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The new Arctic *Chlorella* species: growth and lipid contents

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ABSTRACT

Microalgae are considered as promising sources for biofuel production. In cold temperature, it is difficult to maintain proper culture condition for outdoor mass cultivation. Most microalgae inhabited in the polar region are able to grow at extreme cold temperature. Two *Chlorella* species, ArF0004 and ArF0006 were isolated from the Arctic sea ice and grown at various culture temperatures of 4, 12, and 20℃. The maximal cell densities of ArF0004 and ArF0006 in Bold's Basal Medium (BBM) were 1.3×10^7 cells/ml and 8.3×10^6 cells/ml at 20℃, respectively. The formation of lipid body of two *Chlorella* species were visualized by Nile red staining method and exhibited yellow-gold at 488 nm fluorescence. The fatty acid methyl esters (FAMEs) were analyzed using gas chromatography. ArF0004 and ArF0006 showed similar fatty acid contents, which were dominated by cis-10-heptadecenoic acid methyl ester (C17:1), 5,8,11-heptadecatrienoic acid methyl ester (C17:3) and α-linolenic acid methyl ester (C18:3). ArF0004 and ArF0006 also presented mainly α-linolenic acid methyl ester content (~27.6%) recommended FAMEs for good biodiesel properties. The two Arctic microalgae could grow at a wide range of temperatures and produce lipids at low temperature. Thus, we could expect that two Arctic *Chlorella* species may be suitable candidates for biofuel production. [This work was supported by Bio-industry Technology Development Program, Ministry of Agriculture, Food and Rural Affairs (PN14070).]

INTRODUCTION

Arctic's climate is characterized by cold winter, with the lowest temperature on earth. Despite the harsh conditions, polar region has a rich diversity of microalgal flora. With regards to potential abilities in Arctic microalgae, there have been trying to search polar algae for use in alternative energy and wastewater treatment. We performed screening for desirable candidates for lipid production and found two *Chlorella* species at Arctic ocean. In the present study, potential of two *Chlorella* species as a source of biodiesel was evaluated in terms of fatty acid content and growth temperature range.

MATERIALS & METHODS

Samples were collected from sea ice near the Dasan station located in NyAlesund, Spitsbergen, Norway (78° 50′ N, 11° 56′ E). The algae were purified by serial dilution followed by plating on agar. Individual green colonies were isolated and inoculated into liquid Bold's Basal Medium (BBM). To detect lipid droplets, cells were stained with Nile red (1 μg/ml, 9-diethylamino-5H-benzo[α]phenoxazine-5-one) in acetone. Lipids were extracted in hexane:methyl tert-butyl ether (1:1). Fatty acid methyl esters were analyzed by gas chromatography.

