The Arctic sea ice algae *Chlamydomonas* sp. ArF0032 : Growth and lipid contents at low temperature

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ABSTRACT

Biodiesel produced from algae shows great potential as green alternative energy. In winter season, however, outdoor mass cultivation for biodiesel production has a problem presenting poor growth rate.

The Arctic *Chlamydomonas* species, ArF0032 was isolated from sea ice near the Dasan Station located in Ny-Alesund, Spitsbergen, Norway (78° 50′ N, 11° 56′ E). ArF0032 was revealed to have optimal growth temperature of 4 °C and the maximal cell density of 1.7×10^7 cells/ml at 4 °C (Fig. 1). The formation of lipid body of ArF0032 was visualized by Nile red staining method and exhibited yellow-gold at 488 nm fluorescence. The ArF0032's fatty acid methyl ester (FAME) was analyzed using gas chromatography. The ArF0032 showed fatty acid contents, which were dominated by palmitic acid methyl ester (C16:0), 5,8,11-heptadecatrienoic acid methyl ester (C17:3), oleic acid methyl ester (C18:1), linoleic acid methyl ester (C18:2) and α-linolenic acid methyl ester (C18:3). The ArF0032 presented maximum percentage (20%) of oleic acid and the content of unsaturated fatty acids (72.1%) was higher than that of saturated fatty acid (9%).

The Arctic ArF0032 could grow at low temperature and produce fatty acids (C16:0 and C18:1) as desirable biodiesel. Thus, result of this study suggest that Arctic *Chlamydomonas* ArF0032 may be a source for biodiesel production.

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 *Chlamydomonas* species in Arctic ocean. In the present study, potential of *Chlamydomonas* 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 Ny-Alesund, 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

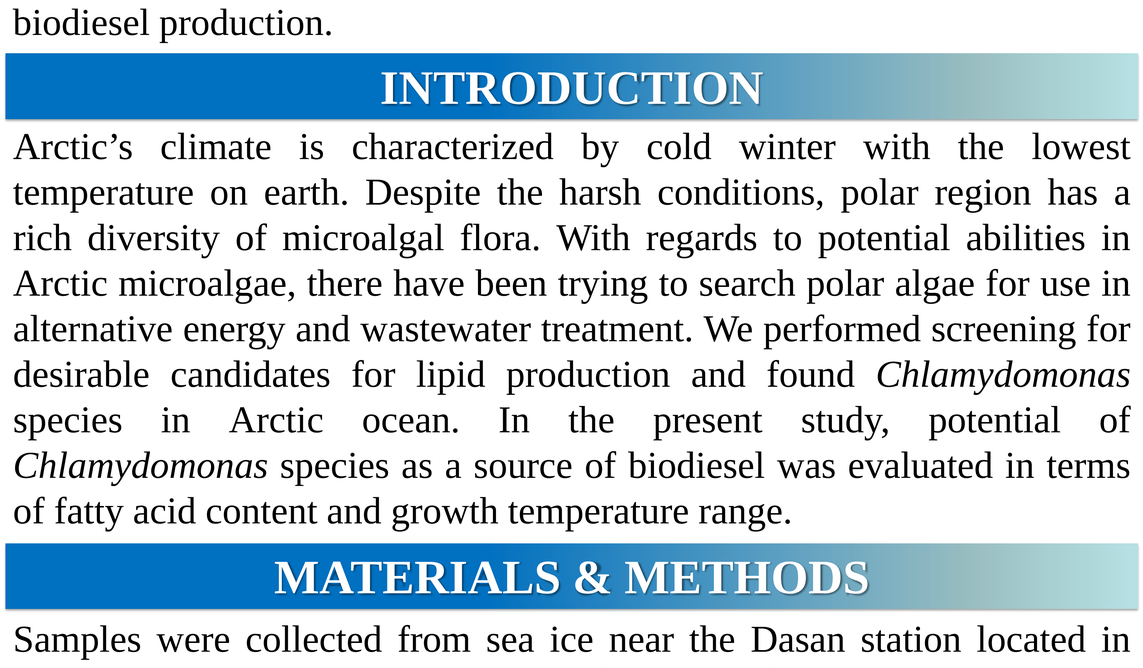


Fig. 1. Phylogenetic tree based on nuclear SSU rDNA sequence data. The Arctic green microalgae analyzed for the SSU alignment are shown in bold. SSU sequences from the Arctic green microalga ArF0032 was newly completed for this study and have been deposited in GenBank.

