Cloning and functional expression of a *Thraustochytrium* Δ5 desaturase gene and its knock out system

Yong-Hoon Lee, Young-Su Jeong, Geun-Joong Kim, and Byung-Ki Hur
Institute of Biotechnological Industry, College of Engineering, Inha University
TEL:+82-32-860-8676, FAX:+82-32-875-0827

Abstract

It is generally known that polyunsaturated fatty acids (PUFAs) are good for anti-ageing and inhibition of oxidation and also are critical constituents of membranes particularly found in the retina and central nervous system. In microorganism-based PUFAs biosynthesis, the genus *Thraustochytrids* is well evaluated for their potential as a promising candidate in the practical production of PUFAs. In this study, we attempted to extract intactly total nucleic acid from this strain and used for cloning. Using the extracted nucleic acid and degenerated primers, we amplified a putative Δ5 desaturase gene that contained 1320-nucleotide and encoded 439 amino acids. This gene exhibited an expected function, when expressed in *P. pastoris,* in the presence of appropriate exogenous substrate DGLA. The conversion product was identified as AA by using GC and mass spectrometer. The resulting gene was also used for the construction of knock-out strains replaced genes with truncated open reading frames. These results and information can provide a basis for the construction of engineered strains suitable for the practical production of PUFAs.

Acknowledgements

This work was supported by the Korean Research Foundation Grant (KRF-2004-005-D00006).

Reference

1. Qui, X., Hong, H. and MacKenzie, S. L. Identification of a Δ4 desaturase from *Thraustochytrium* sp. involved in the biosynthesis of docosahexaenoic acid by heterologous expression in *Saccharomyces cerevisiae* and *Brassica Juncea.* 2001. *J. Biol. Chem.* **276**: 31561-31566.