RESULTS

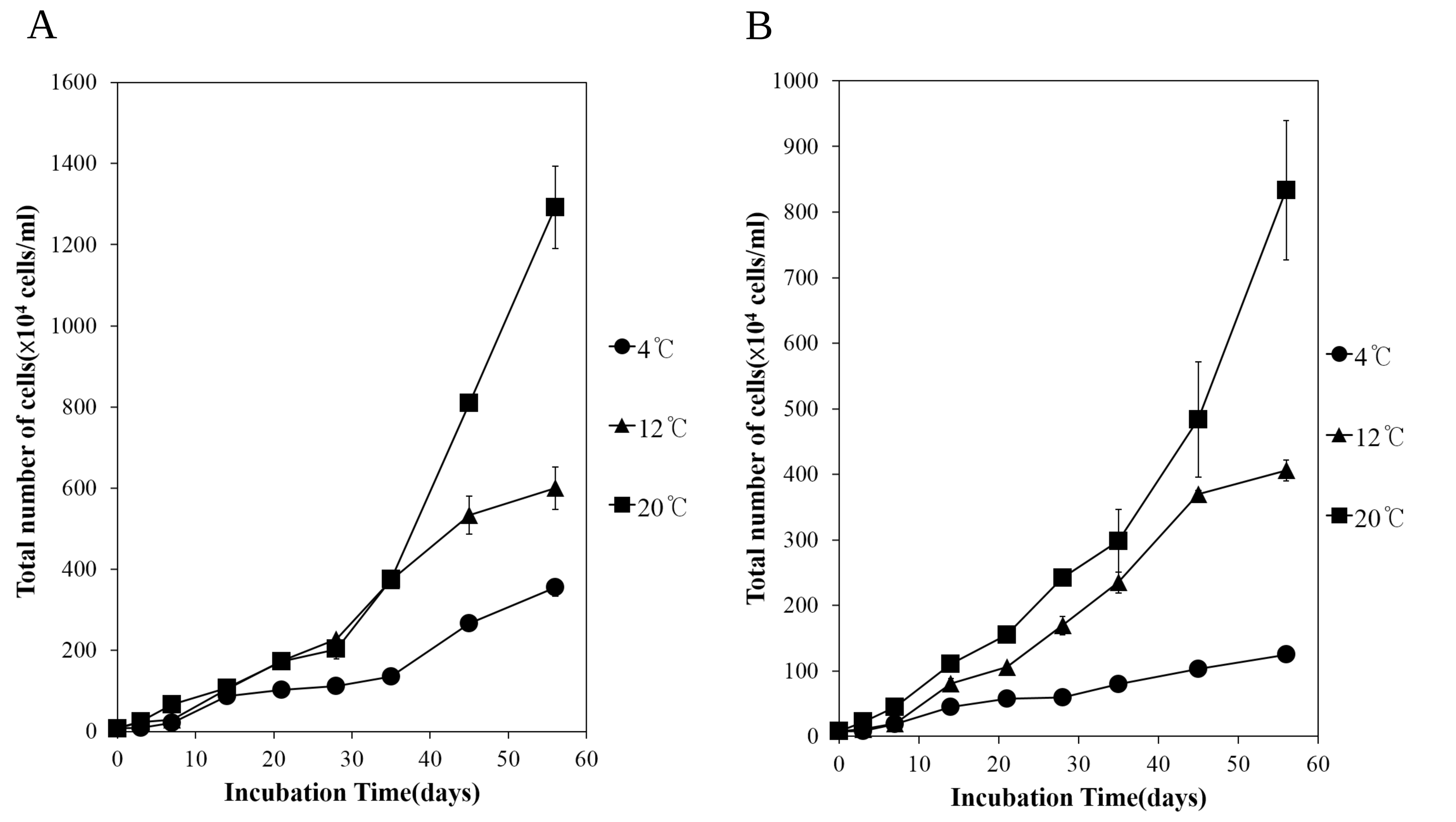

Fig. 1. Growth curves at various temperatures. Microalgae grew in BBM medium and stationary cultured at 4℃, 12℃, and 20℃ under white light intensity of 40 μmol $m^{-2} s^{-1}$

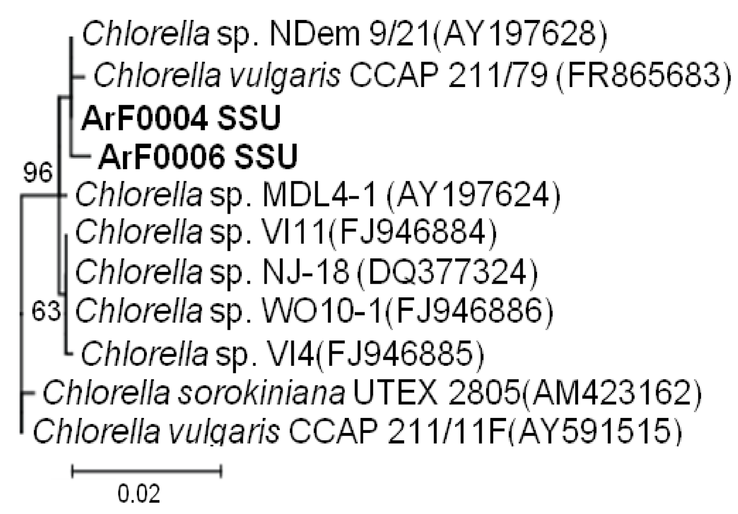

Fig. 2. Phylogenetic tree of SSU sequences from microalgae. Two Arctic green microalgae analyzed for the SSU alignment are shown in bold. SSU sequences from two Arctic green microalgae (ArF0004, ArF0006) were newly completed for this study ranged from 2670 to 1687 bp in length and have been deposited in GenBank.

