We isolated a cDNA clone with homology to known desaturase genes from Oblongichytrium sp., recently classified as a new genus of thraustochytrids (Labyrinthulomycetes), and found that it encoded Δ5-desaturase by its heterologous expression in yeast. The enzyme had higher activity toward 20:4n-3 than 20:3n-6, indicating that this δ5-desaturase can be used in the production of n-3 polyunsaturated fatty acids in transgenic organisms.

Key words: Δ5-desaturase; Labyrinthulomycetes; Oblongichytrium; polyunsaturated fatty acids; thraustochytrid

Thraustochytrids (Labyrinthulomycetes), marine microorganisms with substantial amounts of polyunsaturated fatty acids (PUFAs),1,2) are useful resources in obtaining genes encoding enzymes for the production of PUFAs. In the present study, we isolated a Δ5-desaturase gene from Oblongichytrium sp., which was recently classified as a new genus in thraustochytrids.3) Δ5-desaturase had a sequence fairly distinct from that previously identified in thraustochytrids (Thraustochytrium sp.),4) and had a preference for n-3 PUFAs as substrates. The Oblongichytrium sp. SEK 347 (NBRC 102618) strain was isolated from Gushikawa on Okinawa Island, Japan.3) It was cultured in GPY medium containing 2% glucose, 1% peptone, and 0.5% yeast extract in a half-salt concentration of artificial seawater (Tropic Marine, Aquarientechnik, Wartenberg, Germany). Cultures were grown at 25°C in a rotary shaker (120 rpm) for 5 d. Total RNA was extracted from the cells using the RNaseasy plant mini kit (Qiagen, Hilden, Germany) after cell disruption with a Braun homogenizer (Melsungen, Germany) using glass beads (0.45–0.5 mm in diameter). The extracted RNA was then used to synthesize full-length cDNA using the SMART RACE cDNA amplification kit (Clontech Laboratories, Mountain View, CA). The prepared cDNA was used as a template for PCR amplification with degenerate primers (5'-CAAGCAYCCNGGNGG-3' and 5'-GGGAAANAGRTGTTG-3'), which were designed to hybridize conserved histidine box sequences in fatty acid desaturases. A putative desaturase was screened from amplified products by DNA sequencing. The 5'- and 3'-ends of the putative desaturase were amplified using the SMART RACE cDNA amplification kit with a primer used in 5'-rapid amplification of cDNA ends (RACE) (5'-AAAGAGA-GCATACAGTCCCAACCAGG-3') and a primer used in 3'-RACE (5'-AAGCTTCAAGCGGAAAACCTCCTGCAG-3'). A full-length ORF of the putative desaturase was amplified with primers (5'-ATCTCGAGGACATCAACGTCACT-3' and 5'-GCTCTAGAGGTCAGTACATTACC-3') containing XhoI and XbaI sites (underlined) respectively, which were designed from elucidated sequences by 5'-RACE and 3'-RACE. PCR amplification was carried out using the cDNA from Oblongichytrium sp. as a template with KOD plus (Toyobo, Osaka, Japan). The PCR amplification product was cloned into pL1091-5,3) and verified by DNA sequencing.

Saccharomyces cerevisiae BY4741 strain (Mat a leu2Δ0 his3Δ1 ura3Δ0 met15Δ0) was transformed with pL1091-5 vector with and without the putative desaturase gene. The transformed yeast cells were cultured at 30°C in synthetic medium containing 0.17% Bacto-yeast nitrogen base without amino acids and ammonium sulfate, 2% glucose, 1 g/l of ammonium sulfate, 20 μg/ml of histidine, 20 μg/ml of methionine, 500 μg/ml of leucine, 0.25% Tergitol NP-40, and 20 μg/ml of exogenous fatty acids. Lower amounts of ammonium sulfate and larger amounts of leucine than usual were used to increase lipid production, as described previously.6) The fatty acids were directly transmethy-
lated from dried cells, as described previously. The products of desaturation reaction (20:4n-6 \((\Delta^5,8,11,14)\) and 20:5n-3 \((\Delta^5,8,11,14,17)\)) were assigned by co-migration with authentic standards, and were confirmed by the similarity of spectra of GC/MS to that of authentic standards.

Oblongichytrium sp. SEK347 mostly contained n-3 fatty acids as PUFAs (7.8% eicosapentaenoic acid \((20:5n-3 \,(\Delta^5,8,11,14,17))\), 3.1% docosapentaenoic acid \((22:5n-3 \,(\Delta^7,10,13,16,19))\), and 22.9% docosahexaenoic acid \((22:6n-3 \,(\Delta^4,7,10,13,16,19))\) of the total fatty acids \((5^d\text{ culture at } 25\,^\circ\text{C})\)). The presence of 22:5n-3 \((\Delta^7,10,13,16,19)\) instead of 22:5n-6 \((\Delta^4,7,10,13,16)\) was different from many other thraustochytrids. This observation suggests that desaturation and elongation of PUFAs in this strain preferentially use n-3 fatty acids as substrates. A full-length ORF of putative desaturase from Oblongichytrium sp. SEK347 was obtained by PCR amplification using degenerated primers and RACE reactions, as described above. The entire ORF consisted of 1,308 bp, which encoded a polypeptide of 435 amino acids with a molecular mass of 50 kDa (Fig. 1). The amino acid sequence was significantly homologous to known front-end desaturases, containing three histidine boxes and a cytochrome b5 domain.

