Genetic Isolation and Functional Analysis of Stearoyl-CoA Desaturases and Omega-6 Fatty Acid Desaturase from Polar Psychrophilic Microorganisms

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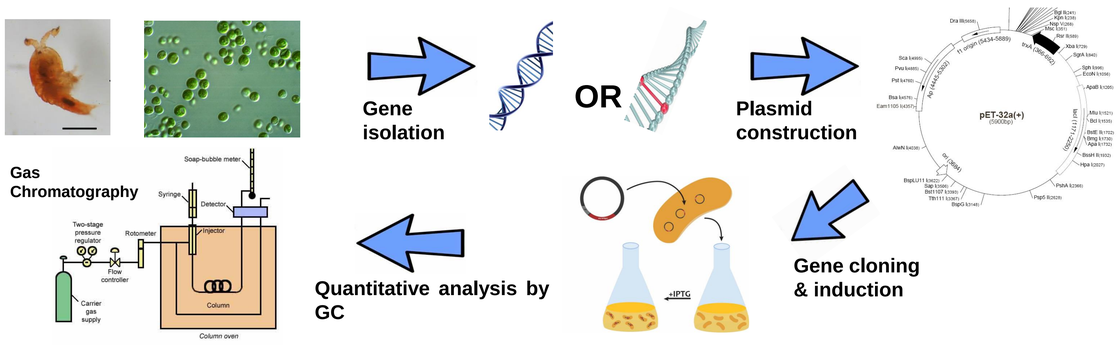
ABSTRACT

Stearoyl-CoA desaturase (SCD, Δ9 desaturases) is a key regulator in fatty acid metabolism that catalyzes the desaturation of stearic acid to oleic acid and controls the intracellular levels of monounsaturated fatty acids (MUFAs). Two SCD genes were discovered from an Antarctic copepod, *Tigriopus kingsejongensis*, which were collected in a tidal pool near the King Sejong Station, Antarctica. The molecular weights deduced from both genes were estimated to be 43.1 kDa (TkSCD-1) and 26.1 kDa (TkSCD-2). The amino acid sequences were compared with those of fatty acid desaturases and sterol desaturases from various types of organisms and used to analyze the relationships among TkSCDs. The enzymatic functions of both SCDs, as assessed by heterologous expression of recombinant proteins in *E. coli*, revealed that the amount of C16:1 and C18:1 fatty acids increased by more than three-fold after induction with isopropyl β-D-thiogalactopyranoside. Especially, 19.36 ± 1.34% and 14.04 ± 1.41% of C18:1 fatty acids were produced in cells expressing TkSCD-1 and TkSCD-2, respectively. The results of this study suggest that both *TkSCD* genes encode a functional desaturase that is capable of increasing the amounts of palmitoleic fatty acids and oleic fatty acids in a prokaryotic expression system.

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. 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. 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) proteins. Quantitative analysis 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) 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 SCD. These results demonstrate that AChFAD6 increases membrane fluidity by enhancing desaturating C18 fatty acids in prokaryotic systems. In addition, mutation 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

