Functional Analysis and Enzymatic Modification by Site-Directed Mutagenesis of an Omega-6 Fatty Acid Desaturase from Arctic *Chlamydomonas* sp.

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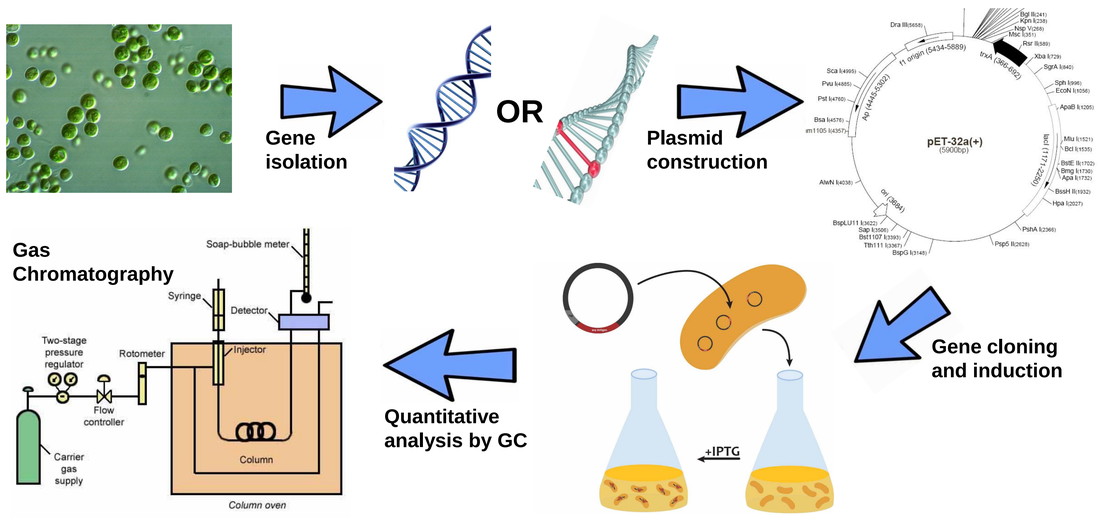
ABSTRACT

Arctic *Chlamydomonas* sp. is a dominant microalgal strain in cold or frozen freshwater in the Arctic region. The full-length open reading frame of the omega-6 fatty acid desaturase gene (AChFAD) was obtained from the transcriptomic database of Arctic *Chlamydomonas* sp. from the KOPRI Culture Collection of Polar Microorganisms (KCCPM). Amino acid sequence analysis indicated the presence of three conserved histidine-rich segments as unique characteristics of omega-6 FADs, and three transmembrane regions transported to plastidic membranes by chloroplast transit peptides in the N-terminal region. Arctic *Chlamydmonas* sp. omega-6 fatty acid desaturase with 48.2 kDa showed enzymatic activity enhancing the concentration of linoleic fatty acid in the *E. coli* expression system. The AChFAD6 desaturase activity was examined by expressing wild-type and V254A mutant (Mut-AChFAD6) heterologous recombinant proteins. Quantitative gas chromatography indicated that the concentration of linoleic acids in *AChFAD6*-transformed cells increased more than three-fold (6.73 ± 0.13 mg g^{-1} dry cell weight (DCW)) compared with cells transformed with vector alone. In contrast, transformation with *Mut-AChFAD6* increased the concentration of oleic acid to 9.23 ± 0.18 mg g^{-1} DCW, indicating a change in enzymatic activity to mimic that of stearoyl-CoA desaturase (SCD). These results demonstrate that AChFAD6 of Arctic *Chlamydomonas* sp. increases membrane fluidity by enhancing desaturating C18 fatty acids and facilitates production of large quantities of linoleic fatty acids in prokaryotic expression systems. Therefore, genetic modification on the membrane-spanning regions of fatty acid desaturases might increase the yield of target fatty acids and modulate enzymatic activities for industrial application.

