Studies of arctic *Chlamydomonas* sp. KNM0029C for biodiesel production

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ABSTRACT

Biodiesel has attracted interest because this fuel not only can replace the conventional diesel as petroleum fuel but has a low effect to environmental issue. Biodiesel produced by polar microalgae could be used in cold countries, because it has advantages that clogging can be prevented in the fuel supply due to high content of polyunsaturated fatty acids which lower the melting point of biodiesel. In this study, we visualized intracellular lipid formations of Arctic KNM0029C, and analyzed contents of fatty acids by using GC. In addition, to enhance the cell mass and lipid production of KNM0029C, we optimized medium component of Tris-acetate-phosphate (TAP). In conclusion, main fatty acids contents were C18:1, C18:2, C18:3, and C20:2 at 4˚C and its maximum lipid production reached 178.6mg L^{-1} which was 2.3-fold higher than that of C. reinhardtii CC-125 as mesophilic strain. When KNM0029C was cultured in optimized TAP medium, total cell numbers and lipid production were increased to ~35% and ~10%, respectively. The results of the present study could potentially contribute toward large-scale lipid production at low temperatures.

MATERIALS & METHODS

Samples were collected from sea ice near the Dasan station located in Ny-Ålesund, 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 BODIPY 505/515 (0.2 μg mL^{-1}, 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene, Invitrogen, USA) in acetone. Lipids were extracted in hexane:methyl tert-butyl ether (1:1). Fatty acid methyl esters were analyzed by gas chromatography.

RESULTS

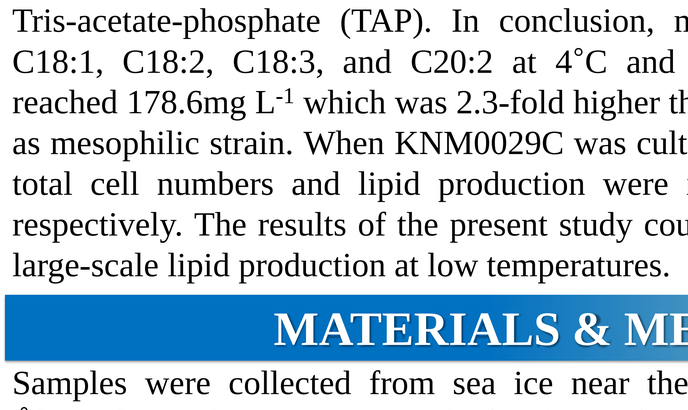
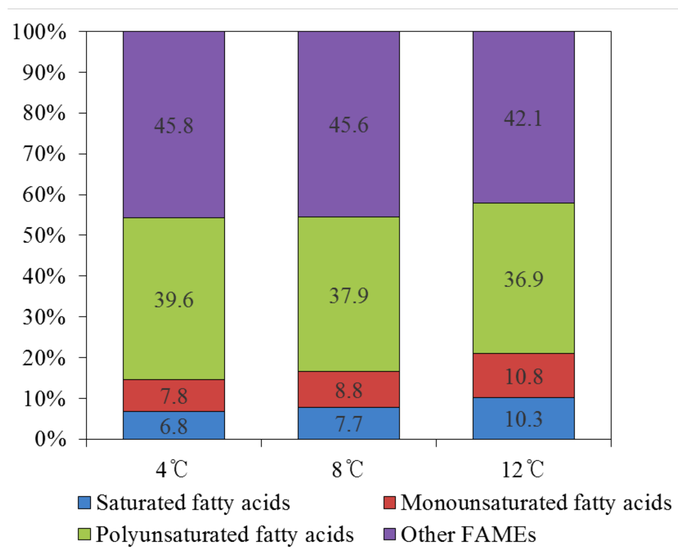


Fig. 1. Confocal microscopy images of BODIPY 505/515-stained *green* microalgae. (A) KNM0029C, (B) *C. reinhardtii* CC-125. Lipid droplet; *false-colored green*. Chlorophyll; *false-colored red*. Merged images of chlorophyll autofluorescence and BODIPY 505/515 images. The two microalgae were cultured in TAP-N for 2 weeks at 4 ˚C. *Scale bar* = 10μm

Table 1. Fatty acid composition (% total fatty acid) of Arctic *Chlamydomonas* sp. KNM0029C at low temperatures.

