Cultivation and Lipid Formation of a Green Microalgae, *Chlamydomonas* sp. ArM0029A, Isolated from Arctic Sea Ice

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

[1]Division of Life Sciences, Korea Polar Research Institute, KIOST, Incheon 406-840, Korea

[2]Department of Polar Sciences, University of Science and Technology, Incheon 406-840, Korea

TEL: +82-32-760-5521, FAX: +82-32-760-5509

ABSTRACT

Chlamydomonas sp. ArM0029A originated from Arctic sea ice was cultivated for the production of biofuel. The pure culture was identified by sequence analysis (18s rDNA, rbcL gene) and the high lipid-producing microalgae *Chlamydomonas* sp. ArM0029A was examined by using nile red and fluorescence microscopy. *Chlamydomonas* sp. ArM0029A was grown at 4℃, 8℃, 12℃and 20℃, the doubling times were 72h, 34h, 18h, and 13h, at 4℃, 8℃, 12℃and 20℃, respectively. The maximal cell densities were 4×10^7, 2.6×10^7, 2×10^7, and 1.5×10^7 cells/mL at 4℃, 8℃, 12℃ and 20℃, respectively. Cells were visualized by nile red staining method and the lipid body dyed yellow-gold at 488nm fluorescence. The total lipid was analyzed by LC-MS and it was found that *Chlamydomonas* sp. ArM0029A contained more phospholipid, triglycerides (TG) and diacylglycerols (DG) than the control strain (*Chlamydomonas reinhardtii* CC-125) in positive mode. Taken together, these results suggest that ArM0029A may be a source of biodiesel and functional lipids. [This work was supported by Bio-industry Technology Development Program, Ministry of Agriculture, Food and Rural Affairs (PN13120).]

INTRODUCTION

Microalgae are considered an attractive source for producing biodiesel due to their high oil content. The key factors that affect algae growth and metabolism are sunlight, CO_2, water, nutrients, and temperature. Cold climates will reduce algae's oil production potential. To resolve this problem, we performed screening for desirable candidates and found *Chlamydomonas* sp. ArM0029A at Arctic ocean. In the present study, potential of ArM0029A 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) in 2008. The algae were purified by serial dilution followed by plating on agar. Individual green colonies were isolated and inoculated into liquid Tris–acetate–phosphate (TAP) medium. To detect lipid droplets, cells were stained with Nile red (1 μg/ml, 9-diethylamino-5H-benzo [α]phenoxazine-5-one) in acetone.

