNF0008 cells were cultivated in BBM at 100 µmol photons m^{-2} s^{-1} of light intensity on a 16:8 h light-dark cycle for 2 weeks. (B) KNF0008 cells were cultivated in BBM at 100 µmol photons m^{-2} s^{-1} of light intensity on a 16:8 h light-dark cycle for 4 weeks. (C) KNF0008 cells were cultivated in nitrogen-deficient BBM at 100 µmol photons m^{-2} s^{-1} of light intensity on a 16:8 h light-dark cycle for 2 weeks. (D) *B. braunii* cells were cultivated in nitrogen-deficient BBM at 100 µmol photons m^{-2} s^{-1} of light intensity on a 16:8 h light-dark cycle for 2 weeks. Top-left: BODIPY 505/515 channel, false colored green; Top-right: black-and-white DIC microscopy image; Bottom-left: chlorophyll autofluorescence channel; Bottom-right: merged images of chlorophyll autofluorescence and BODIPY 505/515. Scale bar = 10 µm. BODIPY 505/515–stained green microalgae.

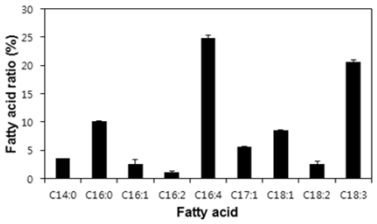


Fig. 5. Comparison of fatty acids produced from KNF0008. Cells were cultivated in nitrogen-deficient BBM at 15 °C for 2 weeks and each FAME was identified using Supelco 37 Component FAME Mix standard solution.

Conclusions

The oil composition and cultivation temperature of microalgae are key factors in determining the commercial usability for the production of functional fatty acids and biodiesels. Thus, the psychrophilic characteristics and formation of high amounts of unsaturated fatty acids can make KNF0008 a good candidate for oil production without necessitating temperature control in the winter season.

Acknowledgements

This work was supported by grants from KOPRI (PE15140, PE19180). This project was also supported by a grant (PN15070) of iPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries), Ministry of Agriculture, Food and Rural Affairs, Korea