Fatty acid	4℃	8℃	12℃
C14:0	2.3	3.0	3.5
C16:0	3.5	3.9	5.8
C16:3	4.5	5.5	4.7
C18:1	6.8	7.7	8.3
C18:2	5.2	5.1	5.6
C18:3	20.7	20.6	20.5
C20:1	1.0	1.2	2.4
C20:2	9.2	6.8	6.0
C22:0	0.9	0.8	0.9
Other FAMEs	45.8	45.6	42.1



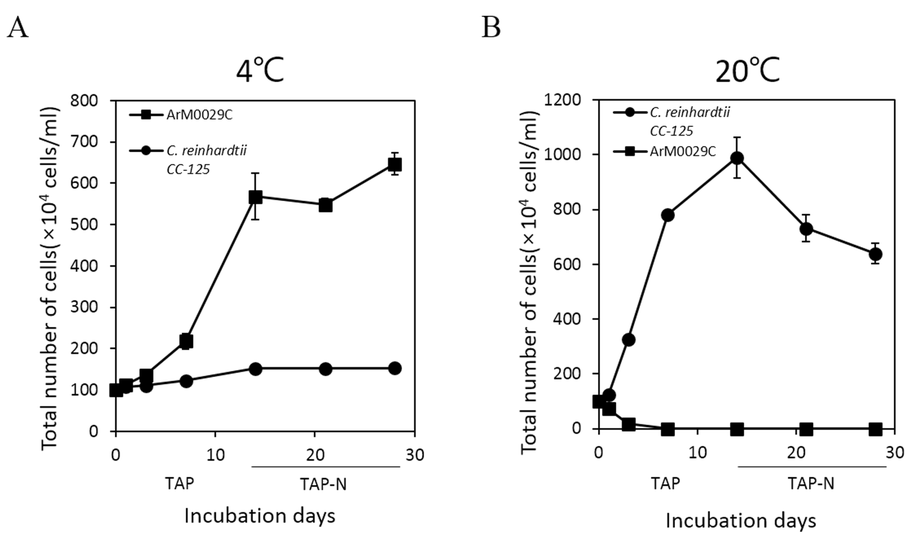


Fig. 2. Effects of temperature on the growth of KNM0029C and *Chlamydomonas reinhardtii* CC-125. (A) 4 ˚C; (B) 20 ˚C. Approximately 1 × 10^6 cells mL^{-1} were inoculated and grown in TAP for 2 weeks, and the cells were then cultivated in TAP-N for an additional 2 weeks. Data shown are the average cell densities ± SD from replicates

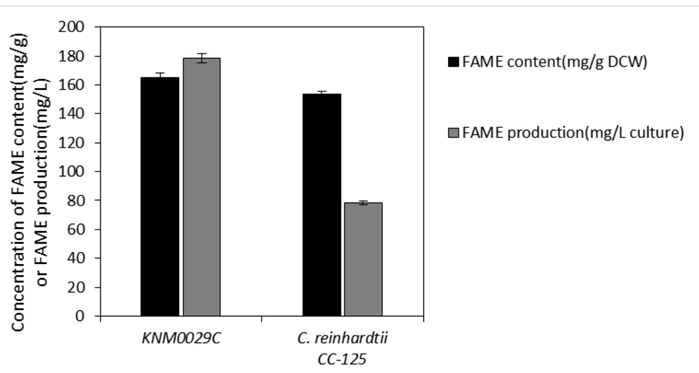


Fig. 3. Total FAME content of KNM0029C and *C. reinhardtii* CC-125 grown at 4˚C for 4 weeks. Cells were cultured for 2 weeks in TAP-N after 2 weeks of culture in TAP. Data shown are the average cell densities ± SD from replicates.