RESULTS

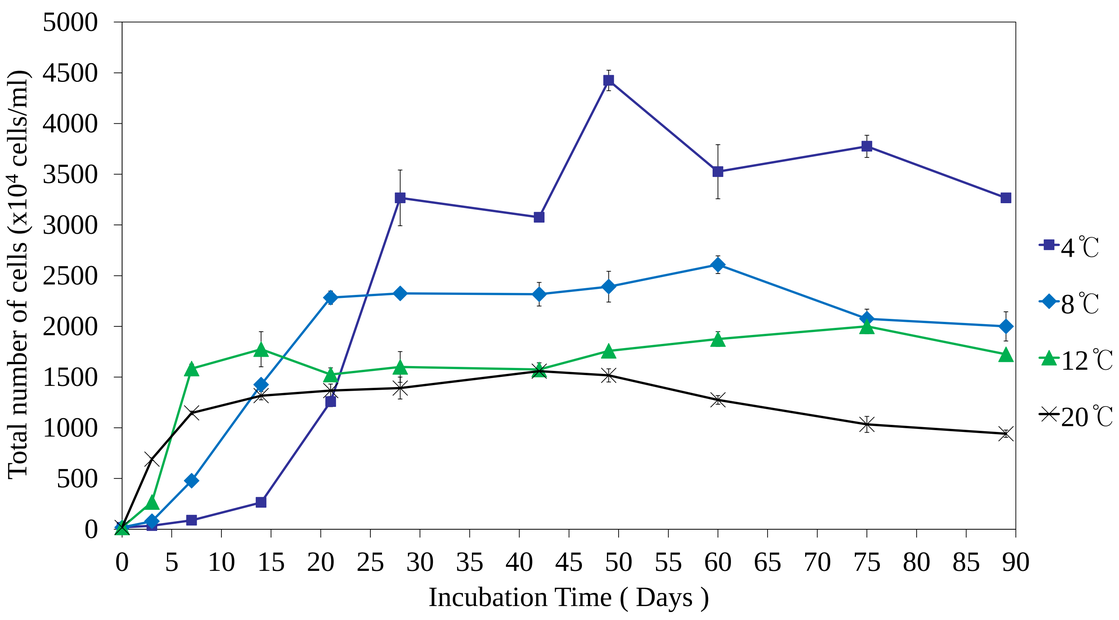

Fig. 1. Growth curves of ArM0029A at various temperatures. The ArM0029A cells grew in TAP medium and cultured at 4℃, 8℃, 12℃, and 20℃ under white light intensity of 40 μmol m^{-2} s^{-1}.

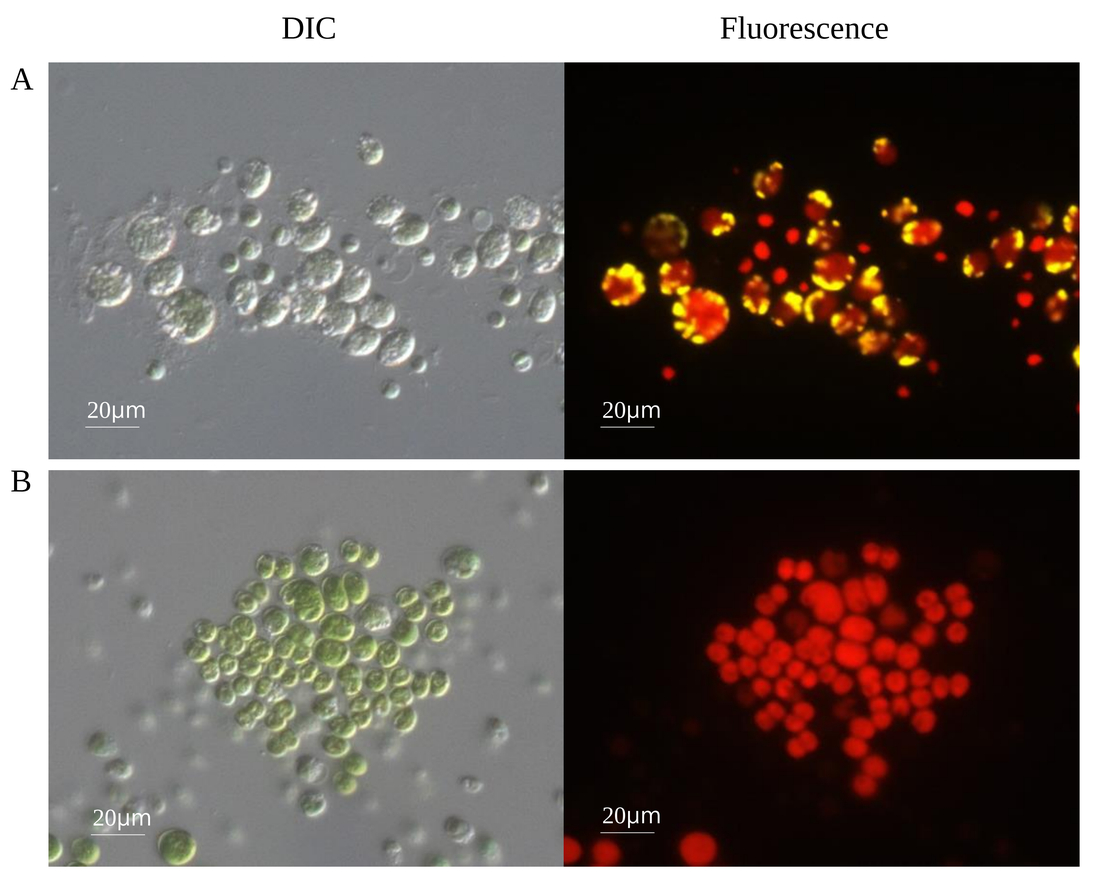

Fig. 2. Detection of lipid body in cell using Nile red staining. Cells were stained with Nile red and viewed for yellow-gold fluorescence at 488nm. A) ArM0029A, B) *Chlamydomonas reinhardtii* CC-125. Yellow and red colors indicate lipid droplets and chlorophyll, respectively.