MATERIALS & METHODS



RESULTS

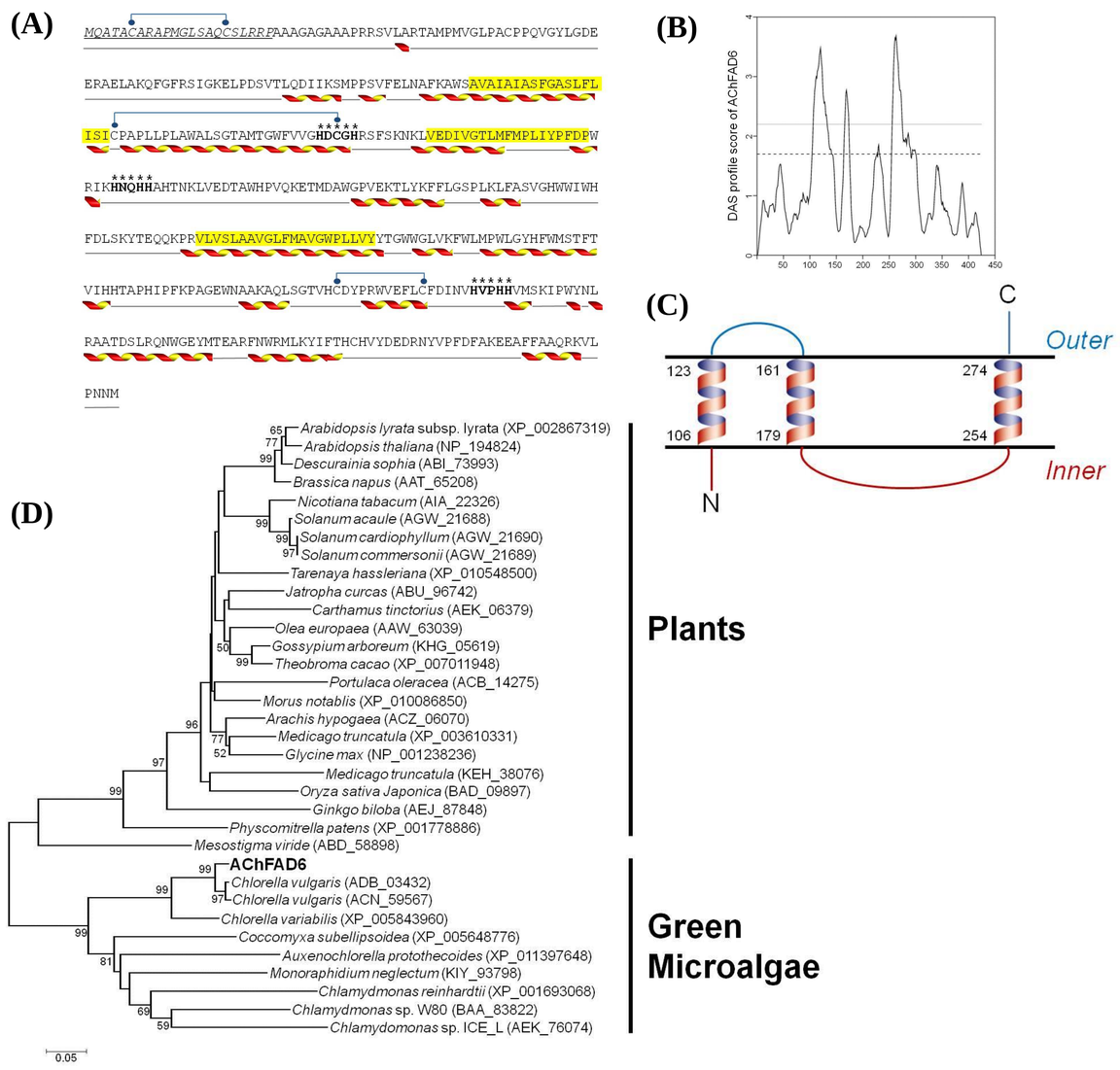


Fig. 1. Information of the secondary structure (A), membraneous topology (B and C) and phylogenetic relationship (D) of Arctic *Chlamydmonas* sp. omega-6 fatty acid desaturase (FAD, AChFAD6). In the figure (A), blue lines, yellow highlights and asterisks indicate disulfide bridges, transmembraneous amino acids and Histidine-rich motifs, respectively.

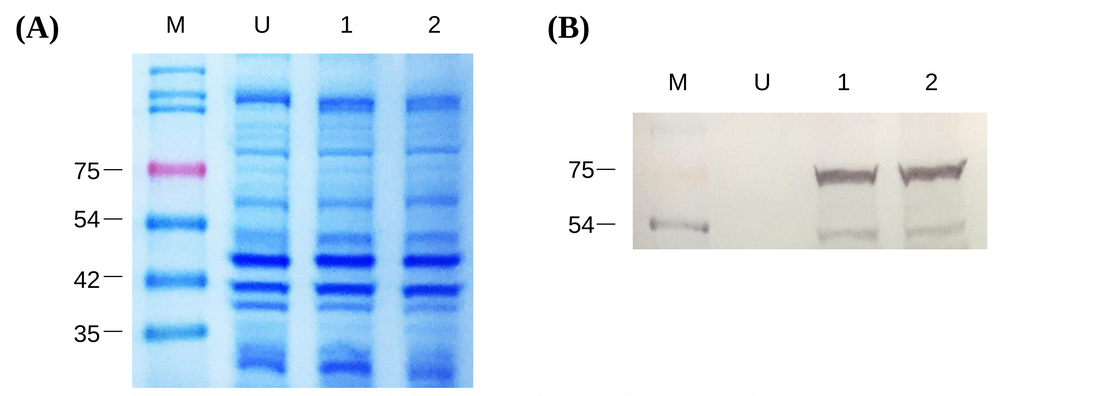


Fig. 2. Heterologous expression and detection of recombinant AChFAD6 in *E. coli*. M, protein ladder; U, Uninduced cells (Vector only); 1 and 2, *E. coli* cells harboring AChFAD6 and Mut-AChFAD6, respectively