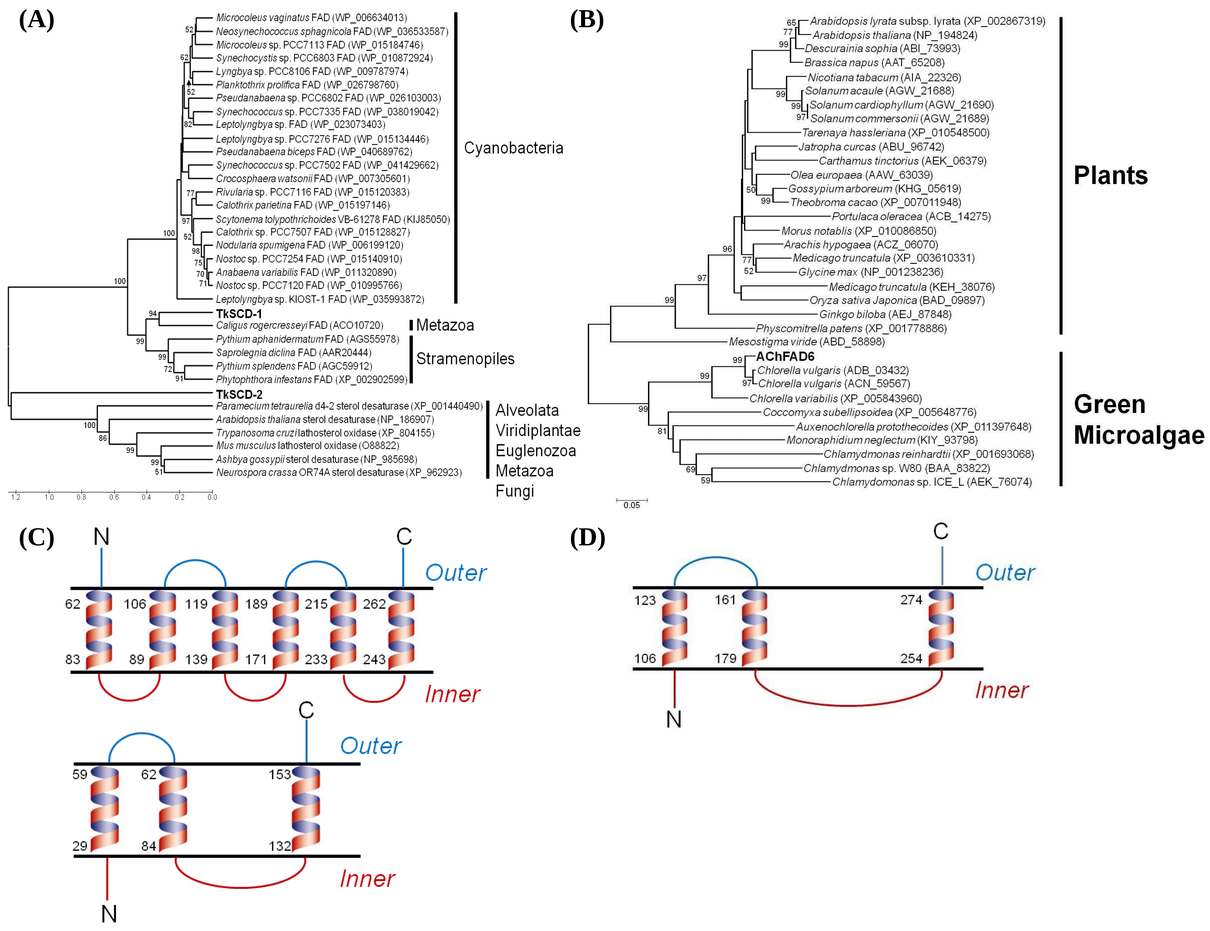


Fig. 1. Phylogenetic relationship (A and B) and membraneous topology (C and D) of TkSCDs and AChFAD6, respectively

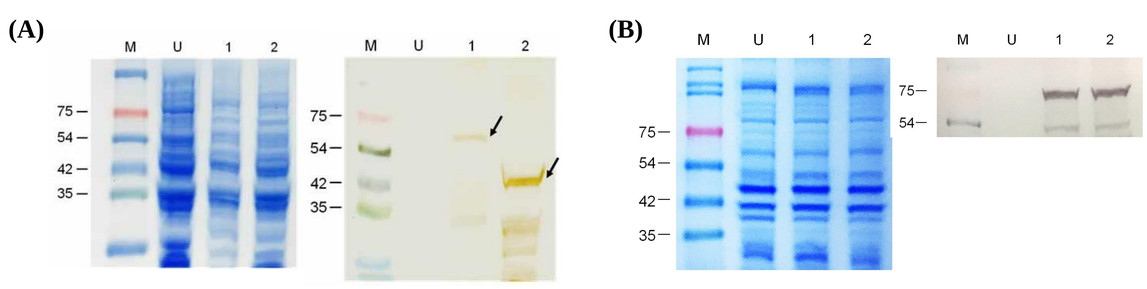


Fig. 2. Heterologous expression and detection of recombinant SCDs and FADs in *E. coli*. M, protein ladder; U, Uninduced cells (Vector only); (A) 1 and 2, *E. coli* cells harboring TkSCD-1 and TkSCD-2, respectively; (B) 1 and 2, *E. coli* cells harboring AChFAD6 and Mut-AChFAD6, respectively