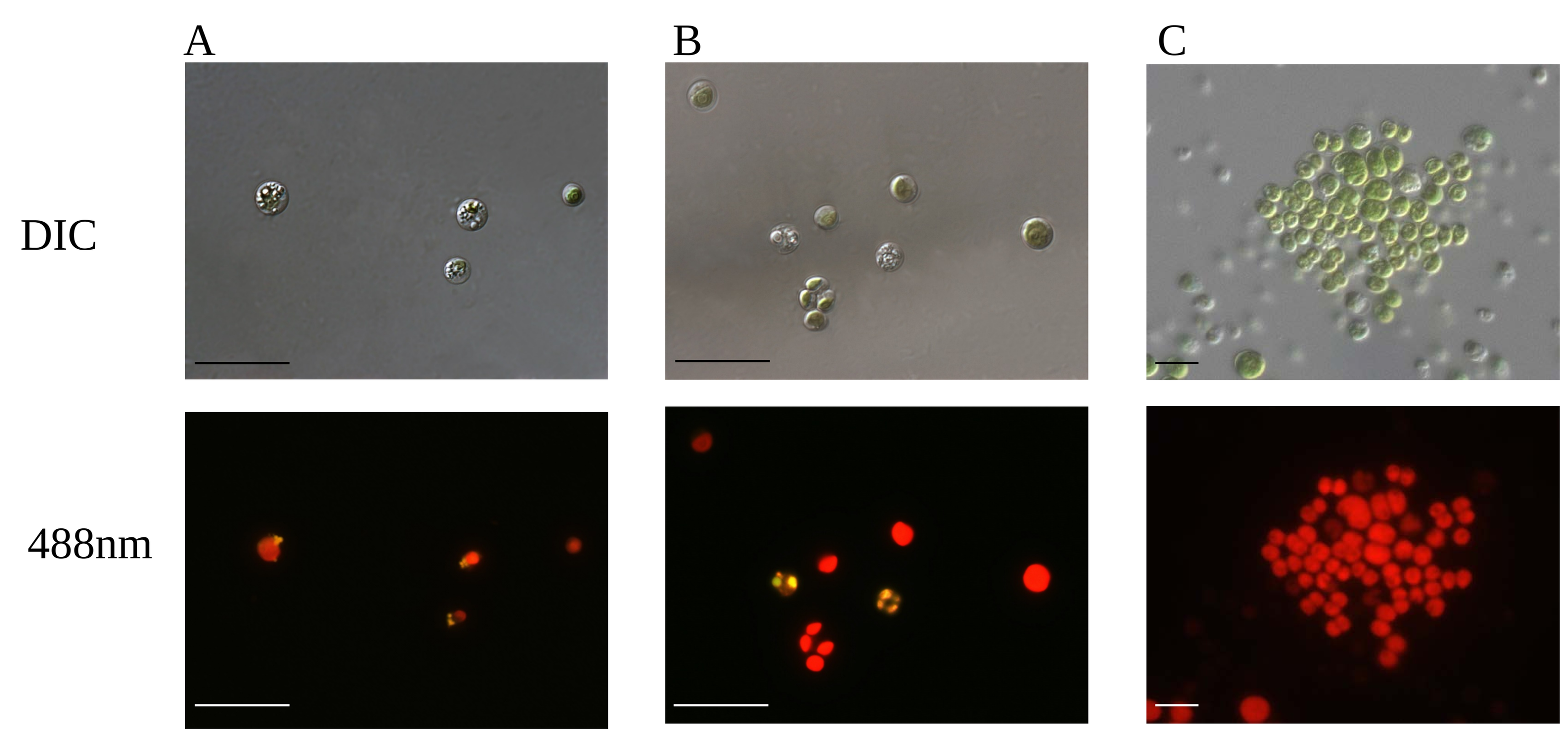

Fig. 3. Detection of lipid body in cell using Nile red staining. Cells cultured in BBM were stained with Nile red and viewed for yellow-gold fluorescence at 488nm. A) ArF0004, B) ArF0006, C) *Chlamydomonas reinhardtii* CC-125. Yellow and red colors indicate lipid droplets and chlorophyll, respectively (scale bar = 20μm).

Table 1. Fatty acid composition (% total fatty acid) of Arctic *Chlorella* sp. ArF0004 and ArF0006.

Fatty acid	ArF0004	ArF0006
C16:0	3.05	2.98
C17:1	20.32	16.07
C17:3	20.22	23.27
C18:1	2.03	–
C18:2	7.26	6.11
C18:3	25.46	27.63

* FAME composition : C16:0, Palmitic acid methyl ester; C17:1, cis-10-Heptadecenoic acid methyl ester; C17:3, 5,8,11-Heptadecatrienoic acid methyl ester; C18:1, Oleic acid methyl ester; C18:2, Linoleic acid methyl ester; C18:3, α-Linolenic acid methyl ester

* – : not detected

CONCLUSION

The two Arctic microalgae showed similar growth trends and fatty acid composition in this study. These microalgae could grow at a wide range of temperatures. Unsaturated fatty acids (UFAs) were dominant over 70% from ArF0004 and ArF0006. It was indicated that the survival of cells is partly due to the predominance of UFAs, which increases membrane fluidity at low temperature. Arctic *Chlorella* sp. ArF0004 and ArF0006 may suggest economic feasibility for lipid production.

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