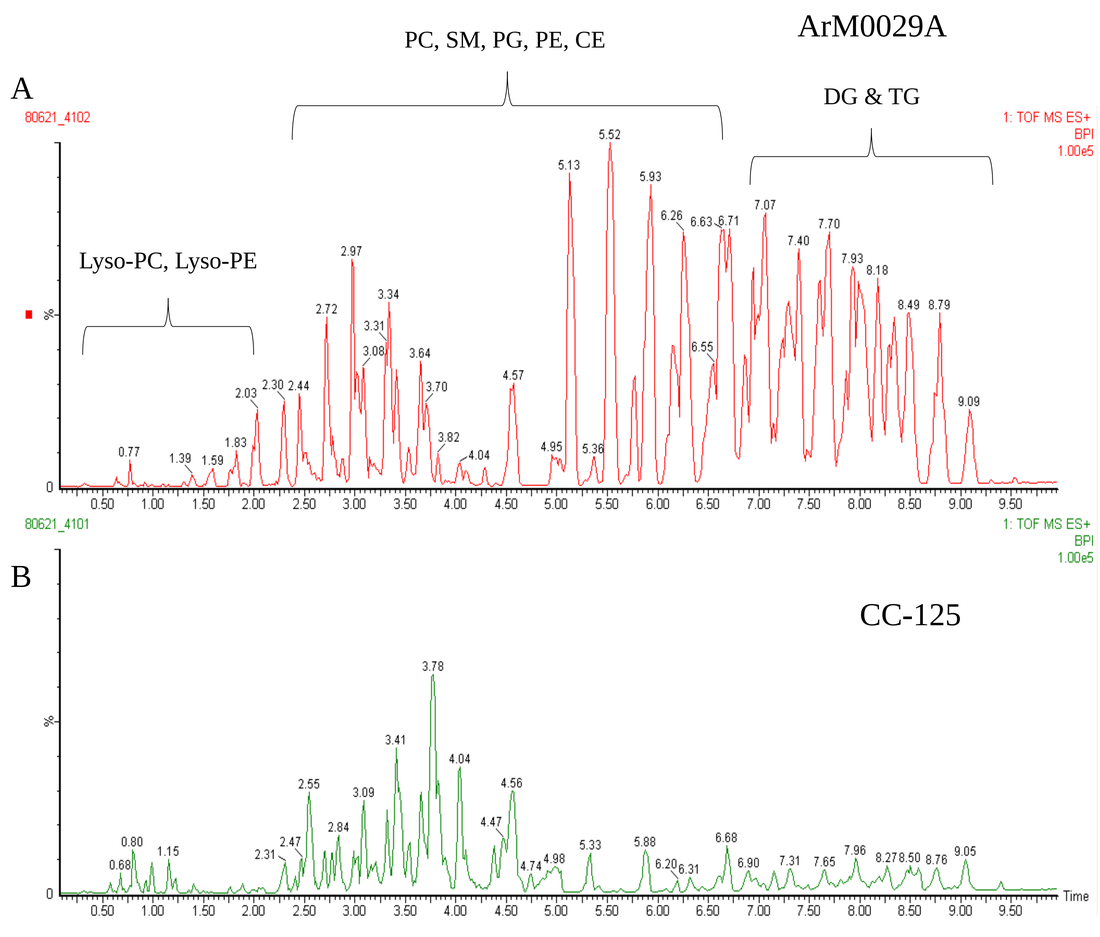

Fig. 3. Analysis of total lipid by LC-MS. A) ArM0029A, B) *Chlamydomonas reinhardtii* CC-125. It was found that *Chlamydomonas* sp. ArM0029A contained more phospholipid, triglycerides (TG) and diacylglycerols (DG) than the control strain (*Chlamydomonas reinhardtii* CC-125) in positive mode.

CONCLUSION

The suitable strains that display high growth and lipid accumulation rates is an important prerequisite for successful cultivation. Arctic ArM0029A may suggest economic feasibility for lipid production. Thus, *Chlamydomonas* sp. ArM0029A may be a source of biodiesel and functional lipids.

REFERENCES

1. Ahn JW et al., A New Arctic Chlorella Species for Biodiesel Production (2012) Bioresour Technol, 125, 340-343.
2. Greenspan P and Fowler S.D., Spectrofluorometric Studies of the Lipid Probe, Nile Red (1985) J Lipid Res, 26, 781-789.
3. Sharma K.K et al., High Lipid Induction in Microalgae for Biodiesel Production (2012) Energies, 5, 1532-1533.