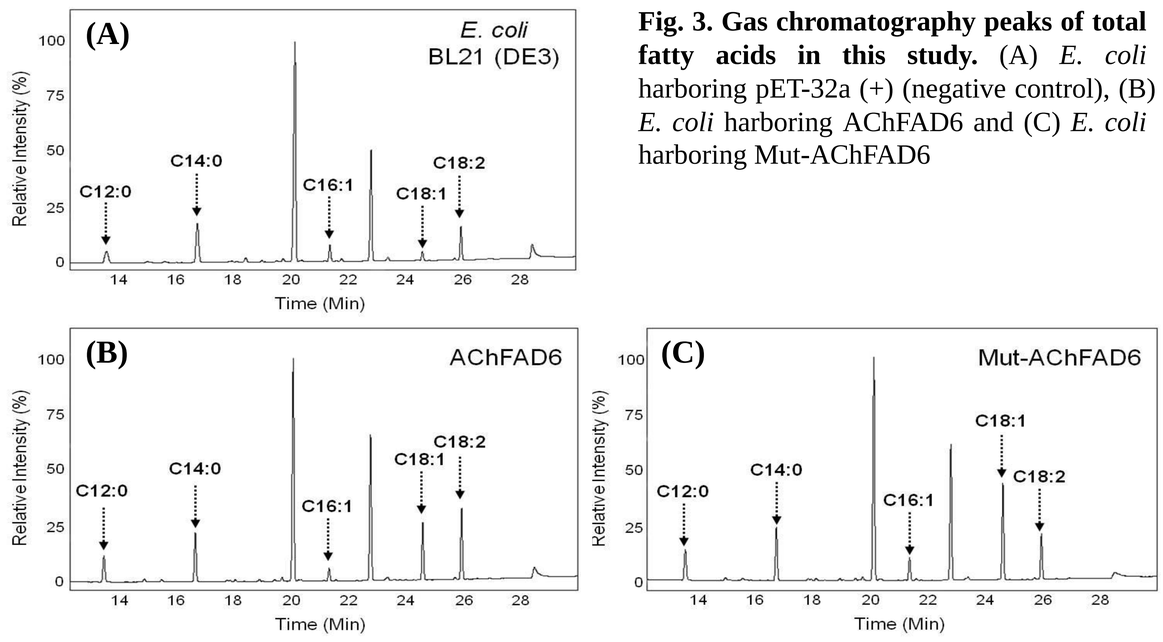


Fig. 3. Gas chromatography peaks of total fatty acids in this study. (A) *E. coli* harboring pET-32a (+) (negative control), (B) *E. coli* harboring AChFAD6 and (C) *E. coli* harboring Mut-AChFAD6

Table 1. Dominant contents of fatty acid methy esters (FAMEs) in expressed *E. coli* cells modified by induction of AChFAD6 and Mut-AChFAD6

	Organisms analyzed			
	E. coli (BL21(DE3))	AChFAD6	Mut-AChFAD6	*C. reinhardtii* cc-125
C12:0 (mg/g, %)	1.54 ± 0.05 (4.06 ± 0.24%)	2.99 ± 0.17 (5.32 ± 0.97%)	3.48 ± 0.17 (5.39 ± 0.35%)	-
C14:0 (mg/g, %)	3.85 ± 0.15 (10.12 ± 0.65%)	5.02 ± 0.25 (8.93 ± 1.58%)	5.38 ± 0.21 (8.32 ± 0.45%)	-
C16:1 (mg/g, %)	1.11 ± 0.06 (2.92 ± 0.23%)	1.26 ± 0.05 (2.24 ± 0.38%)	**2.23 ± 0.07 (3.45 ± 0.16%)**	1.00 ± 0.02 (0.77 ± 0.03%)
C18:1 (mg/g, %)	0.56 ± 0.05 (1.47 ± 0.17%)	**5.45 ± 0.16 (9.67 ± 1.52%)**	**9.23 ± 0.18 (14.27 ± 0.49%)**	1.40 ± 0.04 (1.08 ± 0.05%)
C18:2 (mg/g, %)	2.06 ± 0.12 (5.41 ± 0.45%)	**6.73 ± 0.13 (11.92 ± 1.76%)**	**4.24 ± 0.13 (6.56 ± 0.30%)**	-
Total FAs (mg/g)	38.07 ± 0.97	63.66 ± 1.27	64.71 ± 0.97	130.00 ± 1.56

* FAME composition:C12:0, Lauric acid methyl ester; C14:0, Myristic acid methyl ester; C16:1, Palmotoleic acid methyl ester; C18:1, Oleic acid methyl ester; C18:2, Linoleic acid methyl ester; -: not detected

CONCLUSION

- The gene and the deduced amino acid sequences of omega-6 fatty acid desaturase (FAD) were isolated and enzymatic activity was investigated by gas chromatography.
- The omega-6 FAD with 48.2 kDa showed typical activity of the enzyme improving the concentration of linoleic fatty acid in the *E. coli* expression system.
- An V254A as substitution of the first amino acid in the third transmembrane domain resulted in a enzymatic change from FAD to stearoyl-CoA desaturase.
- The first amino acid in third membrane-spanning region is crucial to maintain omega-6 FAD activity. Therefore, genetic modification on the membrane-spanning regions of FADs can increase the yield of target fatty acids and modulate enzymatic activities for industrial application.

ACKNOWLEDGEMENT

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