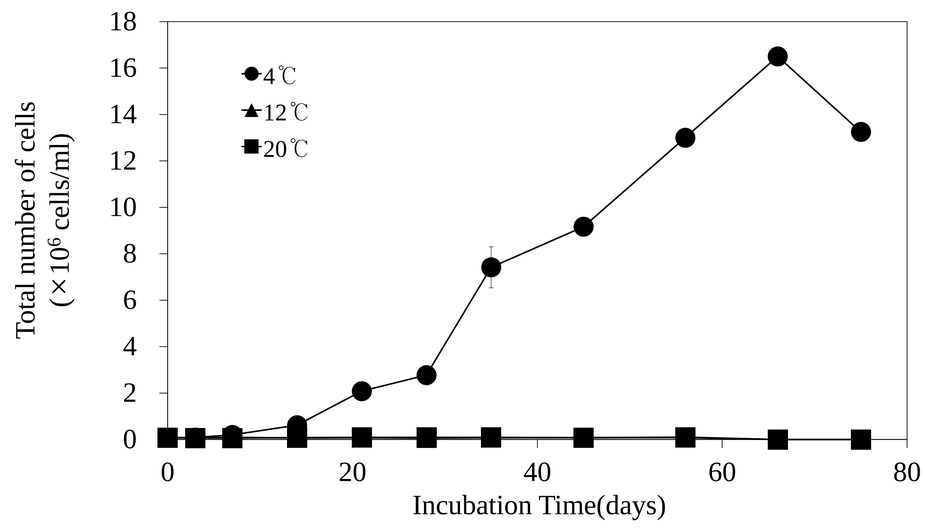


Fig. 2. Growth curves at various temperatures. Microalgae grew in BBM medium and stationary cultured at 4 °C, 12 °C and 20 °C under white light intensity of 40 μmol m^{-2} s^{-1}

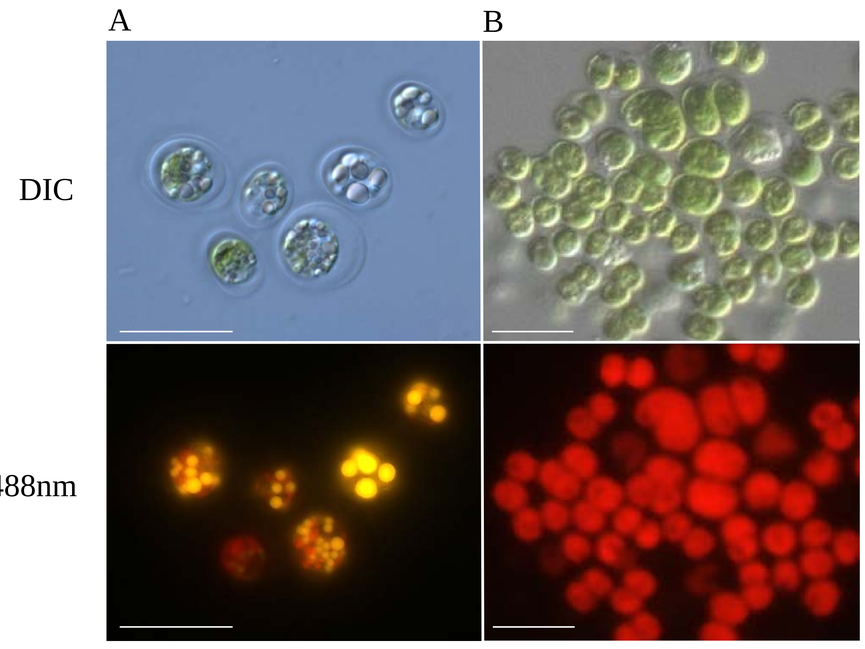
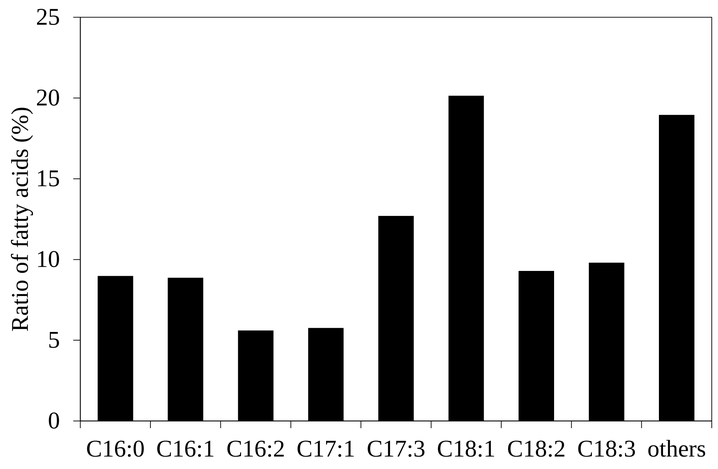


Fig. 3. Detection of lipid body in cell using Nile red staining. Cells cultured in BBM were stained with Nile red and viewed for yellow-gold fluorescence at 488 nm. A) ArF0032, B) *Chlamydomonas reinhardtii* CC-125. Yellow and red colors indicate lipid droplets and chlorophyll, respectively (scale bar = 20 μm).

Table 1. Fatty acid composition (% total fatty acid) of Arctic *Chlamydomonas* sp. ArF0032.

Fatty acid	ArF0032 (%)
C16:0	8.97
C16:1	8.86
C16:2	5.59
C17:1	5.75
C17:3	12.69
C18:1	20.13
C18:2	9.28
C18:3	9.79



* FAME composition : C16:0, Palmitic acid methyl ester; C16:1, Palmitoleic acid methyl ester; C16:2, Hexadecadienoic 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 Arctic microalga ArF0032 was shown to be psychrophilic microorganisms from its cardinal temperatures for growth. Unsaturated fatty acids (UFAs) were dominant over 70% from ArF0032. It was indicated that the survival of the cells was partly due to the predominance of UFAs, which increased membrane fluidity at low temperature. Arctic *Chlamydomonas* sp. ArF0032 may suggest economic feasibility for lipid production.

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