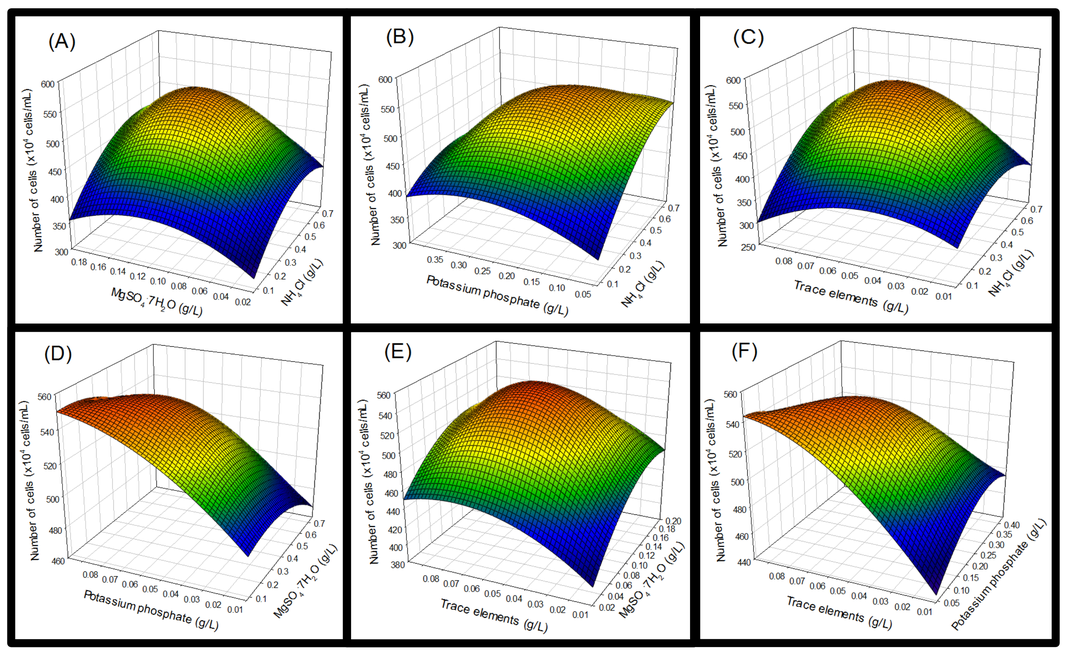


Fig. 4. Optimization of medium component of Tris-acetate-phosphate (TAP). Three-dimensional response plot showing the effect of **(A)** NH_4Cl and $MgSO_4 \cdot 7H_2O$, **(B)** NH_4Cl and potassium phosphate, (**C**) NH_4Cl and trace elements, (D) $MgSO_4 \cdot 7H_2O$ and potassium phosphate, (E) $MgSO_4 \cdot 7H_2O$ and trace elements, and (F) Potassium phosphate and trace elements

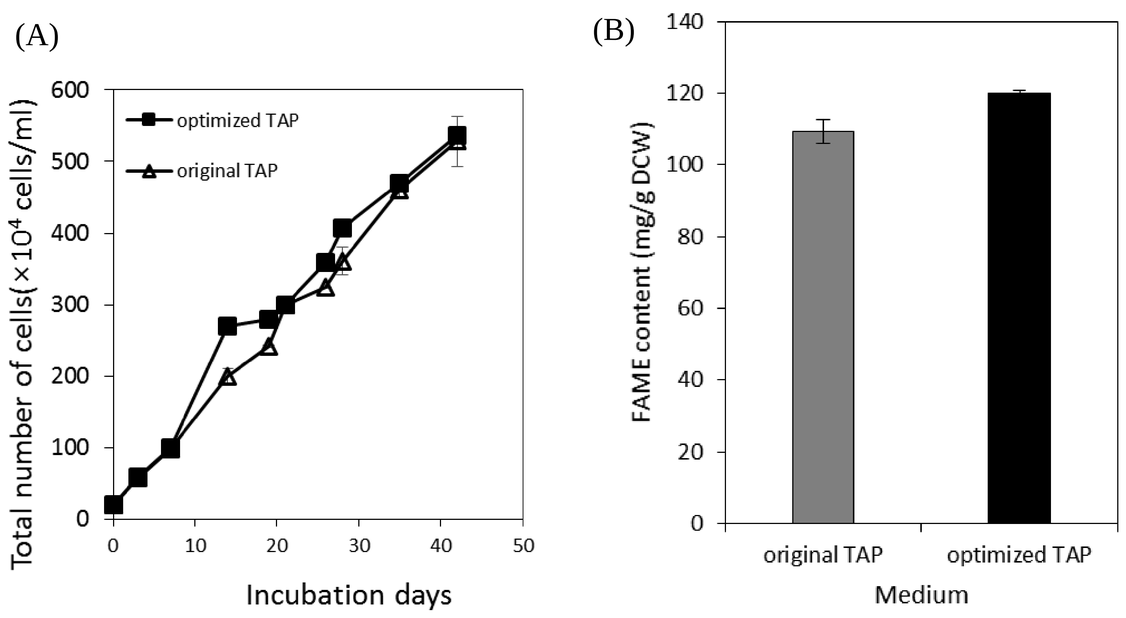


Fig. 5. Cell growth and lipid production in optimized TAP medium. (A) Time profile of KNM0029C cell growth. Open triangle conveys original TAP and solid square represents optimized TAP condition. **(B)** Cells were cultured in original TAP and optimized TAP, respectively. To compare lipid production of cells in each condition, total FAME of those was quantified by using GC.

CONCLUSION

A high level of unsaturation of fatty acids contributes toward lowering the cold filter plugging point (CFPP), which is used for evaluating the flow of biodiesel at low temperatures. In cold-climate countries, the use of CFPP values is crucial when selecting fuels because the clogging phenomenon can result in mechanical damage to vehicle engines. The fatty acid composition of KNM0029C makes it a promising candidate for the production of biofuels in cold environments. In conclusion, an Arctic microalgal strain identified as a *Chlamydomonas* sp. showed a growth temperature range from 4˚C to 12˚C and produced high concentrations of intracellular oils. This research provides suitable candidate and optimized medium at low temperature for biodiesel production and use.

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