To determine the enzyme activity of the putative desaturase, the entire ORF was inserted into yeast expression vector pL1091-5 containing an ADH1 promoter, and the vectors containing the putative desaturase gene were used to transform yeast cells. The transformed yeast cells were cultured in the presence of exogenously added fatty acids. The fatty acid profiles of the yeast cells as measured by GC analysis revealed that the putative desaturase catalyzed the conversion of 20:3n-6 \((\Delta^5,8,11,14)\) and of 20:4n-3 \((\Delta^5,8,11,14,17)\) into 20:4n-6 \((\Delta^5,8,11,14)\) and 20:5n-3 \((\Delta^5,8,11,14,17)\) (Fig. 2), indicating that the cloned gene encoded a \(\Delta^5\)-desaturase. The \(\Delta^5\)-desaturase had higher activity toward 20:4n-3 \((\Delta^5,8,11,14,17)\) than 20:3n-6 \((\Delta^5,8,11,14)\). This result suggests that this \(\Delta^5\)-desaturase can be used in the production of n-3 polyunsaturated fatty acids in transgenic organisms.

The putative desaturase did not catalyze the conversion of linoleic acid \((18:2 \,(\Delta^9,12))\), \(\alpha\)-linolenic acid \((18:3n-3 \,(\Delta^9,12,15))\) or 22:5n-3 \((\Delta^7,10,13,16,19)\) (data not shown), indicating that the enzyme did not have \(\Delta^6\)- or \(\Delta^4\)-desaturase activity. Instead, it catalyzed the conversion of 20:2n-6 \((\Delta^6,11,14)\) and of 20:3n-3 \((\Delta^6,11,14,17)\), although the conversion ratios (product fatty acid/substrate and product fatty acids in yeast cells; 5\%:6\%\% and 6\%:1\%\% for 20:2n-6

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**Fig. 1.** Comparison of the Deduced Amino Acid Sequence of \(\Delta^5\)-Desaturase from Oblongichytrium sp. with Related Sequences. The conserved HPQG motif of the cytochrome b5 domain is marked with asterisks and three histidine boxes are underlined. The conserved amino acids are shaded. The DDBJ/EMBL/GenBank accession nos. for \(\Delta^5\)-desaturase from Oblongichytrium sp. (ObD5), Phaeodactylum tricornutum (PtD5), Thalassiosira pseudonana (TpD5), Phytophthora megasperma (PmD5), and Thraustochytrium sp. (ThD5) are AB432913, AY082392, AY817152, AJ510244, and AF489588.
In conclusion, we isolated and characterized Δ5-desaturase from Oblongichytrium sp., recently classified as a new genus of thaustochytrids. It had a preference for n-3 PUFAs as substrates. This might provide a useful tool for the production of n-3 PUFAs in transgenic organisms.

References

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(Δ11,14) and 20:3n-3 (Δ11,14,17) respectively) were much lower than that of 20:3n-6 (Δ8,11,14) or 20:4n-3 (Δ8,11,14,17) (24.8 ± 0.6% and 36.6 ± 4.1%). These products did not have the same retention times as 20:3n-6 (Δ8,11,14) and 20:4n-3 (Δ8,11,14,17) respectively, indicating that the enzyme did not have Δ8-desaturase activity. We assume that they were 20:3n-6 (Δ5,11,14) and 20:4n-3 (Δ5,11,14,17), since many Δ5-desaturases introduce a double bond at the Δ5 position of fatty acids that do not contain a double bond at the Δ8 position.8–11

In thaustochytrids, Δ5-desaturase has been sequenced in *Thraustochytrium* sp., but the sequence identity between Δ5-desaturase from *Oblongichytrium* sp. and from *Thraustochytrium* sp. was only 21%. Instead, the sequence from *Oblongichytrium* sp. showed higher similarity to that of Δ5-desaturase from a diatom, *Phaeodactylum tricornutum* (Δ9 (36%)) and *Thalassiosira pseudonana* (Δ9 (30%). The substrate specificity such as the preference for n-3 PUFAs of Δ5-desaturase in *Oblongichytrium* sp. was similar to Δ5-desaturase from an oomycete fungus, *Phytophthora megasperma* (sequence identity, 26%).

References