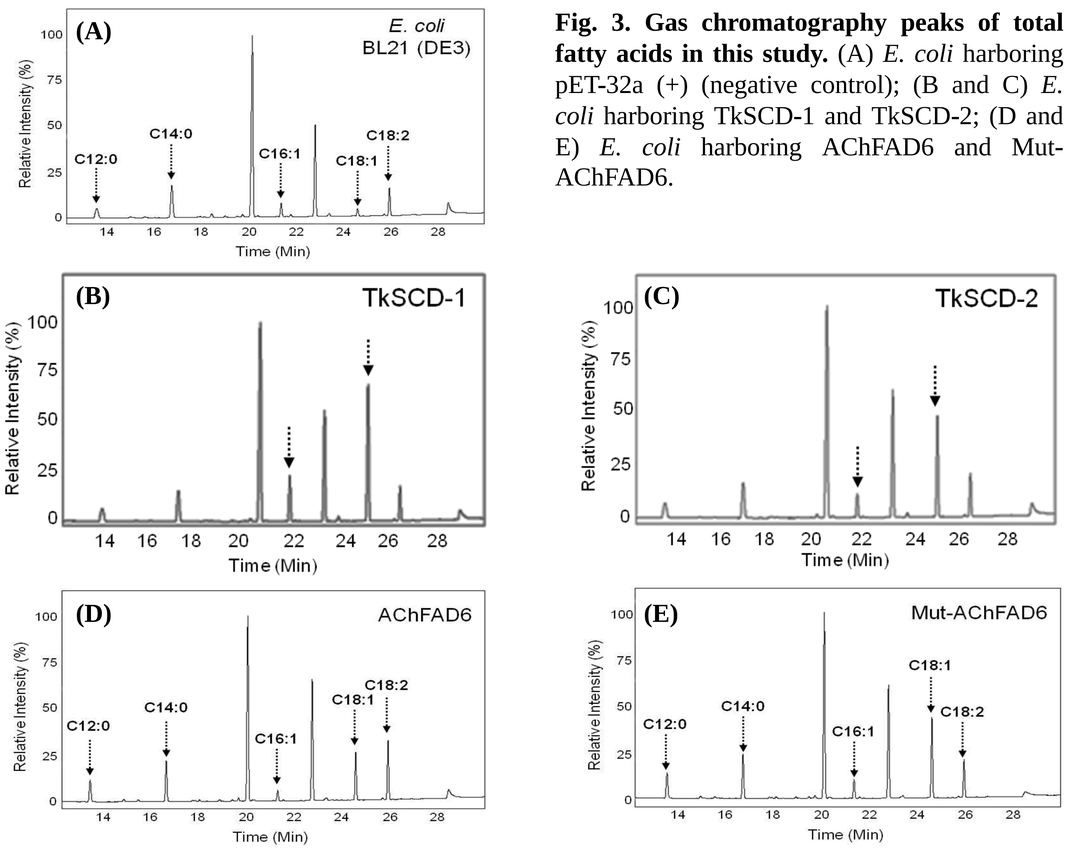


Fig. 3. Gas chromatography peaks of total fatty acids in this study. (A) *E. coli* harboring pET-32a (+) (negative control); (B and C) *E. coli* harboring TkSCD-1 and TkSCD-2; (D and E) *E. coli* harboring AChFAD6 and Mut-AChFAD6.

Table 1. Dominant contents of fatty acid methy esters (FAMEs) in expressed *E. coli* cells modified by induction of TkSCD-1 and TkSCD-2

	Organisms analyzed			
	E. coli (BL21(DE3))	TkSCD-1	TkSCD-2	*C. reinhardtii*
C16:0 (mg/g, %)	13.99 ± 0.60 (40.74 ± 1.73%)	15.90 ± 0.41 (33.14 ± 0.87%)	12.68 ± 0.39 (33.45 ± 1.02%)	27.75 ± 0.38 (21.34 ± 0.29%)
C16:1 (mg/g, %)	1.11 ± 0.06 (2.92 ± 0.23%)	3.34 ± 0.28 (6.95 ± 0.57%)	1.24 ± 0.22 (3.27 ± 0.58%)	1.00 ± 0.02 (0.77 ± 0.03%)
C18:0 (mg/g, %)	0.33 ± 0.02 (0.96 ± 0.06%)	0.37 ± 0.03 (0.76 ± 0.05%)	0.28 ± 0.03 (0.73 ± 0.07%)	1.43 ± 0.13 (1.10 ± 0.10%)
C18:1 (mg/g, %)	0.56 ± 0.05 (1.47 ± 0.17%)	**9.40 ± 0.58 (19.59 ± 1.20%)**	**5.34 ± 0.31 (14.08 ± 0.80%)**	1.40 ± 0.04 (1.08 ± 0.05%)
Total FAs (mg/g)	38.07 ± 0.97	47.96 ± 3.66	37.89 ± 2.54	130.00 ± 1.56

Table 2. 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*
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; C16:0, Paltimic acid methyl ester ; C16:1, Palmotoleic acid methyl ester; C18:0, Stearic 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 stearoyl-CoA desaturases from Antarctic copepod and an omega-6 fatty acid desaturase (FAD) from Arctic microalga were isolated and enzymatic activity was investigated by gas chromatography.
- The both genes of *T. kingsejongensis* was analyzed to be related to FADs from Metazoa, Alveolata and Euglenozoa. The omega-6 FAD of Arctic *Chlamydomonas* sp. was shown to have close relationship with those from green microalgae.
- From the analysis of secondary structure, it was strongly predicted that TkSCD-1 and TkSCD-2 were belong to the integral proteins with six and three transmembraneous domains. The AChFAD6 was analyzed to show three transmembraneous regions and was predicted to be located into chloroplastic membranes.
- The TkSCD-1 demonstrated remarkably 17.7-fold increment (9.40 ± 0.58 mg/g, 19.59± 1.20%) of C18:1 (oleic acid) compared with that of the negative control (vector-only).
- An V254A as substitution of the first amino acid in the third trans-membrane 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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